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Original Research Article

High Level Aminoglycoside Resistance among Clinical Enterococcal Isolates in a Tertiary Care Centre of North East India

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ABSTRACT

Background: Enterococci are organisms whose low virulence is well compensated for by their intrinsic resistance to antibiotics and their ability to acquire resistance to several broad spectrum antibiotics. Although Enterococci are moderately resistant to aminoglycosides, synergistic combination therapy with a cell wall active agent often provides effective therapy for these infections. However, occurrence of high level aminoglycoside resistance (HLAR) among enterococcal isolates results in the failure of such combinations. Moreover simultaneous occurrence of vancomycin resistance compounds the problem of treating such infections.
Aim: To assess routine enterococcal isolates for their antibiotic resistance patterns with special reference to high level aminoglycoside resistance.
Materials and Methods: All Enterococcal isolates from urine, pus/exudate and lower respiratory samples were screened for their HLAR status. The MIC of the HLAR isolates to gentamicin and streptomycin were determined. These isolates were also screened for Vancomycin resistance.
Results: Enterococci were isolated in 586 out of 14,416 samples. E. faecalis accounted for 84.9% and E. faecium15.1% of the total Enterococcal isolates. Among the isolated Enterococci, 43.5% (n=255) showed high level aminoglycoside resistance to gentamicin or streptomycin or to both streptomycin and gentamicin by the disc diffusion method using high content discs. E. faecalis constituted 69.4% and E. faecium 30.6% among the HLAR isolates.
Conclusion: This study shows a high occurrence of HLAR among clinical Enterococcal isolates warranting routine screening for HLAR in order to guide therapy of Enterococcal infections

Key words: Enterococcal infection, High Level Aminoglycoside Resistance (HLAR). E. faecium
Key message: HLAR screening in routine bacteriology helps clinician save valuable time in deciding appropriate therapy for an enterococcal infection. Study points towards need for increased vigilance for VRE among such isolates. High content disc screening appears to be an easy and reliable method to detect HLAR in the routine laboratory.

INTRODUCTION

Enterococci cause a wide variety of infections, most commonly urinary tract infection, bloodstream infection, endocarditis, infections of the abdomen, biliary tract, burn wounds and indwelling

foreign devices. Enterococcus faecalis cause 80-90% of human Enterococcal infections while E. faecium accounts for majority of the remainder. [1]

The inherently low virulence of Enterococci is well compensated for by their intrinsic resistance to antibiotics. Further, they also exhibit the ability to acquire resistance to several broad spectrum antibiotics. [2] Traditionally, a combination of penicillin/ampicillin with an aminoglycoside is the treatment of choice for Enterococci with vancomycin as last resort. [3]

Although Enterococci are moderately resistant to aminoglycoside, 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, thereby giving rise to high level aminoglycoside resistance (HLAR), the synergism of aminoglycosides with cell wall active agents is lost. The presence of vancomycin resistance along with HLAR is making the treatment of these infections extremely difficult. [3,4]

Therefore, the determination of HLAR status of an Enterococcal isolate is needed to determine the best course of antimicrobial chemotherapy.

The present study was undertaken considering the paucity of data on high level aminoglycoside resistance (HLAR) in Enterococci spp. especially from the north-eastern region of India.

MATERIALS AND METHODS

This retrospective study was conducted over a period of one year from July 2013 to June 2014.

All Enterococcal isolates from urine, pus/exudate and lower respiratory samples were included in the study.

Enterococci were identified using standard protocol. [5] The isolates were screened for their HLAR status. The MIC of the HLAR isolates to gentamicin and streptomycin were determined. These isolates were also screened for Vancomycin resistance.

Screening for HLAR

Inocula of the isolates corresponding to 0.5 McFarland were prepared and these were swabbed onto Mueller-Hinton agar plates to provide lawn cultures of the organisms. High content discs containing 120µg gentamicin and 300µg streptomycin, prepared in-house, were placed on the inoculated media. Enterococcus faecalis ATCC 29212 was used as control. The plates were incubated at 35±2°C for 18hrs. The zones of inhibition were measured. Zone size ≤ 6mm was considered resistant, 7-9mm inconclusive and ≥ 10mm sensitive. [6] All isolates were subjected to MIC determination for confirmation of their HLAR status.

Determination of Minimum Inhibitory Concentration (MIC) of gentamicin and streptomycin by agar dilution

MIC of gentamicin and streptomycin were determined by agar dilution method. Mueller-Hinton agar plates with concentrations of antibiotics from 1 µg/L to 2048 µg/L were prepared. Inocula of the isolates corresponding to 0.5 McFarland were prepared and spot- inoculated onto the plates. The plates were incubated at 35±2°C for 18 hrs. Visible growth in the areas inoculated was taken as resistance. [6]

E. faecalis ATCC 29212 was used as control.

Vancomycin resistance screening of HLAR isolates

Brain heart infusion agar plates with 6µg/mL Vancomycin were prepared. Suspensions of the isolates corresponding to 0.5 McFarland were prepared and inoculated as spots onto the plates. The plates were incubated at 35±2°C for 24 hrs. Visible

growth was taken as resistance. [6] E. faecalis ATCC 29212 was used as control.

RESULTS

Enterococci were isolated in 586 out of 14,416 samples.

Table 1: Type of samples and isolates

	Urine	Pus / exudate	Respiratory sample	Total
Total samples	10,528	1475	2413	14,416
Enterococcus spp	491	55	40	586
HLAR*	211	29	15	255

* High Level Aminoglycoside Resistance

E. faecalis accounted for 84.9% and E. faecium15.1% of the total Enterococcal isolates. Among the HLAR isolates, 69.4% were E. faecalis and 30.6% E. faecium.

