
**E-ISSN:** 2706-9575
**P-ISSN:** 2706-9567
IJARM 2021; 3(1): 331-335
Received: 10-07-2020
Accepted: 15-09-2020

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# Pattern in Minimum Inhibitory Concentration of Vancomycin and Tigecycline on Clinical Isolates of *Enterococcus* Species Isolated from Clinical Samples at our Tertiary Care Hospital

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**DOI:** https://doi.org/10.22271/27069567.2021.v3.i1f.160

**Abstract**
**Background**: Genus *Enterococcus* have emerged as nosocomial pathogens. Urinary tract infections followed by intra-abdominal abscesses, Wounds, usually intra-abdominal or pelvic, are the next most common sites. Bacteremia is the third most common infection caused by *Enterococci*. Enterococcal meningitis is rare and is seen primarily in neonates and inpatients who have undergone complicated neurosurgical procedures [4]. There is an increasing role of *Enterococci* in nosocomial infections in the recent years mostly because of their intrinsic resistance to many clinically useful antibacterial drugs. One of the leading cause of nosocomial infection is Vancomycin-resistant Enterococci (VRE). For good therapy an effective, accurate and early detection of VRE along with Minimum Inhibitory Concentrations (MIC) is necessary. Tigecycline has a broad spectrum of activity against enterococci (vancomycin-susceptible). Haemolysin is a cytolytic protein and the producing strains of *Enterococci* have been shown to be associated within creased severity of infection [9]. In order to know the current scenario of minimum inhibitory concentration of Vancomycin and Tigecycline and the production of Haemolysin by some strains of Enterococci, the present study has been done.
**Methods:** This study was performed on Clinical isolates of Enterococcus which were received from tertiary care hospitals and processed at Department of Microbiology, Kasturba Medical College, Mangalore and Microbiology diagnostic laboratory (KMC hospital, Ambedkar circle), Mangalore. The study was done for a period of 6 months from Nov 2017-May2018. Convenient non-random sampling method was followed to collect clinical isolates. All isolates of Enterococcus from various clinical specimens like pus, deep tissue, urine, blood and body fluids were included in the study.
**Result:** Out of 100 Enterococcus isolates collected, majority were of *E. faecium*. Among all the isolates, 5% were resistant to vancomycin, but 100% sensitivity to tigecycline. Hemolysin produced by 46% of the total isolates. It has shown its association of the Enterococcus species to various antibiotics and their resistant pattern.

**Keywords:** MIC, Enterococcus, VRE

## Introduction
Genus *Enterococcus* is Gram positive, ovoid shaped cocci which are arranged in pairs or short chains. [1] Though they are the normal flora of the intestinal tract, oral cavity and vagina, but have emerged as nosocomial pathogens [2]. This genus is composed of 38 species, the most important of which are *Enterococcus faecalis* and *Enterococcus faecium* – both human intestinal colonizers [3]. Urinary tract infections followed by intra-abdominal abscesses are by far the most common enterococcal infections in humans majority being nosocomial. Wounds, usually intra-abdominal or pelvic, are the next most common sites. Bacteremia is the third most common infection caused by *Enterococci*. Enterococcal meningitis is rare and is seen primarily in neonates and in patients who have undergone complicated neurosurgical procedures [4].
*E. faecalis* is isolated from approximately 80-90% of human infections and *E. faecium* from most of the rest. *Enterococcus* is mainly isolated from urine, pus, blood and body fluids. [5] There is an increasing role of *Enterococci* in nosocomial infections in the recent years mostly because of their intrinsic resistance to many clinically useful antibacterial drugs including

*β*-lactam antibiotics, aminoglycosides and glycopeptides like vancomycin. The unyielding response to the treatment owe to the high mortality rate of Enterococcal infections [5].
Tigecycline is a new, semi synthetic glycylcycline [6]. Resistance to the tetracycline class is mediated by ribosomal protection mechanism or by efflux. Tigecycline has more potent activity against tetracycline-resistant organisms. Based on *in vitro* susceptibility data, tigecycline has a broad spectrum of activity against enterococci (vancomycin-susceptible) [7]. Quinupristin/dalfopristin, linezolid, daptomycin, tigecycline and vancomycin are FDA approved agents as appropriate choices for treatment of Gram-positive pathogens [7].
The third leading cause of nosocomial infection is Vancomycin- resistant Enterococci (VRE). Occurrence of VRE is a persisting clinical problem in all geographic areas and continues to be exacerbated by clonal dissemination within the health care facility leading to limited therapeutic options. Steady pandemic spread of VRE along with acquisition of resistance to newer antimicrobials warrants continued surveillance of these versatile pathogens. For good therapy an effective, accurate and early detection of VRE along with Minimum Inhibitory Concentrations (MIC) is necessary [8].
Haemolysin is a cytolytic protein capable of lysing human, horse and rabbit erythrocytes [9]. Haemolysin producing strains of *Enterococci* have been shown to be virulent in animal models and human infections and to be associated with increased severity of infection [9].
In order to know the current scenario of minimum inhibitory concentration of Vancomycin and Tigecycline and the production of Haemolysin by some strains of Enterococci, the present study is undertaken to determine the Minimum Inhibitory Concentration (MIC) of Tigecycline and Vancomycin on clinical isolates of Enterococcus and to correlate between hemolysin production and drug resistance pattern.

