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Original Research Article

Determination of vancomycin, linezolid and daptomycin resistance among *Eenterococcus* isolates from a tertiary care hospital

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ABSTRACT

Introduction: The clinical importance of the genus *Enterococcus* species relates to its intrinsic antibiotic resistance. Synergistic activity of penicillin and aminoglycosides against *Enterococcus*is preferred treatment. However, Infectious Diseases Society of America (IDSA) recommends usage of linezolid and daptomycin against emerging vancomycin resistant *Enterococcus*(VRE) isolates. In this study, we sought to measure the prevalence of vancomycin, linezolid and daptomycin resistance in *Enterococcus* clinical isolates from patients in a tertiary hospital of Western Maharashtra.

Materials and Methods: A total of 363 clinical isolates of *Enterococcus*species isolated from different clinical samples over the last three years and three months study period were analyzed for antibiotic susceptibility pattern against vancomycin, linezolid and daptomycin.

Results: Study demonstrated low prevalence of vancomycin (4.95%) and linezolid resistance (1.10%) among *Enterococcus* clinical isolates. Combined resistance to vancomycin and linezolid was also noted in few isolates. No daptomycin resistance was detected in this study, however in vitro daptomycin susceptible and vancomycin heteroresistance isolates may not respond to daptomycin monotherapy.

Conclusion: Our study, demonstrated relatively low prevalence of vancomycin and linezolid resistance, but emergence of combined newer drug resistance in *Enterococcus* species is cause of concern and reiterates the importance of importance of judicious use of antibiotics.

1. Introduction

Enterococcus species constitutes normal intestinal microflora in high proportion of healthy adults. *Enterococcus faecalis* and *Enterococcus faecium* are the two most common species of enterococci isolated from clinical samples.[1] *E. faecalis* are more prevalent than *E. faecium*, however increase in *E. faecium* clinical isolation have been reported recently. *E. faecium* infections are of clinical relevance, due to high percentage of resistant to vancomycin and ampicillin.[2]

Enterococcus are opportunistic pathogens associated with endocarditis and urinary tract infections, intra-abdominal infections and surgical wound.[3] These bacteria are intrinsically resistant to low concentrations of β-lactams, trimethoprim–sulfamethoxazole, aminoglycosides, and clindamycin. Furthermore, they readily acquires foreign genetic material with antibiotic resistant or undergo mutation for antibiotic resistance.[4] Few recent studies have described resistance to higher concentration of penicillins, aminoglycosides, macrolides-lincosamides-streptogramins, fluoroquinolones, rifampin and glycopeptides.[5,6]

In year 1988, *Enterococcus* resistance to high levels of vancomycin and teicoplanin was reported for the first time. Since then, number of infections with vancomycin-resistant enterococci has increased in hospitals all over the world. Infectious Diseases Society of America (IDSA) recommends using linezolid or daptomycin for *Enterococcus* strains resistant to ampicillin and vancomycin.[7] However, very limited studies are available on antibiotic susceptibility pattern of *Enterococcus* species to vancomycin, linezolid and daptomycin and these studies results has inter regional

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and intraregional variations.[8]

This study was undertaken to determine the antibiotic susceptibility pattern of *Enterococcus* species to vancomycin, linezolid and daptomycin, in a tertiary care hospital of western Maharashtra.

2. Materials and Methods

This study was a retrospective study conducted by Department of Microbiology, of a tertiary care hospital. Total of 363 consecutive, non-repetitive clinical isolates of *Enterococcus* species from different clinical samples were included in the study. The study period was from 01 Jan 2016 to 31 Mar 2019.

Enterococcus species were isolated and identified in accordance with standard procedures.[9] Antibiotic susceptibility testing (ABST) of all isolate was conducted by both disc diffusion method and Minimum inhibitory concentration (MIC) on automated Vitek (BioMérieux) system.[10] *Enterococcus* ATCC strains E. faecalis ATCC 29212 were used as standards for antibiotic susceptibility testing. Strains resistant to vancomycin, linezolid and daptomycin were confirmed further tested by E test strip (Himedia, Mumbai). For automated ABST and E test manufacturer instructions were followed. Minimum inhibitory concentration (MIC) interpreted was as per criteria laid by Clinical Laboratory Standards Institute (CLSI) guidelines.[11]

