www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i2.129

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

MDR Enterococci- Race for Supremacy between Enterococci and Antibiotics

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Abstract

Introduction: *Enterococci, considered a normal commensal of intestinal tract, are fast emerging as a pathogen causing serious and life threatening hospital borne infections. During the last few years, enterococci have acquired resistance to a number of important antibiotics like Penicillin, Aminoglycosides including glycopeptides which pose a serious challenge not only for clinicians but also for health care institutions. It results in treatment failure, selection and spreading of resistant strains in the health care institution.*

Aims & Objective: *In view of this, the present study was undertaken to know the prevalence of multi drug resistant Enterococcus Species with special reference to Vancomycin.*

Materials & Methods: *During the study period of 6 months from January 2015 to June 2015, from 3131 clinical samples received by Microbiological laboratory, 207(6.61%) Enterococci were isolated and identified by using standard microbiological techniques and their antimicrobial susceptibility was performed according to CLSI guidelines. E-test was performed on Vancomycin resistant strains to detect the MIC of Vancomycin.*

Results: *Out of 3131 samples, 207(6.61%) samples were positive for Enterococcal species. of which 123 (59.42%) isolates were E.faecalis, 55(26.57%) E.faecium, & 29 (14.01%) other Enterococcus Spp. Maximum isolation was from Urine 143(69.08%) followed by Pus 43(20.77%) and 08(3.86%) broncho-alveolar lavage samples. 102 (49.28%) isolates were multidrug resistant Enterococci. Of which 52(50.98%) were MDR E.faecium and 50 (49.02%) MDR E.faecalis. Two isolates, both urine samples and Vancomycin resistant (VRE), were Entercoccus faecium. They showed (100%) sensitive to Linezolid, (99.03%) sensitivity to Vancomycin. Other resistant drugs in our study were Nitrofurantoin (60.84% used only in urinary isolates), Amikacin (74.40%), Gentamicin(74.88%), Ciprofloxacin (78.74%), Erythromycin (82.61%) and Penicillin (93.72%).*

Conclusion: *Empirical therapy for Enterococcal infections should be guided by local patterns of drug resistance. Regular screening of Enterococcal isolates for Vancomycin resistance detection should be implemented along with the infection control measures, screening of health care workers and surveillance of cultures which can control spread of multidrug resistant Enterococci.*

Keywords: *Enterococci, Multidrug resistant (MDR), E-Test, Vancomycin.*

INTRODUCTION

Enterococci are Gram-positive cocci, often in pairs or short chains, and difficult to distinguish from Streptococci on physical characteristics alone [1]. It's a common gastrointestinal and biliary tract commensal, and in lower number also resides in vagina and lower urethra [2]. Two species are common commensal in the human intestine: E. faecalis (80–90%) and E. faecium (10-15%) [2]. In recent times it has emerged as troublesome nosocomial pathogen causing significant morbidity and mortality [3,4] and a cause of nosocomial superinfection in patients receiving antimicrobial agent [5].

There is growing evidence that these bacteria frequently possess several specific traits that enable them to survive in the hospital environment, colonize patients, and cause infections such as bacteraemia, peritonitis, endocarditis and urinary tract, wound, and device-related infections.[6] The most common nosocomial infections caused by Enterococci are urinary tract infections[7,8]. Also there has been an increase in the rate of VRE (Vancomycin-Resistant Enterococci), which may be intrinsic or acquired through gene transfer [3].

In USA alone, eight lakh cases of Enterococcal infections occur each year [9]. The National Nosocomial Infection Surveillance (NNIS) of USA has reported the increase in the prevalence of invasive nosocomial Enterococcal strains showing high level of Vancomycin resistance [10]. 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 [11].

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 drugresistance [12] Therefore we conducted the study to find out prevalence of multi drug resistant Enterococcal isolates with reference to Vancomycin resistance in our set up.

MATERIALS & METHODS

The present prospective study was conducted for 6 months from January 2015 to June 2015 in Microbiology laboratory of Pad.Dr.D.Y.Patil Medical College & Research center.

