



## Vancomycin-Resistant Enterococci Detected in Clinical Samples in Tertiary Centre of North Zone of India

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### Abstract

Enterococcus are emerging as an important cause of nosocomial infections and are gaining clinical importance because of multi drug resistance especially the increasing prevalence of Vancomycin Resistant Enterococci (VRE). The present study was conducted in a tertiary care centre in India with the aim to study prevalence of enterococcal infections in various clinical specimens and their antibiotic susceptibility pattern, especially against Vancomycin. A total of 50 isolates of Enterococci were identified from various clinical specimens. Maximum VRE isolates were from urine samples followed by pus and blood. Also, most sensitive drug for VRE was Linezolid. Least sensitivity was seen with Penicillin and Ampicillin.

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### Keywords

Enterococcal infections,  
Vancomycin Resistant Enterococci,  
Linezolid.

### Introduction

Enterococcus were originally classified as enteric gram positive cocci but later included in genus *Streptococcus*. But on basis of various studies on fatty acid composition and nucleic acid hybridization, they were assigned a separate genus called as *Enterococcus*.

Enterococcus considered a normal commensal of intestinal tract are emerging as an important cause of nosocomial infections, ranking only second to staphylococci<sup>(1)</sup>. Species most common implicated in causing clinical human infections: *E. faecalis* (80-90%) and *E. faecium* (10-15%)<sup>(2)</sup>. The most common nosocomial infections produced by these organisms are Urinary tract infections (associated with instrumentation and antimicrobial resistance, intra-abdominal, Pelvic infections, also cause surgical wound infections,

bacteraemia, endocarditis, neonatal sepsis and rarely meningitis<sup>(3)</sup>. A major reason why these organisms survive in hospital environment is the intrinsic resistance to several commonly used antibiotics and perhaps more importantly, their ability to acquire resistance to all currently available antibiotics, either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons<sup>(4)</sup>. Also there has been increase in the rate of VRE especially in hospital environment, which may be intrinsic or acquired by gene transfer<sup>(5)</sup>. Their virulence is contributed by several proteins secreted in extracellular medium like Hemolysin-cytolysin, extracellular serine proteinase and protease gelatinase<sup>(6)</sup>. The infections caused by *Enterococcus* are usually seen in previously colonized patients and spread through hands of health care workers and the environment.

Severe infections caused by enterococcus are often very difficult to treat because of tremendous capacity of organism to acquire resistance to Penicillin, High concentration of aminoglycosides and Vancomycin. The increased prevalence of VRE infections poses a great challenge for the clinicians as well as the healthcare institutions. Epidemiology of VRE varies from one hospital to other depending on the hospital size, patient characteristics, antibiotic usage patterns and geographic location. The rates of VRE infections are highest among critically ill patients in ICUs having limited treated options<sup>(7)</sup>.

The injudicious use of antimicrobial agents and the rising colonization pressure are the largest contributors to selection of vancomycin resistance. Environmental surfaces and medical equipment items in the patient's room frequently become contaminated with VRE and may also serve as a reservoir especially in the rooms of patients who have diarrhea<sup>(7)</sup>. Advanced age, severity of illness, inter- institutional transfer of the patient, prolonged hospital stay, gastrointestinal surgery, transplantation, exposure to medical devices, especially central venous catheters, and heavy exposure to broad-spectrum antimicrobial drugs are risk factors for colonization and infection with VRE<sup>(8)</sup>.

First report of glycopeptides resistance was observed in 1980s and total six phenotypes of glycopeptides resistance have been observed in enterococci, Van A and Van B being most important. Van A with high level inducible resistance to Vancomycin and Teicoplanin, Van B with variable resistance to Vancomycin and sensitive to Teicoplanin and Van C with intrinsic low level resistance to Vancomycin and susceptible to Teicoplanin. Van A and Van B are usually associated with *E. faecalis* and *E. faecium*<sup>(8)</sup>. Since the first isolation of vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium* in 1988<sup>(9)</sup> vancomycin resistant enterococci (VRE) have become one of the major threats to public health in many parts of the world.

VRE were first detected in Europe in 1986, but now they have also been reported from Australia, Belgium, Canada, Denmark, Italy, Malaysia<sup>(10)</sup>. In AIIMS New Delhi, India 5 resistant isolates of *E. faecalis* were reported by disk diffusion and agar screen methods, these were further identified into van types by PCR.

The increasing spread of VRE along with acquisition of resistance to newer

antimicrobials warrants continued surveillance of these versatile pathogens.

Keeping all the scenario of VRE in mind, present study was conducted to isolate enterococci from various clinical specimens and find out *in vitro* antimicrobial susceptibility and rate of VRE isolates.

