Prevalence and Antibiotic Resistance Pattern of Isolated Enterococcus by Standard Techniques

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ABSTRACT

Introduction: Enterococci have emerged as important nosocomial pathogens and appearance of resistance to many of the antimicrobials used for Gram-positive organisms has made the management of infections due to Enterococcus species difficult.

Aim: Aim of the study was to observe the prevalence rate of Enterococcus species and observe its antibiotic resistance pattern in our hospital.

Method: Enterococci strains were isolated from various clinical samples by culture and biochemical methods and its antibiotic susceptibility testing was seen by Kirby Bauer method as per CLSI guidelines. Minimum inhibitory concentration (MIC) determination for detecting Vancomycin resistance was done by HiMedia E strip test.

Result: In 200 clinical isolates of Enterococcus, 170 were Enterococcus faecalis and 30 species were Enterococcus faecium. Out of 200 Enterococcus isolates 29 were VRE.

Conclusion: The prevalence of Enterococcus species was 5.70% and vancomycin resistance among Enterococci isolates in this study was 14.5% and. Treating serious infections caused by vancomycin-resistant Enterococci has emerged as one of the leading clinical challenges for physicians because of limited therapeutic options.

Keywords: Enterococcus, Vancomycin resistant Enterococcus, MIC

INTRODUCTION

Enterococci are gram-positive anaerobes that live as commensal inhabitant in the alimentary canal of a person(1). There is growing evidence that these bacteria frequently possesses several specific traits that enable them to survive in the hospital environment, colonize patients and cause infection in patients such as bacteraemia, peritonitis, endocarditis and urinary tract, wound, and device-related infections(2). This organism is considered as second leading cause of hospital acquired infections (3,4). Serious Enterococcal infections are often difficult to treat since the organisms have a tremendous capacity to acquire resistance to penicillin, high concentration of amino glycoside and vancomycin. Enterococci with high level resistance to amino glycosides (HLAR), beta lactamase production and glycopeptides resistance including vancomycin resistance are posing a great therapeutic challenge, not only for clinicians but also for healthcare institutions (5). VRE is making the treatment of various infections extremely difficult and pose a great challenge to clinicians.(6) Different types of genes that impart resistance to
vancomycin have been reported in Enterococci. Out of nine recognized genotypes of vancomycin resistance in Enterococci; vanA-E, vanG, vanL, vanM and vanN, transferable vancomycin resistance in clinical isolates of Enterococci is primarily linked to the acquisition of vanA or vanB gene clusters. The vanA cluster is carried on Tn1546-like mobile genetic elements which are typically located on conjugative plasmids and mediates high-level resistance to both vancomycin and teicoplanin (VanA-type) (7). Because of the diverse antimicrobial resistance mechanisms, both intrinsic and acquired as well, successful treatment and control of Enterococcal infections are becoming increasingly difficult. Present options of antimicrobials for treating Vancomycin resistant Enterococci (VRE) include Linezolid, Quinupristin/Dalfopristin and Teicoplanin. Two newer antimicrobials came to act upon Enterococcal strains including VREs namely Daptomycin, and Tigecycline2. Daptomycin is currently the Drug of Choice for VRE Strains.(8) however The proposed study is an effort to find out to determine the prevalence of Enterococcal infections and their antimicrobial resistance pattern with regards to Vancomycin resistance in our set up.

MATERIAL AND METHOD
The present study was conducted on 200 pure isolates of Enterococci isolated from various clinical samples like Pus, Blood; wound Swab, Sputum, urine, etc. received at Department of Microbiology of RNT Medical and associated Hospitals, Udaipur for bacteriological culture and sensitivity. Specimen were cultured on blood agar, MacConkey agar and chrome agar (urine sample). The isolates were identified by colony morphology, Gram’s staining, catalase production, growth in nutrient broth containing 6.5% NaCl, aesculin hydrolysis in presence of 40% bile salts, growth at 10°C, 37°C and 45°C and other biochemical reactions(9).

Antibiotic Susceptibility Testing:
Antibiotic susceptibility testing for ampicillin, norfloxacin, high gentamicin, Nitrofurantoin, ciprofloxacin, teicoplanin, vancomycin, linezolid, were done by Kirby-Bauer disc diffusion method on Mueller-Hinton agar and results were interpreted as per CLSI guideline. Enterococcus faecalis ATCC 29212 [Hi Media Laboratories, Mumbai] was used as quality control strain. Minimum inhibitory concentration (MIC) determination was done by Vancomycin E test strips (HiMedia Laboratory, Mumbai) (10).