During the study period, 3131 clinical samples like Urine, Pus, Swabs, Peritoneal fluid, Blood, Pleural fluid, CSF, Stool, Bronchoalveolar lavage, Endo-tracheal tube tip were received and processed according to standard microbiological techniques and the Antimicrobial susceptibility testing of pathogens were done according to CLSI guidelines.[2]

The Enterococcal isolates were identified by colony morphology, Gram's staining, 3% catalase production, 40% Bile Esculine test, Growth in 6.5% growth in NaCl & Sugar fermentation tests like Mannitol, Sorbitol, Arabinose for speicies identification were used.[13,14]

Antibiotic susceptibility testing for Ampicillin (10µg), Amoxyclav (20/10µg), Erythromycin (15µ), Cotrimoxazole (1.25/23.75µg), Gentamicin (10µg), Ciprofloxacin (5µg), Vancomycin (30µg), Linezolid (30µ), was done by Kirby-Bauer disc diffusion method on blood agar and results were interpreted as per CLSI guidelines.[15] Enterococcus feacalis ATCC 29212 [Hi Media Laboratories, Mumbai] was used as quality control strain.

Minimum Inhibitory Concentration (MIC) determination was done on the Enterococcal strains which showed resistant zone / no zone of clearance with KBDDM, by Vancomycin E test strips (Ezy MIC^{TM} strips from HiMedia Laboratories, Mumbai) on Muller Hinton agar with 5% sterile defibrinated blood, incubated at 37^{0}C for 18 hrs. 1.0, 2.0, 4.0 µg/ml (< S, I, >R) were the Standard Quality Control reference MIC values for Vancomycin for the standard ATCC Culture of E. faecalis 29212

RESULTS

207(6.61%) Enterococcal isolates were obtained from 3131 clinical samples over a period of six months from January 2015 to June 2015. Out of 207 Enterococcal isolates, 123 (59.42%) isolates were E. faecalis , 55(26.57%) were E.faecium and 29(14.01%) were other Enterococcus Spp.

Table 1: Prevalence of Enterococci.

Total no. of Clinical Samples	Total Positive Sample of Enterococci	Percentage (%)	95% CI for prevalence
3131	207	6.61	(5.77% - 7.54%)

The observed prevalence was 6.61% with a 95% confidence interval (5.77% - 7.54%).

Table: 02: Sample wise distribution.

Sample	Total No.	Percentage of total positive isolates(207)	No. of MDR	Percentage of Total MDR(102)
Urine	143	69.08	73	71.57
Pus	43	20.77%	20	19.61%
Pleural Fluid	03	1.45%	01	0.98%
Peritoneal Fluid	02	0.97%	01	0.98%
BAL Fluid	08	3.86%	02	1.96%
ET-Tube Tip	05	2.42%	04	3.92%
ICD-Tube Tip	01	0.48%	00	00
Ear swab	02	0.97%	01	0.98%
Total	207	100%	102	100%
p-value	Chi-square = 611.45, P< .001, Significant		Chi-square =349.14, P< .001, Significant	

Among (207) Enterococcal isolates, 143(69.08%) were from urine samples, 43(20.77%) were pus samples, 08(3.86%) were Bronchio-alveolar lavage, 5(2.42%) ET tubes, 03(1.45%) Pleural fluid, 02(0.97%) peritoneal fluid, 02(0.97%) ear swabs and 01(0.48%) intercostals drainage tube tip.

The analysis using Chi-square test for goodness of fit shows a significant difference in the proportion of positive isolates (p<.001) and number of MDR (p<.001) according to samples.

Table: 03: Ward wise distribution.

OPD/IPD		Total No. of Positive Sample	Percentage (%)of total positive isolates(207)	
OPD		41	19.81%	
IPD	Medicine	55	26.57	80.19%
	ICU	36	17.39	
	Surgery	28	13.53	
	Pediatrics	23	11.11	
	OBGY	16	07.73	
	Orthopedic	08	03.86	
Total		207	100%	
p-value		Chi-square = 51.19, df=6, p < .001, Significant.		

The above table shows ward-wise distribution of total positive isolates. The analysis using Chi-square test for goodness of fit shows a significant difference in the proportion of positive isolates (p<.001) according to samples.

