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Prevalence of vancomycin resistant *Enterococcus* and its antimicrobial resistance pattern in clinical isolates

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Abstract
Enterococci are the member of healthy human intestinal flora but are also leading causes of highly antibiotic resistant infections. Serious enterococcal infections are often difficult to treat since the organism has a tremendous capacity to acquire resistance to penicillin, high concentration of aminoglycoside and vancomycin. Aim of the study to check the prevalence rate of enterococcus species in our hospital. A total 125 enterococcus were studied, species identification was done by conventional methods. Antibiotic susceptibility testing was by done by Kirby-Bauer disc diffusion method. Vancomycin resistance were detected by disc diffusion and E-test MIC method. In 125 clinical isolates of Enterococcus, 88 species are *Enterococcus faecalis* and 37 species are *Enterococcus faecium*. Out of 125 enterococcus isolates 16 were Vancomycin resistant. Regular screening of enterococcal isolates for vancomycin resistance detection should be implemented. It is very important to implement infection control measures, screening of health care workers in intensive care units which can control spread of multidrug resistant enterococci.

Keywords: vancomycin resistant enterococcus, E-test MIC

Introduction
Enterococci are the member of healthy human intestinal flora but are also leading causes of highly antibiotic resistant infections in hospital settings [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]. Serious enterococcal infections are often difficult to treat since the organisms have a tremendous capacity to acquire resistance to penicillin, high concentration of aminoglycoside & vancomycin [3]. Enterococci with high level resistance to aminoglycosides (HLAR), beta lactamase production & glycopeptides resistance including vancomycin resistance are posing a great therapeutic challenge, not only for clinicians but also for healthcare institutions [4].

Multidrug resistance complicates treatment of enterococcal infections and the therapeutic spectrum of these cases is limited. Careful review of *in vitro* susceptibility data is required to treat infections caused by MDR *E. faecium*, the most commonly found group of VRE. Empiric therapy of enterococcal infections should be guided by local patterns of drug resistance. Nowadays emergence of MDR enterococci is thought to be due to antibiotic selective pressure. This organism is considered as second leading cause of hospital acquired infections [5]. The present study was aimed to detecting the antimicrobial resistance pattern among enterococcus isolates obtained from clinical specimen in a tertiary care hospital in Jaipur with special emphasis on vancomycin resistant in enterococcus and prevalence of vancomycin resistant enterococcus spp. in our hospital.

Material and Methods
The study was carried out of one year December 2016 to January 2018 at the Department of Microbiology, National Institute of Medical Sciences & Research, NIMS University Jaipur (Rajasthan). Various clinical specimens Urine, Pus, Blood, Body fluids were taken from patients attending NIMS hospital. Specimen were collected in a sterile, proper labelled container with aseptic precautions and processed as per standard microbiological procedures.

Organism Identification by (Henry *et al*., 1998) [6]
All samples were screened for the pus cells and organism. Specimens were culture on Blood agar & MacConkey and incubated for 37ºC for 24 hours. Growth was then processed for gram staining and catalase test. Gram positive cocci arranged in pairs showing catalase negative were considered as *streptococcus species*. Speciation of enterococcus species done by grams staining, colony morphology, culture characteristics of the colonies and biochemical tests (Bile esculin hydrolysis test, Pyrrolidonyl Arylamidase, resistance to bacitracin and Optochin, growth at 6.5% NaCl, growth at 37ºC and 45ºC, Hippurate hydrolysis test, sugar fermentation test). Grams stain smear shows gram positive cocci 1-1.5 x 0.5µm, oval shaped arranged in pairs and short chains.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing for ampicillin, Nitrofurantoin, ciprofloxacin, teicoplanin, vancomycin, linezolid, Pristinomycin was done by Kirby-Bauer disc diffusion method on Mueller-Hinton agar and results were interpreted as per CLSI guidelines 2018 [7]. *Enterococcus feacalis* ATCC 29212 [Hi Media Laboratories, Mumbai] was used as quality control strain. Minimum inhibitory concentration (MIC) determination was done by Vancomycin E test strips (Hi-media Laboratory, Mumbai) [8]. Since E test is convenient to perform, MIC determination of Vancomycin against VRE were done by E test (Hi-media Laboratory, Mumbai).

Results

In our study a total of 125 *Enetrococcus* spp. were isolated in a period of one year 2016 to January 2018. Among 125 *Enterococcus* species, 88 (70.4%) species are *Enterococcus faecal is* and 37 (29.6%) species are *Enterococcus faecium.*

Highest prevalence of *Enetrococcus* was seen in females 86 (68.8%) followed by males 39 (31.2%). The maximum percentage of isolation was seen among the age group 40-60 years. In our study maximum *Enterococcus* isolate from urine specimen 79 (63.2%), followed by Pus 24 (19.2%), Blood 14 (11.2%), Others 08 (6.4%). Maximum isolation of *Enterococcus* isolates was isolated from urine specimen. It indicates that urinary tract infections are the most common infections caused by *Enterococci* in this set up.

All the enterococcal isolates were subjected to test for vancomycin resistance by two methods name disc diffusion method and E-test MIC method. We Consider E test (MIC) method is gold slandered method.