RESULT AND DISCUSSION
In our study total 200 Enterococci were isolated from 3464 clinical samples, accounting for a prevalence of 5.7% [as per Table-1]. The prevalence rate of our study was in accordance to the study conducted by Rupali S Shinde et al(13) (2012 ) with 5.5% prevalence in Mumbai. In present study as per figure - 2, out of the 200 Enterococcal isolates, the maximum isolates were from urine 150(75%) followed by blood 26(13%), pus 18 (9%)and others6(3%). This might reflect the role of Enterococci as the most common uropathogens. Previous studies have also supported the urinary tract as the commonest site of Enterococcal infection conducted by Karmarkar et al in Mumbai showed maximum isolates from urine (50%) followed by blood and pus swabs. Figure-2 showed, In present study Enterococcus faecalis was predominant species. This finding of our study was similar with the findings of Gangurde N. and Raj HJ et al. [5,8]. But there were few studies shows Enterococcus faecium as predominant species by Karmarkar et al,[11]. Antibiotic resistance pattern was observed table -2 in the Enterococcus isolates for various antibiotics by species wise. For E. faecalis isolates ampicillin (62.35%), amoxicillin (60.58%), tetracycline (65.68%), norfloxacin (75%) drugs had higher resistance in comparison to other drugs. Linezolid showed highest sensitivity
for all E. faecalis isolates except one. Other drugs which showed high sensitivity in our study were teicoplanin, vancomycin, and nitrofurantoin. Our results were comparable to those of Purohit G et al., where norfloxacin are highly resistant along with ampicillin. linezolid was resistant for one isolate in their study which was similar to our study. However, our result contradicts with their as they had very high resistance to nitrofurantoin which was highly sensitive in our study and in their study, ciprofloxacin was also show very high resistance as compare to our study.

For E. faecium isolates highest resistance seen in ciprofloxacin (83.33%) followed by Norfloxacin (71.42%) and doxycycline. Jada SK, Purohit G, Naruka HS support ciprofloxacin and norfloxacin resistance in E. faecium to our study. Linezolid was highly sensitive but resistance observed in three case (10%). Naruka HS et al., Jain S et al and Rana D et al also observed in (5.55%, 5%, 4.7% respectively). Nitrofurantoin and teicoplanin also have good sensitivity (78.57% and 70% respectively). Meena et al comparable to our study observed 88.8% sensitivity to each drug. Rana D et al also observe high sensitivity for these drugs. Whereas contradicts to Naruka HS study as observed higher resistance to nitrofurantoin.

In the present study, it was noted that, E. faecium showed more resistance to the tested drugs compared to the E. faecalis. The emergence of Vancomycin resistant Enterococci poses a serious threat to hospitalized patients with impaired host defences. In India, the prevalence of VRE has been reported to be between 0 - 30 percent [10]. Our study revealed, 29(14.5%) isolates were found to be resistant to Vancomycin with E. faecium (30%) showing higher resistance than E. faecalis (10.5%). Similar findings by Telkar et al. had also reported greater resistance among E. faecium isolates [18].

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>E. faecalis (n=170)</th>
<th>E. faecium (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>106(62.35)</td>
<td>17(56.66)</td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>103(60.58)</td>
<td>16(53.33)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>83(48.82)</td>
<td>25(83.33)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>112(65.88)</td>
<td>19(63.33)</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>98(57.64)</td>
<td>20(66.66)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>10(5.88)</td>
<td>3(10.00)</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>18(10.58)</td>
<td>9(30.00)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>25(14.70)</td>
<td>11(36.66)</td>
</tr>
<tr>
<td>Norfloxacin*</td>
<td>102(60.00)</td>
<td>10(71.42)</td>
</tr>
<tr>
<td>Nitrofurantoin*</td>
<td>27(19.85)</td>
<td>2(14.28)</td>
</tr>
</tbody>
</table>

*Norfloxacin and Nitrofurantoin are used only for urine samples (E. faecalis 136 and E. faecium 14)
CONCLUSION
The present study indicated an increase in Vancomycin resistance of the Enterococcal isolates. Such strains pose therapeutic dilemmas for clinicians. The Vancomycin resistance in Enterococci not only leaves fewer options for the disease management, but it is also important due to the potential risk of the Vancomycin resistance gene transfer from the Enterococci to Staphylococcus aureus. Our study emphasizes on the proper infection control in clinical practice and empirical use of drugs like Vancomycin.

Acknowledgement: None
Conflict of Interest: None

Table -3 Showing Vancomycin Resistance of Enterococcus species by E-Test

<table>
<thead>
<tr>
<th>Enterococcus species</th>
<th>No. of isolates</th>
<th>E-test MIC (R)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecalis</td>
<td>170</td>
<td>20</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>30</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>29</td>
</tr>
</tbody>
</table>

REFERENCES

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Ethical Approval: Approved
10. Sharma S, Gupta P, Rishi S. Prevalence of vancomycin resistant Enterococcus and its antimicrobial resistance pattern in clinical isolates International Journal of Medical and Health Research Volume 4; Issue 12; December 2018; Page No. 94-96

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