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Original Research Article

Identification of vancomycin resistance in enterococcus & determination of minimal inhibitory concentration of vancomycin by E-test

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ABSTRACT

Introduction: Infections due to Enterococcal species is 2^{nd} prevalent cause of Nosocomial Infections in health care settings. They are crucial because to their inherent resistance to penicillins, cephalosporins, and low level aminoglycosides & their tendency to develop higher level of aminoglycoside and vancomycin resistance, which leaves few therapeutic alternatives for therapy.
Objectives: Isolation and identification of Enterococci with their Antibiogram & determination of Minimal Inhibitory Concentration (MIC) of Vancomycin by E-test.
Materials and Methods: The antibiogram generated by Kirby Baur disc diffusion method and standard microbiological procedures were used to identify enterococcal isolates from distinct clinical samples. MIC level for Vancomycin was tested for all the isolates using E-test from BIOMERIEUX.
Results: Total of 108 isolates (62 E.faecalis and 46 E. faecium) were tested by E-test for MIC of Vancomycin, of which 40 (37.03%) (22 E.faecalis & 18 E. faecium) were Vancomycin Resistant (VRE) strains, having MIC level >32 μg/ml. Nine strains out of 40 (45%) were found to have MIC >256 μg/ml. The enterococcal isolates were observed to be sensitive to Linezolid (80%), Ofloxacin (74%), Amoxyclav (74%) and Cefaperazone (70%).
Conclusion: For the Indian medical community, the rise of antibiotic resistance among Enterococcal species has been a significant concern. So this is need of present era that our laboratories must be well-equipped to report the resistance patterns displayed by enterococci in order to practise evidence-based medicine and reduce hospital acquired infections.

1. Introduction

Group D streptococci include enterococci which are facultative anaerobes that naturally present in human intestine.[1,2] Enterococci have grown in importance not only due to their capability to develop life threatening infections, but also due to their growing resistance to several antibacterial agents. Antibiotic treatment for severe enterococcal infections frequently fails due to frequent resistance to antimicrobial agents & patient death rate is extremely high.[2]

The first glycopeptide antibiotic to be identified was vancomycin. Staphylococcus epidermidis isolates were the first to be linked to vancomycin resistance.[3] The first report of enterococci resistant to vancomycin was made in Europe & it spread rapidly to a huge section of globe.

Vancomycin works by disrupting the trans-glycosylation process, which then affects the trans-peptidation, in order to impede the creation of peptidoglycan precursors of the cell wall of bacteria.[4,5] For the cross-linking of the bacterial cell wall both trans-glycosylation as well as trans-peptidation processes are required.

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Although the genus Enterococcus contains 12 species, E. faecalis and E. faecium are the most prevalent ones. Prevalence of vancomycin resistance in E. faecium caused the frequency of its isolation to rise as it is a more resistant species than E. faecalis.[6]

Taking these factors into account, the current research was carried out in our hospital to isolate and identify vancomycin-resistant enterococcal strains with an antibiogram and to establish their MIC via an E-test.

2. Materials and Methods

The current research was conducted in department of Microbiology. Total of 54 enterococci isolates from different samples (urine, blood, pus, and wound swabs) received for microbiological investigation at the Institute's Bacteriology Laboratory in the previous 12 months was examined. Conventional methods were used to identify all of the isolates.[7]

Antimicrobial susceptibility to linezolid cefaparazone, amoxyclav, cefotaxime, clindamycin, erythromycin, amikacin, ofloxacin, vancomycin was determined by Kirby Bauer disc diffusion Method[8] on MHA(Muller-Hinton agar). The MIC level for vancomycin was determined for each isolate using the E-test from BIOMERIEUX, India, and ranged 0.016 g/ml to 256 g/ml.