## Materials and Methods

This study was performed on Clinical isolates of Enterococcus which were received from tertiary care hospitals and processed at Department of Microbiology, Kasturba Medical College, Mangalore and Microbiology diagnostic laboratory (KMC hospital, Ambedkar circle), Mangalore. The study was done for a period of 6 months from Nov 2017-May 2018.
Convenient non-random sampling method was followed to collect clinical isolates. All isolates of Enterococcus from various clinical specimens like pus, deep tissue, urine, blood and body fluids were included in the study. All the media and chemicals used in the study were procured from HI Media Laboratories Pvt Ltd. Mumbai, India. Urine samples were cultured on UTI chrome agar and Cysteine Lactose Electrolyte Deficient medium by semi –quantitative method. Direct microscopic examination of the urine samples was also done. Blood samples were processed in automated systems (BacT/ALERT3D). They were then inoculated on Blood agar and MacConkey's agar. All the other samples were inoculated on Blood agar and MacConkey's agar. The plates were incubated at 37°C for 24 hours and growth was examined. Identification of genus Enterococcus was done by colony morphology, Gram's staining, Bile Esculin test and speciation was done by Arabinose fermentation test.
Enterococcus isolates were subjected to antibiotic susceptibility testing by modified Kirby-Bauer disc diffusion method, and results were interpreted according to Clinical Laboratory Standards Institute (CLSI) guidelines. [12]The antibiotics tested included Ampicillin (10µg), Penicillin (10units), Teicoplanin (30µg), Gentamicin (10µg), Tetracycline (30µg), erythromycin (15µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Vancomycin (30µg), Nitrofurantoin (300µg) and Streptomycin. All antibiotic susceptibility tests were conducted using ATCC *E. faecalis* 29212 as control. The antibiotics were purchased from Hi Media Laboratories, Mumbai, India.

Dilution susceptibility testing methods are used to determine the minimal concentration, usually in micro gram per milliliter, of an anti-microbial agent required to inhibit or kill a microorganism.
In the agar dilution method for minimum inhibitory concentrations, a standardized suspension of bacteria was inoculated onto a series of Mueller-Hinton agar plates, containing different concentrations (two fold serial dilutions) of Vancomycin and Tigecycline. Enterococcus isolates were inoculated in Mueller-Hinton broth and incubated for 4 hours at 37°C and adjusted to 0.5 McFarland Standard which were then inoculated onto the MHA plates. MIC control strain ATCC *E. feacalis* 29212 and ATCC *E. feacalis* 51299 was used. MIC of both Vancomycin and Tigecycline was interpreted for all VRE isolates by agar dilution method according to the guidelines established by the CLSI [25].
MIC of Vancomycin and Tigecycline was determined by E test for all *Enterococci isolates*. A lawn culture of *Enterococci*, 0.5 McFarland's standard was made on 5% Mueller Hinton agar. The E strip obtained from HI Media were placed on the inoculated plates. The plates were then incubated at 37°C in 5% $CO_2$ for 24 hours. The MIC was read where the elliptical zone intersected the MIC scale on the E-strip. Vancomycin sensitive strain of *E. feacalis* ATCC 29212 was used as a negative control and *E. feacalis* ATCC 51299, vancomycin resistant strain was used as a positive control (CLSI2017). The results for Vancomycin were interpreted as sensitive (MIC<4), intermediate (MIC 8-16) and resistant (MIC>32) based on CLSI guidelines (2017) and for Tigecycline MIC≤0.5 was considered as sensitive.
Hemolysin production was detected by inoculating enterococci samples onto freshly prepared Mueller-Hinton blood agar supplemented with 5% human blood. Plates were incubated overnight at 37°C in a carbon dioxide chamber and evaluated at 24 and 48 hours. A clear zone of β-hemolysis around the streak on human blood agar was considered to be a positive indication of hemolysin production.