### Aims and objectives

To study the prevalence of enterococci and its antibiotic resistance pattern at a tertiary care hospital in Jammu from January 2019 to April 2019.

### Materials and Methods

The present study was conducted in Bacteriology section of Microbiology Lab in GMC, Jammu. Total 50 samples of Enterococcus were isolated from various clinical isolates like urine, pus, blood, wound swab, vaginal swab, sputum by inoculating the sample on blood agar at 37°C for 24hours. Furthermore, following biochemical tests were done for confirmation of gram positive catalase negative cocci: Bile esculin agar test and sugar fermentation tests like glucose, maltose, sucrose.

Antibiotic susceptibility testing was done by Kirby Bauer method as per the CLSI guidelines using commercially available discs from HiMedia.

Colonies were inoculated into peptone water and turbidity was adjusted at 0.5 McFarland standard. Broth culture was spread on the plate to make lawn culture on Mueller Hilton agar. Discs were applied on surface of agar and plates were incubated overnight at 30-35°C in ambient air. Result was interpreted using CLSI guidelines.

The following antibiotics were tested: Penicillin, Ampicillin, Gentamycin, Ciprofloxacin, Vancomycin, Clindamycin, Linezolid, Norfloxacin

### Results and Discussions

In the present study total 50 samples of Enterococci were isolated from various samples: Urine (28), Blood (7), Pus (13), Sputum (2) as shown in table 1. Maximum sensitivity was seen with Linezolid followed by least sensitivity was seen for Penicillin, Ampicillin (Table 2).

Maximum Vancomycin Resistant Isolates were seen in urine isolates followed by pus and blood as shown in table 3.

**Table.1** Number of isolates from various samples

| SAMPLE | NUMBER OF ISOLATES | % OF ISOLATES(n=50) |
|--------|--------------------|---------------------|
| Urine  | 28                 | 56                  |
| Blood  | 7                  | 14                  |
| Pus    | 13                 | 26                  |
| Sputum | 2                  | 4                   |

**Table.2** Antibiotic Resistance pattern of Enterococci

| Antibiotics                                  | Number of Resistant isolates | Number of sensitive isolates |
|--|------------------------------|------------------------------|
| Penicillin                                   | 46 (92%)                     | 4 (8%)                       |
| Ampicillin                                   | 47 (94 %)                    | 3 (6%)                       |
| Ciprofloxacin                                | 46 (92%)                     | 4 (8 %)                      |
| Clindamycin                                  | 46 (92 %)                    | 4 (8%)                       |
| Vancomycin                                   | 22 (44%)                     | 28 (56 %)                    |
| Linezolid                                    | 12 (24%)                     | 38 (76%)                     |
| FOR URINE,<br>(ISOLATES(n=28)<br>Norfloxacin | 18 (64%)                     | 10 (36%)                     |

**Table.3** Number of VRE in various samples

| SAMPLE | NUMBER OF VRE |
|--------|---------------|
| Urine  | 9             |
| Pus    | 6             |
| Blood  | 5             |
| Sputum | 2             |

Present study focuses on increasing role of enterococci in nosocomial infections and also their tendency to show remarkable resistance to antimicrobial agents<sup>(11)</sup>. Our study showed that maximum enterococcal isolates were from urine samples followed by pus and blood.

Chakraborty *et al.*, conducted a study in Kolkata which also showed similar results with maximum isolates from urine (66%), followed by wound swab (19.6%) and blood (8.5%) which correlate to present study<sup>(12)</sup>. Similarly study conducted by Karmarkar *et al.*, in Mumbai showed maximum isolates from urine (50%) followed by blood and pus swabs<sup>(13)</sup>.

Antimicrobial resistance is one of the major public health problems especially in developing countries where relatively easy availability and higher consumption of medicines have led to disproportionately higher

incidence of inappropriate use of antibiotics and greater levels of resistance compared to developed countries.

In the present study maximum Vancomycin Resistant Enterococci (VRE) isolates were seen from urine specimens followed by pus and blood. Most sensitive drug was Linezolid while highest resistance was seen against Penicillin and Ampicillin.

Shrivastava *et al.*, observed similar results showing more no. of VRE in urine isolates with 27% resistance to vancomycin in urine enterococci with along with multiple drug resistance<sup>(14)</sup>.

Shah *et al.*, also observed 8% Vancomycin resistant enterococci in various clinical isolates with drug resistance to other antibiotics. All the studies including ours showed high level resistance to Penicillins<sup>(15)</sup>.

The present study concludes the occurrence of multiple drug resistance in enterococci in various clinical samples with emerging VRE strains hereby limiting the use of these drugs for therapeutic use. So *in-vitro* antibiotic susceptibility for clinical samples should be done. This study also emphasizes on the proper infection control in clinical practice and empirical use of drugs like Vancomycin to avoid further resistance.

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