3. Result

In a total of 108 patients who had enterococci, 78 (72%) were female and 30 (28%) were male. (Figure 1)

Age group of 16 to 30 years saw highest prevalence of enterococci (61.2%), followed by 31 to 45 years (16.8 %), 46 to 60 years (14 %), >60 years (8.4 %), and up to 15 years (3.7%).(Table 1)

Out of total 108 enterococci isolates most of were isolated from urine 56(51.8%) followed by pus 24(22.2%) by conventional method. (Figure 2)

Out of total 108 enterococci isolates maximum resistance was seen in clindamycin62, followed by erythromycin 60, amikacin 44, ofloxacin 28, amoxyclav 28 and linezolid 22 by Kirby Bauer disk diffusion method.(Table 2, Figure 3)

Out of total 108 isolates VRE (vancomycin resistant enterococci) were detected in 40 (37.1%) patients, by E-test for MIC of Vancomycin, among them 14 (35%) were males and 26 (65%) were females.

Age wise distribution of VRE isolates shown in Table 3.

Out of 40 VRE isolates 22(55%) were isolated from urine samples followed by 12(30%) from pus and 6(15%) from swab.

E-test was used to test 108 isolates for vancomycin MIC, of which 40 (37.03%) (22 E. faecalis & 18 E. faecium) were determined to be vancomycin resistant (VRE) strains, having MIC level >32 g/ml. It was discovered that 18 strains out of 40 (or 45%) had MIC >256 g/ml. (Figure 3)

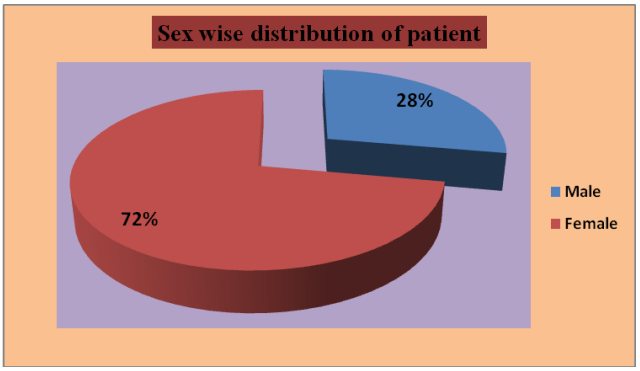

Figure 1: Sex wise distribution of patient

Table 1: Age-based distribution of isolates of enterococci

Age (years)	No. of isolates
Up to 15	04(3.7%)
16-30	66(61.2%)
31-45	16(14.8%)
46-60	14(13%)
More than 60	08(7.4%)
Total	108

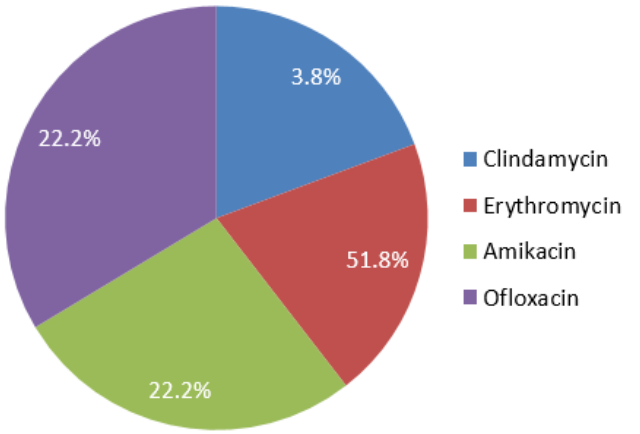

Figure 2: Distribution of enterococci in various clinical specimens

Table 2: Antibiotic sensitivity pattern by KirbyBaur disk diffusion method in enterococci isolates

Antibiotic tested	Sensitive	Resistant
Clindamycin	46	62
Erythromycin	48	60
Amikacin	64	44
Ofloxacin	80	28
Amoxyclav	80	28
Linezolid	86	22
Cefaparazone	76	32