3. Result

A total of 363 *Enterococcus* were isolated during the study period. Of these, there were 250 (68.87%) isolates were of *Enterococcus faecalis* and 113 (31.12%) of *Enterococcus faecium*. No other *Enterococcus* species were isolated. Clinical samples included urine, blood, pus, sputum and other samples like body fluids etc. Majority of isolates were from urine sample followed by blood and pus (Table 1). Among isolates of *Enterococcus*- 11(3.03%), 18(4.95%) and 334(92.01%) were intermediate, resistant and sensitive to vancomycin respectively. Linezolid intermediate and resistant was identified in 3(0.82%) and 4 (1.10%) isolates of *Enterococcus faecium* only. Among 2 (50%) linezolid resistant isolates were also resistant to vancomycin, however they were all sensitive to daptomycin (Table 1). MIC to vancomycin ranged between 0.25-256 μg/ml, MIC of linezolid ranged between 0.25- 16μg/ml and MIC for daptomycin was less than 1 μg/ml for all the isolates (Figure 1) [Table 2].

4. Discussion

In the hospitals, the real challenge in management of enterococcal infection lies in its intrinsic and acquired resistance to numerous antimicrobial agents. Researchers recommend combination therapy for high load enterococcal infection, like endocarditis.[12] Combination therapy includes ampicillin for susceptible *Enterococcus* isolates and vancomycin for penicillin resistance isolates. However, many researcher have reported emergence of vancomycin resistance in *Enterococcus* species, which pose an immense challenge to the clinicians.[13] For vancomycin resistant clinical isolates, Infectious Diseases Society of America (IDSA) recommends linezolid or daptomycin antibiotics especially in bacteremia.[14] In views of these recommendation, in vitro susceptibility testing for newer antimicrobials, such as daptomycin and linezolid, is essential for the management of VRE infections.

In our study, Vancomycin resistance was noted among 10.61% *Enterococcus faecium* and 2.40% *Enterococcus faecalis* isolates. The prevalence of VRE in our study is 4.95% which similar to other published studies.[15,16] Hospitals laboratory should monitor for any evidence of creeping MIC, which indicates heteroresistance. These heteroresistant isolates often leads to treatment failure.[17]

Six different types of vancomycin resistance are shown by *Enterococcus*: Van-A, Van-B, Van-C, Van-D, Van-E and Van-G. Van A confers high degree of resistance to both vancomycin (MIC$\geq$64μg/ml) and teicoplanin (MIC$\geq$16μg/ml), whereas Van B and Van E confers varying level resistance to vancomycin (MIC 4 -1000 μg/ml), but are susceptible to teicoplanin.[18] Van A is the most common mechanism for resistance among clinical isolates, so identification of any intermediate susceptibility to vancomycin warrants detailed molecular investigation.[19,20] In this study, Vancomycin intermediate susceptibility was noted among 11 (3.03%) isolates, which is in concordance with other studies.[19,20]

Linezolid resistance was detected in 4 (1.10%) *Enterococcus* isolates, whereas 3(0.82%) isolates demonstrated intermediate susceptibility. Linezolid resistance in *Enterococcus* has been reported earlier by other researchers also.[21] In this study, prevalence of linezolid resistance is relatively lower compare to other publisher report, due to robust hospital antibiotic policy. Further, in this study, two *Enterococcus* isolates demonstrated combined linezolid and vancomycin resistance. Combined resistance to both vancomycin and linezolid is very rare.[21,22] These results are in concordance with findings reported by other researchers.[21,22] In- vitro detection of linezolid resistance in Enterococcus, has poor correlation between various methods. Therefore, in our study, disc diffusion method was initially used, which was followed by MIC based automated antimicrobial susceptibility testing by Vitek 2 system (bioMérieux) and confirmation by the E-test method.[23]

Daptomycin is lipopeptide antibiotic used in the treatment life-threatening infections caused by gram-positive organisms. Daptomycin activity in media requires presence of divalent cations, especially calcium ions. E- strips of