Table 4: Species wise distribution

Species	Total No.	% of Total +ve Isolates	Total No. of MDR	% of Total MDR Isolates	Total VRE	% of VRE to Respective Species
E. faecalis	123	59.42	50	49.02	00	00
E. faecium	55	26.57	52	50.98	02	3.64
Other Enterococci	29	14.01	-	-	-	-
Total	207	100	102	100		
p-value	p < .001, Significant.		P=0.843, NS		p>.05, NS	

102(49.28%) were multidrug resistant Enterococci. Out of 102 MDR isolates, 50(49.02%) were MDR- E. faecalis, 52 (50.98%) were MDR-E. faecium.

The analysis using Chi-square test for goodness of fit shows a significant difference in the proportion of positive isolates (p<.001) according to species. The total number of MDR (p =.843) and total VRE (p >.05) shows no significant difference according to species.

Table: 05: Antimicrobial susceptibility of Enterococcal isolates (n=207)

Antibiotic	Total No. of Sensitive Isolates	%	Total No. of Resistant Isolates	%
Linezolid (LZ)	207	100	00	00
Vancomycin (VA)	205	99.03	02	0.97
Amikacin (Ak)	53	25.60	154	74.40
Gentamicin (G)	52	25.12	155	74.88
Erythromycin (E)	36	17.39	171	82.61
Co-Trimoxazole (COT)	00	00	207	100
Penicillin (P)	13	6.28	194	93.72
Ciprofloxacin (CIP)	44	21.26	163	78.74
Nitrofurantoin* (NIT)	56*	39.16*	87*	60.84*
p-value	P< .001, Significant		P< .001, Significant	
*: Nitrofurantoin was used only for Urinary Isolates (143 samples).				

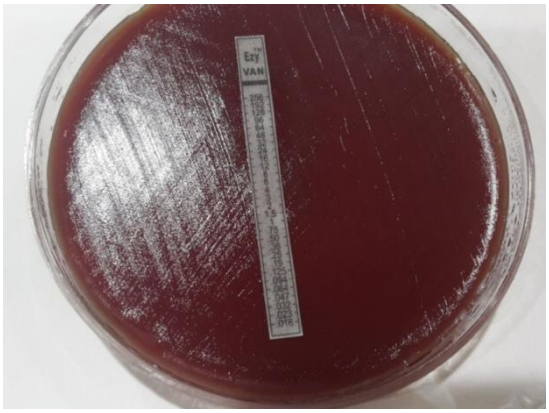
The analysis using Chi-square test for goodness of fit shows a significant difference in the proportion of Total No. of Sensitive Isolates (p<.001) and Total No. of Resistant Isolates (p<.001) according to antibiotic.

All Enterococcal isolates were (100%) sensitive to Linezolid, whereas sensitivity to vancomycin was (99.03%) followed by 39.16% were sensitive to Nitrofurantoin (60.84% used only in urinary isolates), 25.60% sensitive to Amikacin, 25.12% sensitive to Gentamicin, 21.26% were sensitive to Ciprofloxacin,17.39% sensitive to Erythromycin and only 6.28% strains were sensitive to Penicillin. For detection of the MIC of Vancomycin , E-test was performed on 02 Vancomycin resistant strains →showing no zone of clearance on Blood Agar with KBDDM) showed the zone of clearance coinciding / touching the Vancomycin Ezy MIC Strip at 8µg/ml which is more than 4µg/ml indicating a resistant strain to Vancomycin.

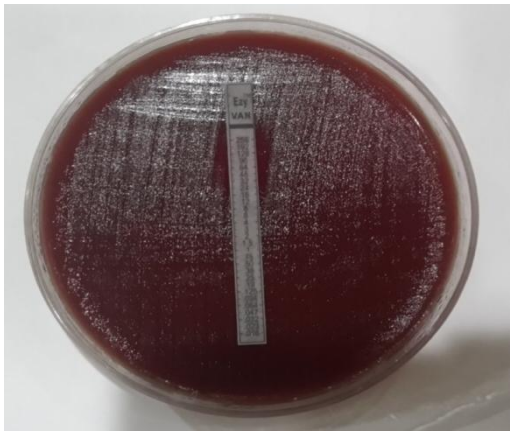
Two isolates, both were urine samples and Vancomycin resistant, were Entercoccus faecium. Both the patients were in-patient (ICU) and immune-compromised, one male and another female, of the age of 70yrs. No mortality was reported in our study.

