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Original Research Article

High Level Aminoglycoside Resistance in Enterococcus Species Isolated from Tertiary Care Hospital of South India - An Update

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ABSTRACT

Background and Objectives: The emergence of Enterococcus species as a causative agent of Health care associated infections poses a formidable challenge to clinicians. Enterococci causing plethora of notorious infections are well accorded with intrinsic resistance to antibiotics. Acquired resistance to commonly used antibiotics such as Penicillin, Aminoglycoside and Vancomycin have further complicated the drug resistance scenario. Detection of High Level Aminoglycoside Resistance (HLAR) in Enterococcus species hints to the loss of synergy between cell wall active antimicrobial agents and Aminoglycosides antibiotics. Hence, the current study was undertaken in our institution to detect the incidence of HLAR.

Materials and Methods: A total of 128 clinical isolates of Enterococcus spp were taken up for study and routine biochemical tests were performed for the confirmation of Enterococcus spp. Screening for HLAR in Enterococcus species was detected by Kirby Baeur disk diffusion method using High level Gentamicin (HLG - 120 µg) and High level Streptomycin (HLG - 300 µg) disks in accordance with guidelines laid by CLSI.

Results: Out of total 128 Enterococcus species isolated from different clinical samples, High Level Gentamicin Resistance was detected in 66 (51.56%) Enterococcus strains and High Level Streptomycin Resistance was seen in 62 (48.43%) isolates. Amongst HLGR Enterococcus strains, 45 (439.4%) were E. faecalis and 21 (67.74%) were E.faecium. Similarly, among HLSR Enterococcus strains, 43 (44.32%) were E. faecalis and 19 (61.29%) were E.faecium.

Conclusion: We hereby conclude that Enterococcus strains, isolated from different clinical specimens must be screened routinely for High Level Aminoglycoside Resistance by Laboratories to improve the therapeutic outcome.

Key words: Enterococci, High Level Aminoglycoside Resistant (HLAR), High Level Gentamicin Resistant (HLGR), High Level Streptomycin Resistant (HLSR)

INTRODUCTION

Enterococci are the cause of many significant infections. They are inherently resistant to many antibiotics, and their susceptibility to cell wall active agents, such as beta-lactams and vancomycin, is reduced. Although enterococci are moderately resistant to aminoglycosides, synergistic combination therapy with a cell wall active agent often provides effective therapy for these infections. When enterococci acquire genes encoding aminoglycoside-inactivating enzymes, or mutations resulting in decreased binding (streptomycin), the synergism of aminoglycosides with cell wall active agents is lost. Determination of the high-level aminoglycoside resistance (HLAR) status of an enterococcal isolate is

needed to determine the best course of antimicrobial chemotherapy. [(1)]

MATERIALS AND METHODS

Bacterial Strains

A total of 128 non-repetitive and non-identical isolates of Enterococci were obtained from several clinical specimens during the period of April 2013-March 2014 for our study purpose.

The genus Enterococci were identified and confirmed by Gram stain, catalase test, Bile esculin hydrolysis, Heat tolerance test, Salt tolerance test and PYR hydrolysis. Enterococci were further identified to species level using Facklam and Collins scheme on the basis of carbohydrate fermentation tests, Arginine dihydrolase test, Potassium tellurite reduction test, Pyruvate utilization and appropriate motility and pigment detection.

Detection of High Level Aminoglycoside Resistance by Kirby Bauer disk Diffusion Method (KBDDM)

Screening test for the detection of High level aminoglycoside resistance in Enterococcus species was performed by standard disk diffusion method with antibiotic disks, Gentamicin 120µg and Streptomycin 300µg. Enterococcal isolates were grown overnight in Brain heart infusion broth and their turbidity was adjusted to 0.5 McFarland standard. A lawn culture of these isolates was made on Mueller Hinton Agar (MHA) with antibiotic disks on place. Plates were incubated at 35° C in ambient air for 16-18 hours.

The zone diameters were interpreted in accordance with the CLSI guidelines

6mm=Resistant
7–9 mm=Inconclusive
≥10 mm=Susceptible
MIC correlates:
For Gentamicin;
R=>500 µg/mL S=≤500 µg/m L
For Streptomycin;
R=>1000 µg/mL (broth) and>2000 µg/mL (agar)
S=≤500 µg/mL (broth) and ≤1000 µg/mL (agar)

Enterococcus faecalis ATCC 29212 was used as negative control strain [(2)]

RESULTS

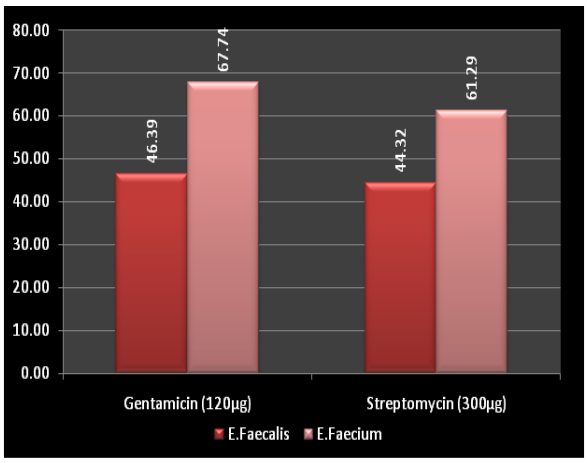
A total of 128 Enterococcal isolates were screened by Kirby Bauer disk diffusion method for high level aminoglycoside resistance.