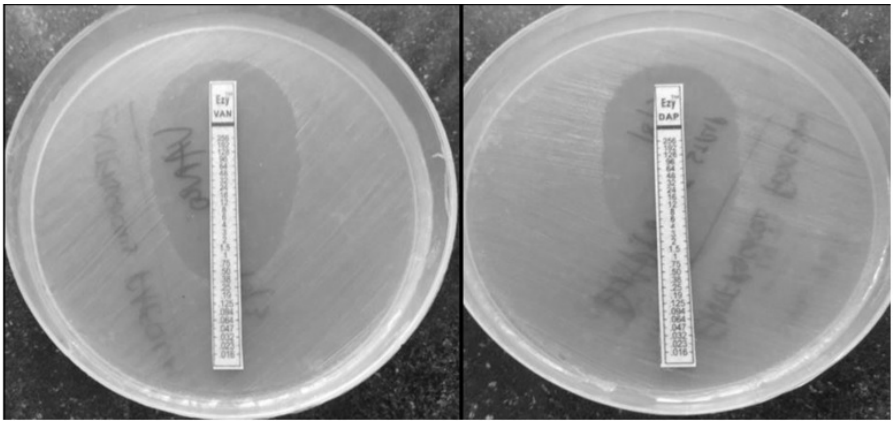

Fig. 1: E test of vancomycin and daptomycin antibiotics against clinical isolates of *Enterococcus*

Table 1: Distribution of *Enterococcus* species in various clinical samples

Samples	Isolated (n/%)	Vancomycin (n)		Linezolid (n)		Daptomycin (n)	
		I	R	I	R	I	R
Urine	320/ (88.15%)	7	18	3	4	-	-
Blood	26/(7.16%)	1	-	-	-	-	-
Pus	10(2.75%)	3	-	-	-	-	-
sputum	4(1.10%)	-		-	-	-	-
others	3(0.82%)	-		-	-	-	-
Total	363	11	18	3	4	-	-

I – Intermediate, R- Resistant

Table 2: MIC interpretative criteria and ABST pattern of *Enterococcus* isolates (μg/ml)

Organism	Vancomycin			Linezolid			Daptomycin		
	S	I	R	S	I	R	S	I	R
	≤4	8-16	≥32	≤2	4	≥8	≤1	2-4	≥8
Enterococcus faecalis	240	4	6	248	1	1	250	-	-
Enterococcus faecium	94	7	12	108	2	3	113	-	-

S: Sensitive, I: Intermediate, R: Resistant

daptomycin with supplemented calcium ions can be applied directly on Mueller Hinton Agar. In our study, calcium supplemented culture media was used for daptomycin activity and all isolates were sensitive to daptomycin. Daptomycin resistance in *Enterococcus* isolates, is rare and reported in cancer patients.[22,24] Researchers have reported association of daptomycin resistant in Enterococci species with earlier exposure to daptomycin and vancomycin resistance. Enterococcal strains with vancomycin hetero-resistance, may fail to respond to daptomycin therapy, despite in vitro susceptibility to daptomycin.[25]

Recommended therapy for serious enterococcal infections consists of synergistic combination of an aminoglycoside and a cell wall–active agent. However, many *E. faecium* isolates are intrinsically resistant to cell wall active antibiotics like penicillin and some acquires resistance to vancomycin. Further, this resistance is mediated by plasmids, which are easily transferable between bacterial species. Newer antibiotics were developed for treatment Enterococci infections resistant to ampicillin, vancomycin, or the aminoglycosides. These antibiotics include linezolid, daptomycin, and tigecycline. However soon after clinical usage of linezolid, resistance to linezolid has also emerged, which is steadily rising.[26]

Present recommendation for *Enterococcus* susceptible to ampicillin, but resistant to aminoglycosides is a combination of ampicillin plus daptomycin or linezolid. For *Enterococcus* isolates that are resistant to ampicillin and susceptible to aminoglycosides, aminoglycoside combined with vancomycin should be used. However, if the isolate is resistant to both ampicillin and aminoglycosides, management should include newer antibiotics daptomycin, linezolid, or vancomycin combined with another susceptible antimicrobial agent.[24,27]

5. Conclusion

Our study demonstrated relatively low prevalence of Vancomycin and Linezolid resistance among clinical isolates of *Enterococcus*. However, persistence of vancomycin pressure on hospital flora and emergence of isolates *Enterococcus* species with combined resistance to newer antibiotics, is a cause of concern. Linezolid and daptomycin are effective antibiotics against VRE. Strict implementation of hospital antibiotic policy with judicious use of antibiotics is a key to prevention of emergence of multidrug resistant strains of *Enterococcus* species.

6. Source of Funding

None.

7. Conflict of Interest

None.

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