Pic.01: ABST on Blood Agar showing

MDR Enterococcus faecium sensitive only to Linezolid(22mm), Resistance to Vancomycin (6mm), Penicillin (8mm), Co-trimoxazole (6mm), Intermediately sensitive to Erythromycin (22mm), Gentamicin (14mm)



Pic.02: E-Test on MH agar with 5% blood showing the MIC of 8µg/ml for Enterococcus faecium (VRE).



Pic.03: E-Test on MH agar with 5% blood showing the MIC of 8µg/ml for Enterococcus faecium (VRE).

DISCUSSION

In the present study, out of the 3131 clinical samples, 207(6.61%) were culture positive for Enterococcus species. In our study, E.faecalis 123 (59.42%) was predominant isolate, whereas the studies by other authors showed similar findings [12, 16, 18, 17, 26, 27]. But few other studies reported E.faecium as a predominant isolate. [19, 21, 28, 29].

Highest isolation of Enterococci was observed in urine 143(69.08%) followed by pus 43(20.77%), In other studies also, urine was the most common sample yielding Enterococci, such as, Mathur et al .[20] obtained 49%, Karmarkar et al[21] obtained 50% and Udo et al[10] obtained 36.6% of Enterococci from urine samples.

166 (80.19%) of Enterococci were isolated from hospitalized (IPD) patients and 41 (19.81%) were OPD patients. Similar findings were also reported by M. M. Bakhit et al [17] where (85.83%) were IPD patients and (14.17%) were OPD patients.

Intensive use of broad spectrum antibiotics is responsible for conversion of enterococci to opportunistic nosocomial pathogens [11, 30]. Enterococci demonstrate both intrinsic as well as extrinsic types of resistance to antibiotics [26]. In our study, 102(49.28%) were Multidrug Resistant Enterococci, lesser than the study by VA Rahang-dale et al (77.23%) [16]. In our study we found E. faecium (50.98%) to be more drug resistant than E. faecalis (49.02%). Similar findings have been reported by other studies. [12, 27, 28, 29]

The emergence of Vancomycin resistant enterococci poses a serious threat to hospitalized patients with impaired host defenses [11]. In India, the prevalence of VRE has been reported to be between 0 - 30 percent [20, 22]. In our study, 2 E.faecium isolates were found to be resistant to vancomycin (3.64%) whereas all E.faecalis isolates were sensitive to vancomycin. Similar findings with greater resistance among E.faecium isolates were also reported by other studies [16, 19, 18, 17]. The Vancomycin resistance in enterococci not only leaves fewer options for the disease management, but it is also important risk of the

Vancomycin resistance gene transfer from the enterococci to S. aureus [30].

All culture positive samples were 100% sensitive to Linezolid similar findings were observed in the studies by V.A. Rahangdale et al [16] and M M Bakhit et al[17] whereas 205(99.03%) isolates were sensitive to Vancomycin. All isolates (100%) were Cotrimoxazole resistant, little higher than the study by Maj. Puneet Bhatt et al (95%) [18]. Maximum resistance was seen in Penicillin (93.72%) followed by Erythromycin (82.61%) , Ciprofloxacin (78.74%), Gentamicin(74.88%) , Amikacin (74.40%) and Nitrofurantoin (60.84% from urinary isolates), the findings were in accordance to the study by Maj. Puneet Bhatt et al (18) and Kapoor et al[22].

The treatment of Enterococcal infections has become difficult because of the inherent as well as acquired resistance shown by Enterococci to several commonly used and currently available antibiotics like Cephalosporins, Aminoglycosides, Trimethoprim-Sulfamethoxazole also the emergence of multidrug-resistant (MDR) strains in hospitals has left the clinicians with very limited treatment option [23,24,25].

CONCLUSION

Thus it can be concluded from our study and other national and international studies that Multidrug Resistant Enterococci have emerged as a serious threat to public health. Vancomycin resistance even though was found to be low; its presence is of serious concern. The drug of choice appears to be Linezolid, followed by Vancomycin. It is important to do regular surveillance of antibiotic susceptibilities to these multi-drug resistant strains to check the changing pattern, and thus guiding the clinicians in treating the infection caused by MDR Enterococci in a best possible way.

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