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Prevalence of Vancomycin and High Level Gentamycin Resistance in Enterococcus Isolates in a Tertiary Care Hospital

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Abstract: ***Enterococcus account for about 12% of health care associated infections (HAI) and ranks third most common multi-drug resistant pathogen causing HAI. Continuous monitoring of the antibiotics susceptibility pattern of Enterococcus species thus became inevitable for control of Hospital Acquired infection. Aim of the study was to determine the prevalence of high level Gentamycin and Vancomycin resistance enterococci among the clinical isolates. All clinical samples (Urine, Blood, CSF and others) received in microbiology lab for bacterial culture and sensitivity were screened for enterococcus, only enterococcus isolates were selected for this study. Primary inoculation was done on Blood agar and mac conkey agar culture media and incubated for 18-24 hrs at 37°C aerobically. Bacterial growth on culture media was identified by colony morphology, gram staining and vitek-2 automated bacterium indentifying system. The prevalence of high level gentamycin resistance in Enterococcus was 74.5% and Vancomycin resistant in Enterococcus was 21.56%. Study concludes that increased prevalence of high level gentamycin and vancomycin resistant in enterococci observed that we need to focus on infection control and prevention majors***

Keywords: eneterococcus,Vancomycin, high level gentamyscin

1. Introduction

The Enterococcus species has emerged as a major nosocomial pathogen, and common difficulty faced in treatment of such infections are attributed to the presence of high level Gentamycin resistance in them. Continuous monitoring of the antibiotics susceptibility pattern of Enterococcus species thus became inevitable for control of Hospital Acquired infection.[1] The alarming thing is the increasing evidence of potential risk of transfer of Vancomycin resistance gene from VRE to various Gram-positive microorganisms especially Staphylococcus aureus through conjugative plasmids, which worsens the scenario further[2].

The emergence of multidrug resistant enterococci to commonly used antibiotics, for example, aminoglycosides and cephalosporin's, is because of their ability to attain and transfer the drug resistance gene, giving rise to enterococci with high level gentamicin (HLGR) and glycopeptide resistance [3].

2. Material and Method

The present study was carried in the Department of Microbiology, Mahatma Gandhi Medical College and Hospital, Jaipur (Rajasthan). All clinical samples (Urine, Blood, CSF and others) received in microbiology lab for bacterial culture and sensitivity were screened for enterococcus, only enterococcus isolates were selected for this study.

All clinical samples were processed as follow:

1) Gram staining was done of all the clinical samples except urine.
2) Direct wet microscopy was done of urine sample.

All culture media were obtained from Hi-Media laboratories, Mumbai, India. Primary inoculation was done on Blood agar and mac conkey agar culture media and incubated for 18-24 hrs at 37°C aerobically. Bacterial growth on culture media was identified by colony morphology, gram staining and vitek-2 automated bacterium indentifying system.

3. Result

A total of about 7679 urine specimens, 1447 Blood specimens, 1196 CSF specimens, and other specimens 5326 specimens were analyzed for Enterococcal growth. A total of about 102 Enterococcal isolates were recovered from the above samples, of which majority were from urine specimens 57, 24 from Blood specimens, CSF 4 specimens and others 17 specimen's isolates.

In table 1 we observe that out of 102 (100%) the specimens were from IPD (66.78%) and (33.22%) specimen were from OPD.Out of 102 (100%) Enterococcus isolates 57 (55.88%) were obtain in urine, followed by blood 24 (23.53%), CSF 4 (3.92%), others 1 (16.66%).

Table 1: Distribution of sample showing growth of Enterococci

Specimen type	OPD		IPD		Total	
	No of sample analyzed	Positive for Enterococcal growth	No of sample analyzed	Positive for Enterococcal growth	No of sample analyzed	Positive for Enterococcal growth
Urine	3117	8 (0.25%)	4562	49 (1.07%)	7679	57 (0.74%)
Blood	00	00	1447	24 (1.65%)	1447	24(1.65%)
CSF	00	00	1196	4 (0.33%)	1196	4 (0.33%)
Others	2081	01(0.04%)	3245	16 (0.49%)	5326	17 (0.31%)
Total	5198 (33.22%)	09 (0.17%)	10450 (66.78%)	93 (0.88%)	15648	102 (0.65%)

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Table 2: Antibiotic susceptibility pattern of isolated Enterococcal spp

Ento. spp	Total no.	CIP		TEI		TETRA		HLG		VA		TGC		LE		LZ	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
E. faecalis	38	8 21%	30 79%	36 87%	2 13%	9 24%	29 76%	12 32%	26 68%	36 95%	2 5%	36 95%	2 5%	9 24%	29 76%	38 100%	0 00%
E. faecium	58	1 2%	57 98%	39 67%	19 33%	20 34%	38 66%	8 14%	50 86%	39 67%	19 33%	8 14%	50 86%	8 14%	50 86%	55 95%	3 5%
Other spp.	6	1 17%	5 83%	0 0%	0 0%	4 67%	2 33%	6 100%	0 00%	5 83%	1 17%	4 67%	2 33%	2 33%	4 67%	5 83%	1 17%

Table 2 represents Antibiotic susceptibility pattern of isolated Enterococcal species. *E. faecium* isolates are resistant to ciprofloxacin (98%), high level gentamicin (86%) and Vancomycin (33%) but sensitive to Tegecyline (97%). *E. faecalis* isolate are resistant to ciprofloxacin (78%) but sensitivity to Vancomycin (95%) and high level Gentamycin (14%).