## Results

A total of 100 Enterococcus isolates were collected, among which 46% were of E. faecalis, and 54% were of *E. faecium* out of which 41 strains were isolated from males and 59 strains from females, as shown in figure 1. All the strains used in this study were isolated from different clinical specimens (Table 1). Figure 2 shows the distribution of Enterococci based on gender.

**Table 1:** Enterococci isolated from various clinical specimens.

| Specimens | Enterococci (%) |
|---|---|
| Urine, Kidney stone | 51 |
| Deep Tissue | 6 |
| Wound swab | 11 |
| HVS, Umbilical swab | 8 |
| Bronchoalveolar Lavage | 1 |
| Pus, peritoneal fluid | 14 |
| Blood, Suction tip, bile | 4 |
| ET aspirate | 5 |

Polymorph nuclear leucocytes (PMNL) was found to be associated with 40 of the urinary isolates, which comprised 20 *E. faecalis* and 20 of *E. faecium*. Among the seven high vaginal swabs that showed growth of Enterococcus spp. 5 had PMNL. These five isolates included 3 of *E. faecalis* and 2 of *E. faecium*.

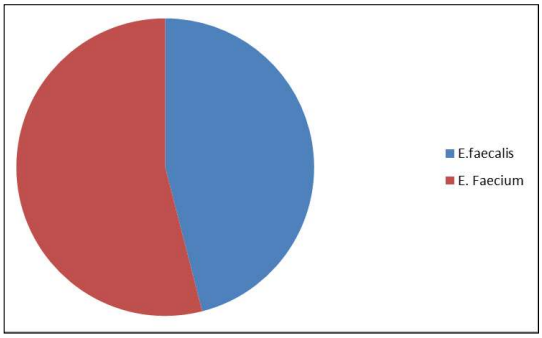


**Fig 1:** Enterococci

1% of *E. faecalis* and 2% of *E. faecium* strains were resistant to vancomycin by disc diffusion method, and these were isolated from pus and deep tissue. MIC values of two isolates showed greater MIC ≥32µg/ml and one with MIC ≥8µg/ml.

Antimicrobial susceptibility pattern for the Enterococcus isolates collected shown in Table 2.Of 100 isolates,

**Table 2:** Antimicrobial susceptibility pattern for Enterococcus.

| Antibiotics tested | Percentage | |
|---|---|---|
| | Sensitive | Resistant |
| Amikacin | 31 | 69 |
| Amoxyclav | 55 | 45 |
| Ampicillin | 51 | 49 |
| Erythromycin | 30 | 70 |
| High-level Gentamicin | 55 | 45 |
| High-level Streptomycin | 44 | 56 |
| Imipenem | 58 | 42 |
| Meropenem | 50 | 50 |
| Nitrofurantoin | 75 | 25 |
| Piperacillin | 35 | 65 |
| Piperacillin-tazobactam | 60 | 40 |
| Teicoplanin | 90 | 10 |
| Vancomycin | 95 | 5 |

Out of 100 isolates, for amikacin, amoxyclav and nitrofurantoin sensitivity was 31%, 55%, and 75%, respectively. 60% of these isolates were sensitive to Piperacillin-tazobactam, but 40% showed resistance. 58% were sensitive to imipenem and meropenem was sensitive to 50% Enterococcus isolates. 5% of the total isolates showed Vancomycin resistance. 44% and 55% of the isolates showed resistance to High-level streptomycin and High-level gentamicin, respectively. Intermediate susceptibility was seen for antibiotics like amikacin (2%), meropenem (2%), piperacillin-tazobactam (2%), High-level gentamicin (2%), erythromycin (2%), piperacillin (5%) and teicoplanin (2%).

| Clinical conditions | No. of samples | Risk factors | Recovery |
|---|---|---|---|
| Acute coronary syndrome | 1 | Sepsis pneumonia Type 2 RR | No (expired) |
| Meningitis | 1 | TB | Yes |
| Hydroureteronephrosis | 1 | Ureteric calculus | Yes |
| Urinary tract infection | 27 | Chronic cervicitis, DM, HTN, primigravida cervical incompetence ARR | Yes |
| Cholangitis | 1 | DM, IHD, BPH | Yes |
| Abscess | 1 | Perianal abscess IHD | Yes |
| Ulcer | 2 | Sepsis Type 2 DM DKA PVD | Yes |
| Pulmonary infection | 6 | BPH Type 2 DM HTN DI Hypertension | Yes |

MIC of Vancomycin and tigecycline was done by E-strip, and the percentage susceptibility of Enterococcus isolates is shown in Table 3. All the strains were within the range and susceptible to tigecycline, whereas, three isolates showed the higher threshold for Vancomycin with two of them MIC of 32µg/ml and one with a MIC of 8µg/ml.