Table 1: Distribution of High Level Gentamicin and Streptomycin Resistance among Enterococcal Isolates (N=128)

	No. of isolates	percentage
High Level Gentamicin Resistance	66	51.56
High Level Streptomycin Resistance	62	48.43

Table 2: High Level Aminoglycoside Resistance In Enterococcus Species

Antibiotic	Species	Number	Sensitive		Resistance		Total No.
			No	%	No	%	
Gentamicin(120ug)	E.*faecalis*	97	52	53.61	45	46.39	128
	E.*faecium*	31	10	32.25	21	67.74	
Streptomycin(300ug)	E.*faecalis*	97	54	55.67	43	44.32	
	E.*faecium*	31	12	38.7	19	61.29	



Graph 1: Screening of High Level Aminoglycoside Resistance in Enterococcus Species

66/128 (51.56%) isolates were High level Gentamicin Resistant 62/128 (48.43%) isolates were High level Streptomycin Resistant (Graph 1).

High level gentamicin Resistance (HLGR) was 46.39% in E.faecalis and 67.74% in E.*faecium.*

High level streptomycin resistance (HLSR) was detected among 44.32% of E.*faecalis* and 61.29% of E.*faecium.*

DISCUSSION

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.

In our study HLGR was more predominant than HLSR. This is in congruence with the studies made by several others. Nonetheless, in a study from Nagpur by Agarwal *et al* reported a prevalence of high level gentamicin resistance in enterococci to be 7.8 % whereas high level streptomycin resistance was reported to be 24.7%. The reason for the higher prevalence of high level streptomycin resistance in comparison to the high level gentamicin resistance in the enterococcal isolates in this study was not clear. It may be related to greater usage of streptomycin in comparison to gentamicin. [(3)]

Table 3: High level Gentamicin resistance among Enterococci in India

Authors	Years 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 [(4)]
Loveena et al	2010	29.45%	56.16%	JK Science 2010,12,157-158 [(5)]
Shinde RS et al	2012	44.68%	60.00%	Ann Trop Med Public Health 2012; 5; 85-8. [(6)]
Fermandes S et al	2013	53.50%	53.00%	Indian J Med Res. May 2013; 137(5): 981-985 [(7)]
Present Study	2014	46.39%	67.71%	-

Studies from New Delhi, Karmarkar *et al* [(8)] and Mumbai, Randhawa *et al* [(9)] reported HLGR as high as 70% and 100% respectively.

Table 4: High level Streptomycin resistance among Enterococci in India

Authors	Years of Study	High level Gentamicin Resistance		Publication
		E.faecalis	E.Faecium	
Mendiratta et al	2008	14.80%	13.60%	Ind J Med Microbiol. 2008.26,369-371 [(4)]
Oberoi et al	2010	28.80%	45.72%	JK Science 2010,12,157-158 [(5)]
Telker et al	2012	57.14%	62.28%	Journal of Clinical and Diagnostic Research 2012; 6: 405-407 [(10)]
Fermandes S et al	2013	48.80%	58.80%	Indian J Med Res. May 2013; 137(5): 981-985 [(7)]
Present Study	2014	44.32%	61.29%	-

In present study, HLSR was detected among 44.32% of E.faecalis and 61.29% of E.faecium. HLSR in our isolates of E.faecium, though considered high, falls within the same range as observed by Fernandes *et al* [(7)]

From our study, it is evident that both HLGR and HLSR are more common in E.faecium. This might perhaps be due to the fact that E.faecium are considered hardier and more drug resistant.

The higher rates of Aminoglycoside resistance in the present study may be ascribed to the source of the isolates being from a tertiary care set up where chronic cases are prevalent and a wider usage of broad spectrum antibiotics occurs as mentioned previously by Randhawa *et al* [(9)]

CONCLUSION

The present study has highlighted that high level aminoglycoside resistance in more common in enterococci and recommends the routine detection of HLAR in hospitals and laboratories.

Aminoglycosides resistance is of great concern, since it eliminates the synergy of aminoglycosides with β -Lactam antibiotics, which is the therapy of choice for most of the Enterococcal infections. Enterococci are naturally resistant to low-level aminoglycosides and since high level aminoglycoside resistance is on the rise, therapy with agents like ampicillin, linezolid, daptomycin and ceftaroline should be considered and evaluations of alternate regimen seem to be the need of the hour.

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