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Research Article

A STUDY OF ANTIMICROBIAL RESISTANCE PATTERN OF MULTIDRUG -RESISTANT ENTEROCOCCI ISOLATED FROM CLINICAL SPECIMENS

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ABSTRACT

Enterococci have emerged as an important etiological agent of nosocomial infections in the recent years. The major reason for this is the increasing number of strains resistant to large number antimicrobial agents. Indiscriminate use of broad spectrum antibiotics contributes to the conversion of Enterococci, the otherwise gut commensal to an opportunistic pathogen and its emergence as important cause of community acquired infections. The propensity of enterococcus species to easily acquire resistance genes and the presence of some unique mechanisms conferring resistance to aminoglycosides and glycopeptides have limited the choice available for treating serious Enterococcal infections. Hence continuous monitoring and determination of antimicrobial susceptibility is quintessential.

Aim: The purpose of the study was to determine the pattern of antimicrobial resistance among Enterococcus species in SRM medical college hospital, Chennai

Materials and methods: This descriptive cross-sectional study was carried out from April2013 - March 2014.A total of 128 isolates of enterococcus species were identified using Facklam and Collins scheme. Antibiotic susceptibility testing was performed by Kirby Baeur disk diffusion method and results were interpreted using CLSI guidelines.

Results: Out of 128 isolates, 97 strains were *E.faecalis* and 31 were *E.faecium*. The isolated enterococcus species showed maximum resistance to commonly used antibiotics like penicillin, ampicillin, ciprofloxacin, and gentamicin .*E.faecium* were more resistant to antibiotics. Vancomycin and teicoplanin resistance was 2.34% and 3.12% respectively. All the strains were sensitive to Linezolid.

Conclusion: A combination of Vancomycin, linezolid and / tigecycline are deemed to be effective against multidrug resistant enterococci. Nonetheless, routine monitoring and regular surveillance of susceptibility pattern of enterococcal infections are absolutely essential for prudent and evidence based use of antibiotics

INTRODUCTION

The accelerated emergence of antimicrobial resistance among the prevalent pathogens is of global health concern. *Enterococci*, is one of such notorious pathogens accounted for causing urinary tract infections blood stream infections and surgical site infections, particularly following manipulation of Gastrointestinal and Gentiourinary tracts. *Enterococcus* resistance to antimicrobial agents to which the genus *Streptococcus* are generally susceptible and its ability to transfer the drug resistance genes from vancomycin-resistant strains to *Staphylococcus aureus* is of concern. The therapeutic challenge of multiple-drug resistant (MDR) *Enterococci*, identifies them as important nosocomial pathogens. *Enterococci* infections have traditionally been treated with cell wall inhibitor agents in combination with an aminoglycoside. Reduced susceptibility to β-lactam antibiotics and vancomycin; in combination with a high level aminoglycoside resistance (HLAR) interferes with the penetration of the aminoglycoside into the bacterial cytoplasm, thus making the antibiotic synergism ineffective (1). Proper in-vitro testing of antimicrobial susceptibility of all isolates of Enterococci, appropriate modification of routine laboratory testing methods, judicial use of antibiotics, proper and systematic surveillance and control of fecal colonisation of resistant enterococci in hospital staff are certain measures to be taken for combating drug resistance in enterococci (2). The objective of the present investigation was to study the antimicrobial resistance in clinical isolates of Enterococci.

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MATERIALS AND METHODS

The present descriptive cross-sectional study was carried out in the department of Microbiology from April –March 2014. microbiology. A total of 128 enterococcal strains were isolated from various clinical samples (urine, blood etc).The strains were identified and speciated according to standard laboratory procedures as per Facklam and Collins Identification scheme. Antimicrobial susceptibility pattern was determined using Kirby Baeur Disk Diffusion method (KBDDM) as per CLSI standards with the following drugs: Ampicillin10U, Pencillin 10U, Gentamicin120µg, Ciprofloxacin5µg, Linezolid30µg, Nitrofurantoin300µg, vancomycin30µg, teicoplanin30µg and tetracycline30µgrespectively. The inoculated plates were incubated at 36 C for 18 hours with an exception to vancomycin which requires 24 hours incubation. The diameter of zone of inhibition for each antibiotic was measured in millimeters and determined as sensitive, intermediate and resistant in compliance with CLSI standards. E.facecalis ATCC 29212 was used as susceptible quality control strain (3).