Table 1: Showing Sample Distribution

Specimen	No. Of Isolates	Percentage (%)
Urine	79	63.2%
Pus	24	19.2%
Blood	14	11.2%
Others (Body fluids, sputum)	08	6.4%
Total	125	100%

Table 2: Showing *Enterococcus* species Distribution

Entero. spp	No. Of Isolates	Percentage (%)
Enterococcus *faecal is*	88	70.4%
Enterococcus faecium	37	29.6%
Total	125	100%

Table 3: Showing Antibiotic Resistance of *Enterococcus* species to other antibiotics

Antibiotic Used	***E. faecal is (n:88)***	*E.* ***faecium (n:37)***
Ampicillin (10µg)	39 (44.3%)	19 (51.3%)
Ciprofloxacin(5µg)	54 (61.3%)	23 (62.1%)
Nitrofurantoin* (30µg)	15 (27.2%)	10 (41.6%)
Vancomycin (30µg)	13 (14.7%)	6 (16.2%)
Teicoplanin (30µg)	0%	0%
Linezolid (30µg)	0%	0%
Pristinomycin (15µg)	0%	0%

*Nitrofurantoin used only for urine samples (n: 79, E. faecal is: 55 & E. faecium: 24 isolates).

Table 4: Showing Vancomycin Resistance of *Enterococcus* species by E-test

Entero.spp	No. Of Isolates	E-test MIC
Enterococcus *faecalis*	88	11 (12.5%)
Enterococcus faecium	37	5 (13.5%)
Total	16	16 (26%)

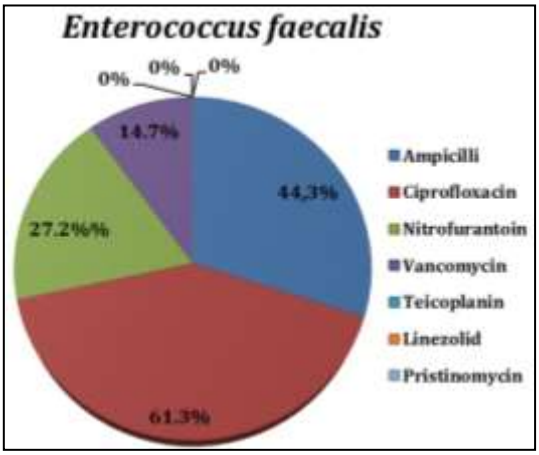

Fig 1: Showing Antibiotic Resistance of *Enterococcus faecalis* to antibiotics

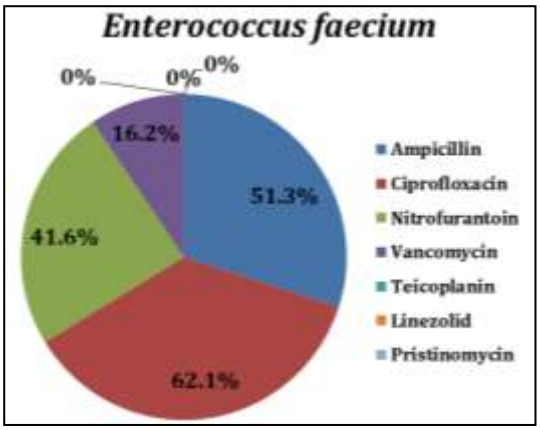

Fig 2: Showing Antibiotic Resistance of *Enterococcus faecium* to antibiotics

Discussion

In our study *Enterococcus faecalis* is predominant species. This finding of our study are similar with the findings of *S. Farnandes et al.*, and *S. Bose et al.* [9, 10]. But there are few studies shows *Enterococcus faecium* as predominant species by S. Jain *et al*., and Karmarkar *et al*. [11, 12].

Enterococcus are widely distributed in nature and are usually part of mixed flora commonly found in gastrointestinal tract (GIT) and remains difficult to differentiate colonization from true infection [13]. Intensive use of broad spectrum antibiotics is responsible for conversion of enterococci to opportunistic nosocomial pathogens (Huycke *et al.*, 1998, Mohanty *et al.*, 2005) [14, 15]. *Enterococci* demonstrate both intrinsic as well as extrinsic types of resistance to antibiotics [16]. Our study revealed presence of multidrug resistant enterococcal species. Our findings are similar with other studies (Mathur *et al.*, A. Telkar *et al*.,) [17, 18]. In our study we found *E. faecium* to be

more drug resistant than *E. faecalis* similar findings have been reported by other studies [19].

The emergence of Vancomycin resistant enterococci poses a serious threat to hospitalized patients with impaired host defences [20]. In India, the prevalence of VRE has been reported to be between 0 - 30 percent [21, 22]. In our study, 16 isolates were found to be resistant to Vancomycin with *E. faecium* (13.5%) showing higher resistance than *E. faecalis* (12.5%). Similar findings by Telkar *et al.* had also reported greater resistance among *E. faecium* isolates [23]. All the VRE isolates in our study found to be sensitive to teicoplanin, linezolid, pristinomycin as treatment options.

Conclusion

The present study indicated an increase in Vancomycin resistance of the enterococcal isolates. 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.* Also now a day there is emergence of Vancomycin resistance, therefore regular screening of enterococcal isolates for Vancomycin resistance detection should be implemented to limit the spread of MDR enterococci. It is very important to implement infection control measures, screening of health care workers, surveillance cultures in intensive care units which can control spread of multidrug resistant enterococci.

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