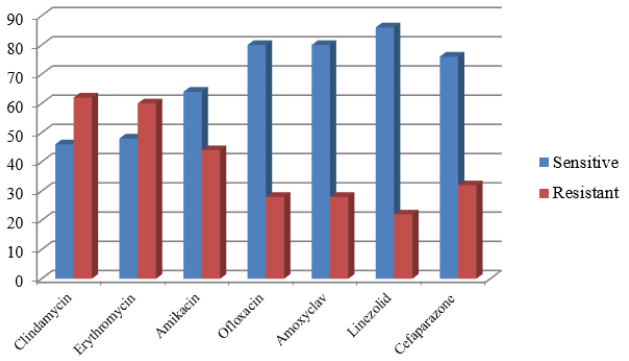

Figure 3: Antibiotic sensitivity pattern in enterococci isolates

Table 3: Age-based distribution of VRE isolates

Age (years)	No. of isolates
Up to 15	02 (5%)
16-30	24 (60%)
31-45	08 (20%)
46-60	02 (5%)
More than 60	04 (10%)
Total	40

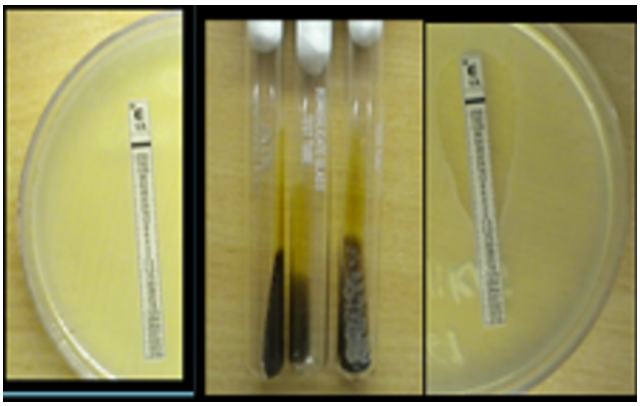

Figure 4: E-test for MIC of vancomycin in enterococci isolates

4. Discussion

One of the most serious global issues is drug resistance. Drug resistant Gram Positive & Negative bacteria has been documented in numerous researches from both India and abroad.[8,9]

This study's objectives were to identify enterococcal infections in a variety of samples to analyze distribution of antibiotic resistance with an emphasis on vancomycin resistance, and to identify vancomycin resistance using the E-test method.

In the current research, enterococcal infections were more prevalent in women (72%). Other researchers have also found higher prevalence in females ranges from 46% to 50%.[10,11]

The majority of the enterococcal isolates in the current study were isolated from urine samples (51.8%). Similar findings from other studies have also been reported.[12,13]

However some studies reported that greatest number of enterococcal isolates were obtained from pus samples.[14,15]

In our investigation, the age group of 16 to 30 years accounted for the majority (61.2%) of the VRE positive isolates from different samples. However, just a few studies found that cases of VRE positivity in people between the ages of 61 and 75 were 5.2% and 12.7%, respectively.[16] According to several researches, patients with highly resistant Enterococci ranged in age from 18 years old to 71 years old.[17]

Through the Kirby Bauer disc diffusion method, the antibiotic sensitivity pattern in our study reveals that clindamycin 31 was the most resistant drug for enterococcus, followed by erythromycin 30, amikacin 22, ofloxacin 14, amoxyclav 14, vancomycin 13, and linezolid 11. However, when 108 isolates (62 E. faecalis and 46 E. faecium) were examined using the E-test for vancomycin's MIC, 40 (37.03%) of them (22 E. faecalis and 18 E. faecium) were discovered to be VRE strains. According to several researches, gentamicin (58.0%), tetracycline (47.1%), and ampicillin (43%) were the drugs against which Enterococci were most resistant, whereas vancomycin and linezolid (18) showed no resistance.[18]

5. Conclusion

For the Indian medical community, the rise of antibiotic resistance among Enterococcal species has been a significant concern. The inherent resistance of Enterococcus to a number of regularly used antimicrobial agents & more importantly, its capacity to develop resistance to all presently available antibacterial drugs, both contribute significantly to their survival in hospital environments. Laboratories must be well-equipped to report the resistance patterns displayed by enterococci in order to practise evidence-based medicine and reduce hospital acquired infections.

6. Source of Funding

None.

7. Conflict of Interest

None.

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