



## VANCOMYCIN RESISTANT ENETROCOCCI-A STUDY ON PREVALENCE OF VANCOMYCIN RESISTANCE ENTEROCOCCI IN A TERTIARY CARE HOSPITAL IN SOUTH KERALA

### Clinical Microbiology

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### ABSTRACT

**Introduction:** Vancomycin-resistant enterococci (VRE), are bacterial strains of the genus *Enterococcus* that are resistant to the antibiotic vancomycin. They have been associated with outbreaks of hospital-acquired (nosocomial) infections around the world. VRE can remain viable in the environment for an extended time period posing a problem for infection control in hospitals and nursing homes. They also pose a major therapeutic challenge as they possess intrinsic resistance to most of the commonly used antibiotics and can acquire resistance to most of the current available antibiotics. Assessment of the prevalence and changing trends of VRE infections is required to help plan infection control measures, which should be implemented in the hospital and also in the community to reduce the mortality and morbidity caused by these VRE infections.

**Objectives:** This study was undertaken to determine the prevalence of Vancomycin resistance among *Enterococcus* species isolates obtained from clinical samples in a tertiary care hospital in southern India and also to detect resistant genes by molecular methods. **Methods:** A descriptive cross-sectional study was done on 150 non-duplicate isolates of *Enterococcus* species from different samples received at the Microbiology laboratory of Sree Gokulam Medical College and Research Foundation included consecutively from January 2021 to December 2021. Identification was based on Gram staining, colony morphologies, and biochemical tests. Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method on Mueller Hinton agar. Vancomycin resistance was determined by Vancomycin screen agar and vancomycin E-test method. Statistical Package for Social Sciences (SPSS) was used for preparing and analysis of data. **Results:** Out of the 150 *Enterococcus* species, 2% (3) isolates were resistant to Vancomycin by Vancomycin screen agar & Vancomycin MIC test in which 2 were *E. faecium* and 1 was *E. faecalis*. Out of 3 VRE, all were sensitive to Teicoplanin, so they were likely of vanB phenotype. **Conclusion:** Vancomycin resistant *Enterococcus* species have emerged as important nosocomial pathogens in our institution with resistance of 2% per cent in our study. Increasing trend in the VRE rates, as seen in India and worldwide, need to be monitored and checked with active surveillance.

### KEYWORDS

*Enterococcus* species; Vancomycin resistant *Enterococci*; glycopeptides; vanA; vanB

Vancomycin-resistant enterococci (VRE) are bacterial strains of the genus *Enterococcus* that are resistant to the antibiotic vancomycin. Vancomycin-sensitive enterococci acquire new DNA in the form of plasmids or transposons which encode genes that confer Vancomycin resistance.

The most common clinically isolated species of enterococcus are *E. faecalis* and *E. faecium*. High-level Vancomycin-resistant *E. faecalis* and *E. faecium* clinical isolates were first documented in France in 1988. Since then, VRE have been associated with outbreaks of hospital-acquired (nosocomial) infections around the world which is probably due to the increasing use of third-generation cephalosporins to which enterococci are naturally resistant.(1)

VRE can remain viable in the environment for an extended time period posing a problem for infection control in hospitals and nursing homes. They also have been detected as part of the enteric flora in non-symptomatic patients.(2) These colonized patients serve as potential sources for transmission of VRE to other patients and medical personnel.

Also, since VRE have intrinsic resistance to most of the commonly used antibiotics and the ability to acquire resistance to most of the current available antibiotics, either by mutation or by receipt of foreign genetic material, they pose a major therapeutic challenge. The possibility of transfer of vancomycin resistance genes to other gram-positive organisms, such as *Staphylococcus aureus*, raises significant concerns about the emergence of vancomycin-resistant *Staphylococcus aureus*.(3)

### MATERIALS AND METHODS:

This study was carried out in the department of Microbiology, Sree Gokulam Medical College & Research Foundation, Venjaramoodu, Thiruvananthapuram, India, from January 2021 to December 2021. All isolates of *Enterococcus* species obtained from urine, blood, pus,

discharge from skin and soft tissue infections, sputum, ear swab, throat swab over the study period were included in the study. Sample processing was done immediately as per standard procedures. The *Enterococcal* isolates grown from the above said samples were taken for further study and identified by standard techniques following Facklam and Collin's phenotypic characterization scheme for *Enterococci*. Antimicrobial susceptibility patterns of all the *Enterococci* isolates were tested by Kirby-Bauer disk diffusion following the standard guidelines issued by the Clinical Laboratories Standards Institute (CLSI). The antibiotics tested were Penicillin A (10 units), Ampicillin (10 microgram), Erythromycin (15 microgram), Tetracycline (30 microgram), High Level Gentamicin (120µg), Chloramphenicol(30 microgram), Vancomycin (30 microgram), Teicoplanin (30 microgram), Linezolid (30 microgram). For urine isolates, susceptibility testing for Norfloxacin (10 microgram), Ciprofloxacin (5 microgram) and Nitrofurantoin (300 µg) was also done. Screening for Vancomycin resistance was done by agar screen method and Vancomycin Ezy MIC™ Strip with MIC ranging from 0.016 µg/ml to 256 µg/ml.