RESULTS

Of the total of 128 isolates of Enterococcus species isolated from clinical samples, 97(76%) isolates were *E,faecalis* and 31(24%) isolates were *E.faecium* (Table 1). Species identification of isolates in our study enabled us to identify species-specific antibiotic susceptibility patterns.

Table 1 Species distribution of Enterococcal isolates from clinical specimen

***Enterococcus* Species**	**Number**	**Percentage (%)**
E.faecalis	97	76
E.faecium	31	24

Table 2 Antibiotic Resistance Pattern of Clinical Isolates of Enterococcus Species

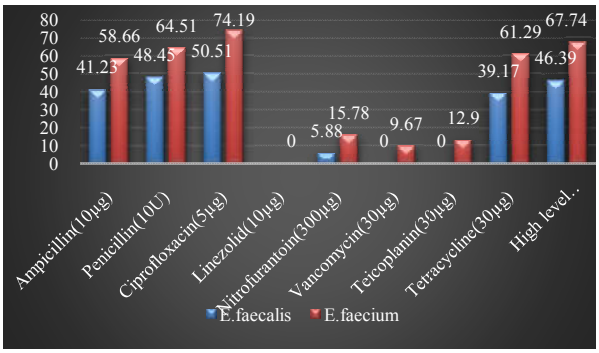

Table 3 Comparison of Antibiotic resistance between *E.faecalis* and *E.faecium*

Antibiotic	**E.faecalis**		**E.faecium**	
	No. Resistant	**Percentage**	**No. Resistant**	**Percentage**
Ampicillin(10µg)	40	41.23	18	58.66
Penicillin(10U)	47	48.45	20	64.51
Ciprofloxacin(5µg)	49	50.51	23	74.19
Linezolid(10µg)	-	-	-	-
Nitrofurantoin(300µg)	3	5.88	3	15.78
Vancomycin(30µg)	-	-	3	9.67
Teicoplanin(30µg)	-	-	4	12.9
Tetracycline(30µg)	38	39.17	19	61.29
High level Gentamicin(120µg)	45	46.39	21	67.74

DISCUSSION

In our study, *E.faecalis* (75.78%) was more frequently isolated than *E.faecium* (24.21%). Similar results have been reported from central and the other parts of South India On the contrary, studies carried out in North india have shown *E.faecium* to be responsible for large number of infections than *E.faecalis* [4]. Predominance of *E.faecalis* in endogenous flora of the body could be the reason behind its high proportion among hospital isolates [5]. Historically the ratio of infections due to *E.faecalis* to those due to other species is approximately 10:1 which plummeted in the recent years [6].

Multidrug resistant Enterococci are increasingly reported all over the world. In this study, 52.34% of isolates were resistant to Penicillin. Rates of penicillin resistance among *E.faecalis* and *E.faecium* were 48.45 % and 64.51% respectively **[Table:4].**

Table 4 Penicillin resistance among Enterococci

Authors	**Year of Study**	***E.faecalis***	***E.faecium***	**Publication**
Adhikari et al	2010	27.69%	70.59%	J Glob Infect Dis 2: 231-235[7]
Shrihari et al	2011	45.09%	66.67%	Int J Biol Med Res. 2011; 2(4): 865 – 869[8]
Jain et al	2011	100.00%		Ind J Path Microbiol 2006; 49 (4):620-2 [9]
Sreeja et al	2012	48.60%	41.60%	J Clin and Diag Res. 2012 Nov, Vol-6(9): 1486-1488 [10]
Deshpande et al	2013	72.50%	83.90%	J Infect Dev Ctries 2013; 7(2):155-158. [5]
Present study	2014	48.45%	64.51%	