**Table 3:** Minimum inhibitory concentrations of vancomycin and tigecycline against Enterococcus by E-test

| No. of dilutions | % of isolates susceptible at the MIC (µg/ml) | |
|---|---|---|
| | Vancomycin Range (1-4) | Tigecycline Range (≤0.25) |
| 0.032 | 0 | 30 |
| 0.064 | 0 | 25 |
| 0.125 | 0 | 5 |
| 0.25 | 0 | 40 |
| 0.50 | 0 | 0 |
| 0.75 | 10 | 0 |
| 1 | 30 | 0 |
| 1.5 | 37 | 0 |
| 2 | 7 | 0 |
| 4 | 12 | 0 |
| 8 | 1 | 0 |
| 16 | 1 | 0 |
| 32 | 2 | 0 |

Table 4 shows MIC of linezolid and tigecycline against MRSA done by agar dilution. All the isolates were within the susceptible MIC range for linezolid. For tigecycline, three strains showed MIC >0.5µg/ml.

**Table 4:** Minimum inhibitory concentrations of Vancomycin and Tigecycline against Enterococcus by agar dilution method

| No. of dilutions | % of isolates susceptible at the MIC (µg/ml) | |
|---|---|---|
| | Vancomycin Range (1-4) | Tigecycline Range (≤0.25) |
| 0.032 | 0 | 5 |
| 0.064 | 0 | 5 |
| 0.125 | 0 | 14 |
| 0.25 | 0 | 76 |
| 0.50 | 12 | 0 |
| 1 | 30 | 0 |
| 2 | 14 | 0 |
| 4 | 40 | 0 |
| 8 | 1 | 0 |
| 16 | 1 | 0 |
| 32 | 2 | 0 |

Haemolysin production among 46% isolates. Hemolytic activity was seen in both the species of Enterococcus. 28% of *E. faecalis* strains and 19% OF *E. faecium* produced the virulence factor.

## Discussion

Enterococcus is one of the major pathogens affecting all age groups. *E. faecium* is more resistant than *E. faecalis*. The isolation rate of *E. faecalis* was more than that of *E. faecium*. However, studies carried out in North India have shown *E. faecium* to be responsible for a large number of infections than *E. faecalis*. In our study, 46% of the total isolates were tested positive for haemolysin. It was also observed that nearly 28% of *E. faecalis* isolates possessed hemolysin, whereas this virulence factor was lesser in case of *E. faecium* isolates. This may be one of the reasons why the species of *E. faecalis* is responsible for a large number of infections. The ability of haemolysin production helps the organisms to acquire nutrition in the host tissues and for the further spread of infection in the host body, thus increasing the infection severity.

Antibiotic resistance among Enterococci is a global problem. In our study, the highest resistance was seen against Erythromycin, which is in agreement with other studies carried out in India. In the current study, 45% and 56% of the total Enterococcus species were resistant to high-level gentamycin and high-level streptomycin respectively.

Agar dilution done for Vancomycin and tigecycline showed MIC values within the susceptible range, except 5% isolates which showed resistance to Vancomycin suggesting they belong to Vancomycin-resistant Enterococcus (VRE). VRE is expected to be a significant problem in the coming years, and hence it is essential that proper measures have to be taken in all healthcare settings to contain the dissemination of the resistant strains. For all the enterococcal isolates, routine testing should be done at least by Vancomycin agar screen test; Vancomycin drug should be used judiciously. Rapid detection and treatment of VRE infected patients will help in limiting the spread of VRE. Studies are necessary to characterize the virulence factors and the drug-resistance genes of enterococcal isolates by the known molecular methods to understand precisely their role in the pathogenesis of nosocomial infections.

## Conclusion

The present study shows the susceptibility pattern trend for vancomycin and tigecycline. It was found that among all the isolates, 5% were resistant to vancomycin, but 100% sensitivity to tigecycline. This conclusion was derived after susceptibility testing was done by both agar dilution and E-test.

Hemolysin production confirmed by a phenotypic method on human blood agar. The results were found to be similar to other studies. This virulence factor was produced by 46% of the total isolates. This test shows the association of the Enterococcus species with various antibiotics and their resistant pattern.

Vancomycin-Resistant Enterococci (VRE) were also isolated during this study. This can be further confirmed by genotypic methods.

The study will help to determine the present trend of MIC values of Vancomycin and Tigecycline for the clinical isolates of Enterococcus species from our tertiary care hospitals.

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