### RESULTS:

A total of 150 enterococci were isolated from various clinical samples and processed during the study period. Maximum number of isolates was from the age group of 61 - 80 years followed by age group of 41 -60 and least number of isolates from 20 and below. Out of the total clinical isolates included, 77(51.4%) belonged to male patients while 73(48.6%) belonged to female patients. Majority of the isolates, 70 were from urine samples (47%), 66 (44%) from pus samples and 14(9%) from blood samples. Majority of the samples, 35.6% were from the General Surgery department, followed by General Medicine 21.7%. The least were from the Pediatric (1.5%) and Dermatology departments (1.1%). Of the 150 enterococci isolates, 140 isolates (93.33%) were identified as *Enterococcus faecalis* and 10 isolates (6.66%) as *Enterococcus faecium*. No other species were isolated. Highest prevalence of resistance was observed against tetracycline

(84%) followed by erythromycin (52.8%) and penicillin (43.2%). High level gentamicin resistance (HLGR) was observed in 37% of the enterococcal isolates in this study. 3 isolates (2%) were found to be resistant to Vancomycin. Resistance was confirmed by Vancomycin screen agar and Vancomycin E- strip method, 2 of which were identified as *E. faecium* and 1 as *E. faecalis*. MIC obtained for vancomycin by E-test for the vancomycin resistant *E. faecalis* isolate was 64µg/ml, while the MIC obtained for the 2 vancomycin resistant *E. faecium* isolates was above >260µg/ml, suggestive of high level Vancomycin resistance. All the VRE isolates were sensitive to Teicoplanin, suggestive of vanB phenotype. The mean age of patients from whom VRE was isolated was 54 years. 2 of the VRE isolates, were isolated from pus samples and 1 from urine sample. All the VRE isolates (100%) were found to be resistant to penicillin, ampicillin, tetracycline and erythromycin. All the three isolates were negative for van A/van B gene by multiplex PCR study.

## ENTEROCOCCUS

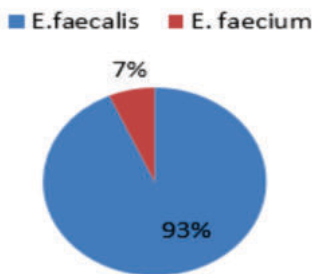


Fig 1. Species distribution among Enterococcal isolates

Table 1: Species Distribution Among VRE Isolates

Enterococcus spp	No. of isolate	Percentage (%) (N=150)
<i>Enterococcus faecalis</i>	1	33.3
<i>Enterococcus faecium</i>	2	66.6
Total VRE	3	100

Table 2: Sample Distribution Among VRE Isolates

SAMPLE	No. of VRE isolates	Percentage (%)
Blood	0	0
Pus	2	66.66
Urine	1	33.3
Total	3	100

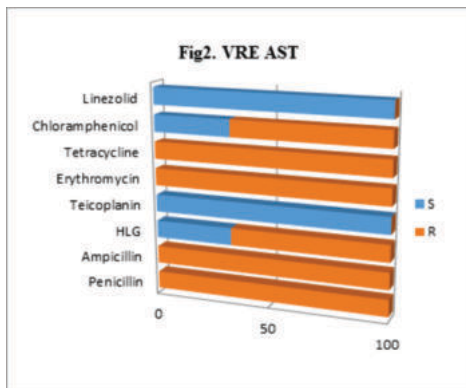


Fig 3. Vancomycin E strip with *E. faecium* MIC >256µg/ml (Resistant)



## DISCUSSION:

*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*. VRE infections may be difficult to cure because the bacteria do not respond to many antibiotics. Sometimes more than one antibiotic is prescribed to help stop the infection. This study was conducted to detect prevalence of vancomycin resistant enterococci in clinical isolates. In this study, *Enterococcus faecalis* and *Enterococcus faecium* were the two most common species of enterococcal isolates, totaling 150 isolates from diverse clinical samples. They were found resistant to multiple antibiotics like penicillin, tetracycline and ciprofloxacin. By using the Vancomycin E-strip and Vancomycin screen agar methods, 3 isolates were determined to be vancomycin resistant enterococci. All the 3 isolates were negative for Van A/van B gene by PCR. Geographical location and the subject population's use of antibiotics affect the prevalence of VRE. Increasing trend in the VRE rates, as seen in India and worldwide, need to be monitored and checked with active surveillance. Although VRE had not previously been identified in this facility, the present prevalence rate of Vancomycin resistant enterococci was found to be 2%. This study emphasises the necessity of running frequent surveillance programmes for timely VRE detection in the community and in hospitals. It furthermore reinforces the significance for the adoption of strict infection control measures, such as the 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.

## CONCLUSION:

VRE has been recognized as a global public health problem as infections due to VRE have been linked with increased rates of morbidity, mortality, a longer length of hospital stays, higher healthcare expenses due to the reduced number of therapeutic options with resistant organisms requiring a higher frequency of surgical interventions for infection control. (4)VRE has been listed under "serious threats" in CDC's 2019 Antibiotic Resistance Threats Report as about 30% of all healthcare-associated enterococcal infections were found resistant to vancomycin. (6) In India, the "National Programme on the Containment of Antimicrobial Resistance", launched under the aegis of the National Centre for Disease Control (NCDC), has recognised *Enterococcus* spp. as one of the pathogens of public health importance and to be tracked. (7) Enterococci has been thus recognized as a priority pathogen included in the national AMR surveillance. The Indian Network for Surveillance of Antimicrobial Resistance (INSAR) reported reduced susceptibility to vancomycin in about 12% of the isolates of *Enterococcus faecalis* in 2018. (7) As per ICMR's AMRSN 2021 report, Vancomycin resistance in Enterococci (*E. faecalis* and *E. faecium*) was 14.9%, thus showing an increasing trend. (8) Scaling up of surveillance and availability of data is essential to identify patterns of resistance, as well as to quantify the nationwide mortality burden owing to AMR. Assessment of the prevalence and changing trends of VRE infections are of immense help in planning infection control measures which should be implemented in the hospital and also in the community to reduce the mortality and morbidity caused by these VRE infections.

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