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Prevalence and Antibiotic Resistance Pattern of Isolated *Enterococcus* by Standard Techniques

Madhubala Mishra[1], Dr. Anshu Sharma[2], Dr. Neelam Chauhan[3], Sandhya Mishra[4]

[1,3]PhD Scholar, Department of Microbiology, RNT Government Medical College, Udaipur, Rajasthan, India
[2]Senior Professor and Head of Department, Department of Microbiology, RNT Government Medical College, Udaipur, Rajasthan, India
[4]Senior demonstrator, Department of Microbiology, RNT Government Medical College, Udaipur, Rajasthan, India

Corresponding Author: Madhubala Mishra

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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 habitant 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 Tn*1546*-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 resistance 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, Puruhit 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 S 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-1 Prevalence of *Enterococcus* (n=3464)

Total samples	*Enterococcal* isolates (%)
3464	200 (5.7%)

Figure no.1 Prevalence of *Enterococcal* isolates among various specimens (n=200)

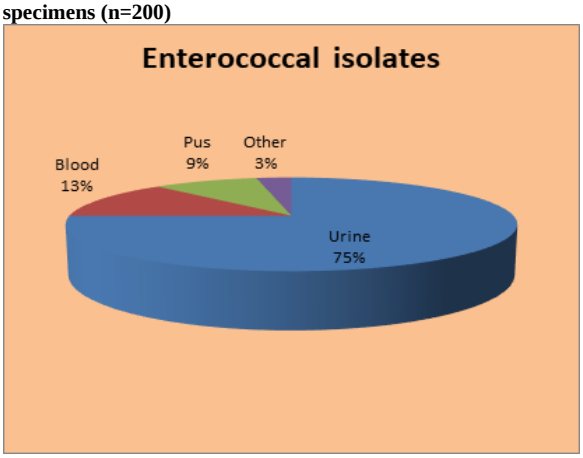

Figure no.2: Species of *Enterococci* isolated from clinical sample(n=200)

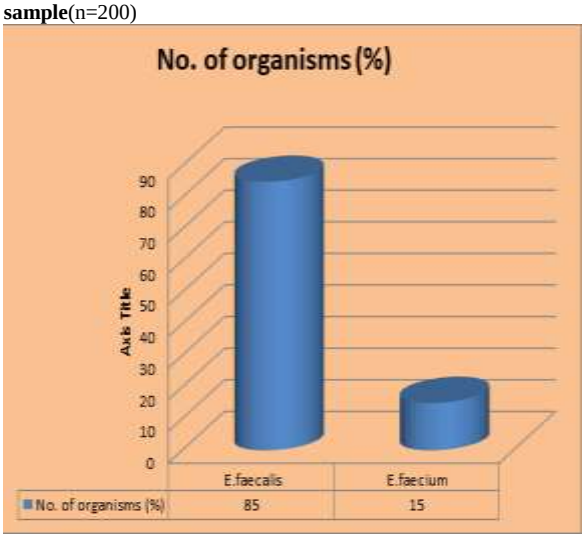

Table 2: Showing Antibiotic Resistance of pattern of *Enterococcus* species

Antibiotics	E.faecalis (n=170)	E.faecium (n=30)
Ampicillin	106(62.35)	17(56.66)
Amoxycillin	103(60.58)	16(53.33)
Ciprofloxacin	83(48.82)	25(83.33)
Tetracycline	112(65.88)	19(63.33)
Doxycycline	98(57.64)	20(66.66)
Linezolid	1(0.58)	3(10.00)
Teicoplanin	18(10.58)	9(30.00)
Vancomycin	25(14.70)	11(36.66)
Norfloxacin*	102(60.00)	10(71.42)
Nitrofurantoin*	27(19.85)	2(14.28)

*Norfloxacin and Nitrofurantoin are used only for urine samples (E.faecalis 136 and E.faecium 14)

Fig 1: Showing Antibiotic Resistance of *Enterococcus faecalis* to antibiotics

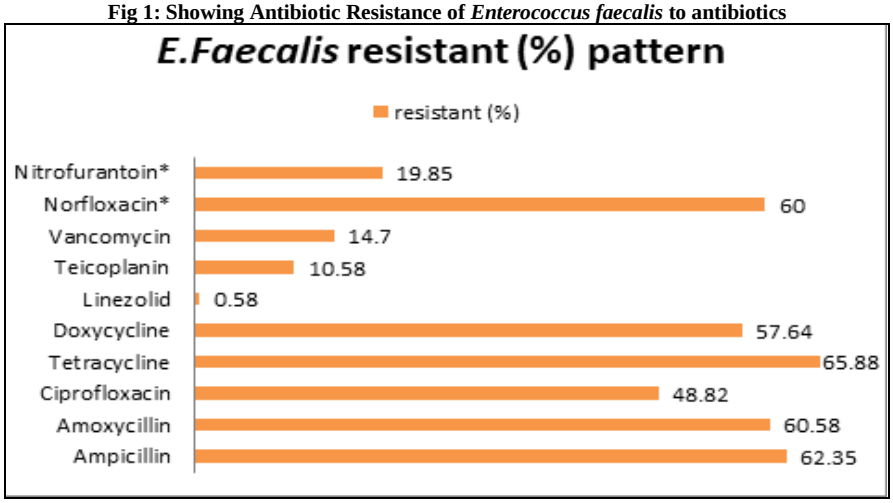

Fig 2: Showing Antibiotic Resistance of Enterococcus faecium to antibiotics

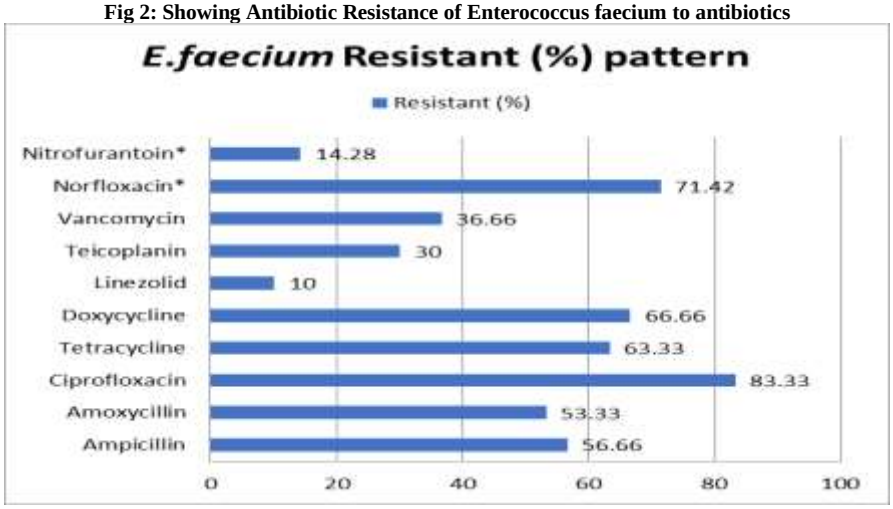

Table -3 Showing Vancomycin Resistance of *Enterococcus* species by E-Test

***Enterococcus* species**	**No. of isolates**	**E- test MIC (R)**
Enetrococcus faecalis	170	20
Enterococcus faecium	30	9
Total	**200**	**29**

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.

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