As apparent from the above table, penicillin resistance in the present study is in agreement with several others. Jain *et al* reported 100% resistance to penicillin among Enterococcal isolates [9]

Table5 Ampicillin resistance among Enterococci

Authors	**Year of Study**	***E.faecalis***	***E.faecium***	**Publication**
Adhikari et al	2010	27.69%	70.59%	J Glob Infect Dis 2: 231-235[7]
Shrihari et al	2011	47.05%	33.33%	Int J Biol Med Res. 2011; 2(4): 865 – 869 [8]
Telkar et al	2012	64.28%	80.00%	Journal of Clinical and Diagnostic Research 2012; 6,405-407[11]
Fernandes et al	2013	36.90%	52.90%	Ind J Med Res. May 2013; 137(5): 981–985 [4]
Present study	2014	41.23%	58.66%	

Recently reports of steady rise in the recovery of Ampicillin resistant Enterococci are available from India. However, Ampicillin resistance in our study was 45.31%.Rates of Ampicillin resistance among *E.faecalis* and *E.faecium* were 41.23% and 58.66% respectively **[Table:5]**

Since Ampicillin is the drug of choice in the treatment of Enterococcal infections, the relatively high resistance of isolates in this study to ampicillin is of great concern especially in treating endocarditis.

Table 6 High level Gentamicin resistance among Enterococci in India

Authors	Year of Study	High level Gentamicin Resistance		Publication
		E.faecalis	*E.faecium*	
Mendiratta et al	2008	14.80%	22.70%	Ind J Med Microbiol. 2008.26,369-371 [12]
Loveena et al	2010	29.45%	56.16%	JK Science 2010,12,157-158[13]
Shinde RS et al	2012	44.68%	60.00%	Ann Trop Med Public Health 2012;5:85-8. [14]
Fernandes S et al	2013	53.50%	53.00%	Indian J Med Res. May 2013; 137(5): 981-985[4]
Present study	2014	46.39%	67.74%	-

According to our study, High level gentamicin Resistance (HLGR) was 46.39% in *E.faecalis* and 67.74% in *E.faecium* **[Table :6].** High level Aminoglycoside resistance in Enterococci is an acquired characteristic and is of great concern since it jeopardizes synergy with cell wall active agents rendering therapeutic difficulty. Therefore it is of vital importance to distinguish these high level aminoglycoside resistant strains from simply intrinsic resistant strains [13].

Table 7 Vancomycin Resistance among Enterococci

Authors	Year of Study	Vancomycin Resistance		Publication
		E.faecalis	*E.faecium*	
Karmarkar et al	2004	23%		Ind J Med Res .2004; 119,22-25. [15]
Ghoshal et al	2006	10%		Ind J Path Microbiol 2006; 49 (4):620-2 [16]
Lathika et al	2011	6%	2%	Nat J Med Res. Jan - March 2012. 2 (1): 25 - 27. [17]
Praharaj et al	2013	9.26%		Ind J Med Res. 2013; 138:549-556 [18]
Gangurdhe et al	2014	4.6%	13.7%	Open Journal of Medical Microbiology 2014,4,11-15. [19]
Present study	2014		9.67%	-

In this study, three isolates of *E.faecium* (9.67%) were identified as vancomycin resistant by disk diffusion method. In our study no vancomycin resistance has been detected in isolates of *E.faecalis*. The emergence of vancomycin resistant Enterococci poses a serious threat to the hospitalized patients with impaired host defense. Mathur *et al* from New Delhi were the first to report VRE from India in 1999 [20]. Athough the prevalence of VRE infections in India is much lower than the western world, it has been increasing since a decade [18].Vancomycin resistance in Enterococci not only leaves fewer options for the disease management but it is also important due to the potential risk of VRE gene transfer from Enterococci to *Staphylococcus aureus*[19].