Table 3: Distribution MIC value vancomycin for the VRE isolates

VRE isolates	Vancomycin MIC values (µg/ml) Resistant (≥32 µg/ml)					Total
	32 µg/ml	64 µg/ml	128 µg/ml	256 µg/ml	512 µg/ml	
E. faecium	15	2	1	1	0	19
E. faecalis	2	0	0	0	0	2
Other spp.	1	0	0	0	0	1
Total	18	2	1	1	0	22

Table 3 shows that Out of the total 22 Vancomycin resistant enterococci(VRE) isolates, the Minimum Inhibitory Concentration (MIC) value of *E. faecium* 19, *E. faecalis* 2 and others 1 isolates fall within resistant range of 32-512 µg/ml.

Table 4: Distribution MIC value High level Gentamycin(HLG) for the VRE isolates

HLG isolates	High level Gentamycin MIC values (µg/ml) Resistant (>512 µg/ml)					Total
	256 µg/ml	384 µg/ml	512 µg/ml	768 µg/ml	1024 µg/ml	
E. faecium	0	0	0	9	41	50
E. faecalis	0	0	0	6	20	26
Other spp.	0	0	0	0	0	0
Total	0	0	0	15	61	76

In table 4 we observed that Out of the total 76 HLG isolates, the MIC value of *E. faecium* 50 and *E. faecalis* 26 isolates fall within resistant range of 256-1024 µg/ml.

In our study in we examined 15648 clinical specimens out of these 102 Enterococci species was isolated. The prevalence of high level gentamycin resistance in *Enterococcus* was 74.5%. The prevalence of Vancomycin resistant in *Enterococcus* was 21.56%.

4. Discussion

Enterococci are emerging as one of the most common agents of nosocomial infections in the hospital and also cause opportunistic infections in immuno compromised individuals. It is essential to detect them early and institute proper therapy based on the antimicrobial susceptibility pattern.

With the emergence of VRE, the situation has worsened leaving very few options of selecting antibiotics for the treatment of this multi – drug resistance organism. At this context, our study has been attempted to assess the prevalence of vancomycin and high level Gentamycin resistance among the clinical isolates of Enterococci recovered from the patients in this area.

A total of about 102 Enterococcal isolates were recovered from a total of about 15648 clinical samples including urine, blood, CSF and others specimens.

The majority of the specimens were from inpatients (91%) than from outpatients (9%) which are in correlation with the findings of Acharya et al. [4], reported 72% specimens from hospitalized patients and 28% specimens from outpatients.

On studying the antibiotic susceptibility pattern we found that most of E. faecium isolates are highly resistant to ciprofolxcin (98%), or high level Gentamycin (86%) and Vancomycin (33%) but a higher sensitivity to Tigecycline (97%) and Linezolid (95%). The E. faecalis isolates have shown higher resistance rates to Ciprofloxacin (78%), but sensitivity to Linezolid (100%), Vancomycin (95%) and high level gentamicin (32%). Kiman Bang et al. [5] observed that 50% resistance rate to ciprofloxacin among isolates.

Out of the 102 Enterococcal isolates, 22 (21.56%) were identified as vancomycin resistant. Vancomycin ≥ 32 µg/ml were subjected to MIC .Genaro A et al. [6] Data shows of MICs revealed vancomycin as the most effective antimicrobial drug, inhibiting 98.6% of the strains in Enterococcus faecalis.

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In our study, the MIC range of most of the VRE isolates fall within 32-512 µg/ml with one isolate showing 512 µg/ml. Findings of Patel R et al. [7] shows the vancomycin MIC range of VRE isolates 256 µg/ml in their study. Praharaj I et al. [8] has reported a case report, van A E. faecalis with Vancomycin MIC 32-512 µg/ml.

Out of the 102, 76 HLG resistant isolates (74.5%) were MIC in resistant range (>512 µg/ml). Thus in our study, the HLG MIC range observed was 256-1024 µg/ml. Kevin R Forward et al. [9] reported that Out of the 27 gentamicin-resistant

E. faecalis strains recovered from blood, 24 were highly resistant to streptomycin Another study by Narges Dadfarma et al.[10] shows out of the 142 isolates, 62 (43.7%) were found to exhibit the HLGR phenotype.

In the presented study Prevalence of Vancomycin resistant in Enterococcus was 21.56% (prevalence of vancomycin resistance in E. faecalis was 5% and in E. faecium was 33%). In a study given by Sangram Singh Patel et al. [11] out of 96 isolates 10 (10.41%) showed resistant to vancomycin. Another study by Wondwossen Abebe et al. [12] vancomycin resistant Enterococci were found to be 11(5.5%) out of 201 specimens. In addition, a report from a hospital in China found the prevalence of Vancomycin resistant E. faecalis in 2012 to 2013 to be higher, at 6.5%, than that of Vancomycin resistant E. faecium, at 2.7% [13] .

Further the prevalence of high level gentamycin resistance in Enterococcus was 74.5%. (Prevalence of high level gentamycin resistance E. faecalis was 68% and E. faecium was 86%). The prevalence of HLG in Sangram Singh Patel et al. [11] study were 46 out of 96 (47.9%). Another study by M. Emaneini et al. [14] concluded that High-level resistance to gentamicin and multiple drug resistance were observed in 96.2% of isolates.

5. Conclusion

The presented study observed increased prevalence of high level gentamycin and vancomycin resistant in enterococci observed that we need to focus on infection control and prevention majors, to reduce high level gentamycin and vancomycin resistance in enterococci.

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