The prevalence of VRE infections in India range from 0-30% [15-19]**[Table: 7]**

Table 8 Teicoplanin Resistance among Enterococci

Authors	Year of Study	Teicoplanin Resistance		Publication
		E.faecalis	*E.faecium*	
Karmarkar et al	2004	9.52%		Ind J Med Res .2004; 119,22-25. [15]
Jain et al	2011	-		Int j App Basic Med Res 2011;1:80-3[9]
Lathika et al	2011	-		Nat J Med Res. Jan - March 2012. 2 (1): 25 - 27. [17]
Praharaj et al	2013	7.6%		Ind J Med Res. 2013; 138:549-556 [18]
Deshpande et al	2013	4.4%	27.6%	J Infect Dev Ctries 2013; 7(2):155-158. [5]
Present study	2014	-	12.96%	-

In the present study, resistance to Teicoplanin (12.96%) was seen in four isolates of *Enterococcus faecium*. Teicoplanin resistance in our study falls within the range mentioned by several authors **[Table:8]**

Table 9 Ciprofloxacin resistance among Enterococci

Authors	Year of Study	*E.faecalis*	*E.faecium*	Publication
Oberoi et al	2010	74.72%		JK Science,12,157-158[13]
Shrihari et al	2011	45.09%	66.67%	Int J Biol Med Res. 2011; 2(4): 865 – 869[8]
Sreeja et al	2012	48.6%	54.1%	J Clin and Diag Res. 2012 Nov, Vol-6(9): 1486-1488 [10]
Praharaj et al	2013	71.38%		Ind J Med Res. 2013; 138:549-556 [18]
Present study	2014	50.51%	74.19%	

Ciprofloxacin has been shown to have good activity against Enterococci but off late resistance became a biggest problem with this drug.

Table 10 Tetracycline resistance among Enterococci

Authors	Year of Study	*E.faecalis*	*E.faecium*	Publication
Jain et al	2011	72%	55%	Ind J Path Microbiol 2006; 49 (4):620-2 [9]
Saraswathy et al	2013	67.34%	20%	Int J Med Res and Health Sci. 2013; 2 (3): 328-333 [21]
Present study	2014	38.23%	50%	-

The total number of Enterococcal isolates considered for study, the minimal use of tetracycline and geographical factors might be responsible for the lower resistance rate of tetracycline observed in our study.

In our study, it is encouraging to note that only 6 (8.57%) urinary isolates were resistant to nitrofurantoin in comparison with the study by Karamarkar *et al* where more than 80% of the isolates were resistant to nitrofurantoin. Our study hints that nitrofurantoin has excellent acitivity against uropathogenic Enterococci as described in a study by Bose *et al* [22].

Overall, *E.faecium* isolates were more resistant than *E.faecalis* in our study. Antibiotic resistance has been consistently reported to be more common in *E.faecium* as compared to *E.faecalis*. All the isolates in our study were susceptible to linezolid. Thus Linezolid can be used as reserve drug for the treatment of serious infections caused by MDR Enterococci.

CONCLUSION

Infection due to multidrug resistant enterococci is not uncommon in our set up. Multi resistance and cross resistance shown by the microorganisms result in limited options of drugs for treatment. This emphasizes the need for speciation and in vitro antibiotic susceptibility testing with alternative chemotherapeutic regimens for treatment of serious enterococcus infections. For enterococcal urinary tract infection, Nitrofurantoin is an excellent choice. Against multidrug resistant enterococcal infection, linezolid, tigecycline and vancomycin are very effective. To have an effective control of multidrug resistant enterococci would require better understanding of the interaction between enterococci, hospital environment and humans, prudent use of antibiotic, better

isolation procedures in hospitals and other patient care environments and improved and rapid surveillance measures[1].

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