Table 2: Distribution of HLAR* isolates

	Urine	Resp sample	Pus/ exudate	Total
OPD	33	0	6	39
IPD	178	15	23	216
Medicine	16	2	0	
Paediatrics	59	0	12	
Neurology	7	0	0	
Intensive Care Unit	16	13	2	
General Surgery	9	0	7	
Obstetrics and Gynaecology	14	0	1	
Othopaedics	11	0	0	
Cardio Thoracic and Vascular Surgery	9	0	0	
Urology	37	0	1	

*High Level Aminoglycoside Resistance

Table 3: Proportion of E. faecalis versus E. faecium

	Urine		Pus / Exudate		Respiratory sample	
	E. faecalis	E. faecium	E. faecalis	E.faecium	E. faecalis	E.faecium
Enterococcus spp	417(85%)	74(15%)	49(89%)	6(11%)	32(80%)	8(20%)
total	491		55		40	
HLAR*	146(69%)	65(31%)	24(83%)	5(17%)	7(46.7%)	8(53.3%)
total	211		29		15	

*High Level Aminoglycoside Resistance

Table 4: HLAR* resistance patterns of the Enterococcal isolates

High level resistance to	Urine		Respiratory		Pus / Exudate	
	E.faecalis	E.faecium	E.faecalis	E.faecium	E.faecalis	E.faecium
Gentamicin only	36	7	3	1	9	1
Streptomycin only	30	8	2	0	4	0
Both Gentamicin and Streptomycin	80	50	2	7	11	4
	146	65	7	8	24	5
Total HLAR*	211		15		29	

*High Level Aminoglycoside Resistance

Table 5: Comparison of E. faecalis and E. faecium with respect to HLAR*

		E.faecalis	E.faecium
Urine	total	417	74
	HLAR* (% of total)	146 (35%)	65 (87%)
Respiratory	total	32	8
	HLAR* (% of total)	7 (21.8%)	8 (100%)
Pus	total	49	6
	HLAR* (% of total)	24(48.9%)	5(83.3%)

*High Level Aminoglycoside Resistance

Among the isolated Enterococci, 43.5% (n=255) showed high level aminoglycoside resistance to gentamicin or streptomycin or to both streptomycin and gentamicin by the disc diffusion method using high content discs.

On performance of MIC for gentamicin and streptomycin, results were concurrent with the screening method (MIC >2000µg/mL) except for four isolates, which showed a low MIC for gentamicin.

Table 6: Distribution of VRE* among HLAR# and total Enterococcal isolates

	Urine	Respiratory sample	Pus/exudate	Total
Enterococci	491	40	55	586
HLAR	211	15	29	255
VRE*+HLAR# (% of HLAR#)	42 (19.9)	2 (13.3)	5 (17.2)	49 (19.2)
Total VRE* (% of Enterococci)	46 (9.3)	3 (7)	5(9)	54 (9)

*Vancomycin Resistant Enterococci
#High Level Aminoglycoside Resistance

Upon subjection to screening with vancomycin screening agar, 19.2% of the HLAR isolates showed resistance to vancomycin in contrast to 9% vancomycin resistance when all Enterococcal isolates were considered.

The antibiograms of the HLAR and non-HLAR isolates were compared

Table 7: Comparison of antibiotic susceptibilities of HLAR* and non-HLAR isolates

	Ampicillin	Amoxicillin	Fluoroquinolone	Aminoglycoside	Nitrofurantoin	Cephalosporin	Carbapenem
HLAR*	85	71.9	95.1	100	57.3	98.7	100
Non-HLAR	59.5	14.9	63.2	84	12.4	73.1	50

*High Level Aminoglycoside Resistance

DISCUSSION

Enterococci exhibit an intrinsic low to moderate level resistance to aminoglycosides (MIC 62 to 500µg/mL) related to the slow uptake or permeability of these agents. High level aminoglycoside resistance in enterococci is acquired either via mutations in existing DNA or by acquisition of new DNA. High level gentamicin resistance is associated with a bifunctional enzyme possessing acetylase and phospho-transferase activities, conferring resistance to all aminoglycosides except streptomycin. High level streptomycin resistance may be ribosomally mediated or due to the production of streptomycin adenyltransferase. [7,8] Since enterococcal resistance to gentamicin and streptomycin occur by different mechanisms, it is important to test susceptibilities to both agents.

This study found a prevalence of 43.5% of HLAR among clinical isolates of Enterococci in our hospital. Various studies in India have reported prevalence of HLAR ranging from 26% [9] to 75%. [3] Some studies have also reported prevalence in the range 46% [10] to 49.59% [11] which is similar to our findings.

The largest number of Enterococci was isolated from urine, UTI being the most common infection caused by Enterococci worldwide. [12] Predictably, the greatest proportions of HLAR isolates (82.7%) were from UTI cases. Prompt and effective treatment of these cases assumes importance as the urinary tract is very often the source of Enterococcal bacteremia and endocarditis. [4] In our study, pediatric patients constitute the largest group of indoor patients (33.1%) in whom HLAR Enterococci were isolated from urine. HLAR Enterococci were also more resistant to nitrofurantoin (57.3%) vs non-HLAR Enterococci (12.4%), thus eliminating a useful treatment option in most UTI cases where HLAR Enterococci were isolated.

Enterococcal HLAR isolates recovered from exudates section were mainly from indwelling devices, viz. DJ stents, chest tube tips, central line tips and endotracheal tube tips. Again, among such samples, the largest number of HLAR isolates were from pediatric inpatients (n= 12, 52.1%). Ten out of these twelve isolates were from indwelling devices.

All the fifteen HLAR isolates from respiratory samples were from inpatients, thirteen of whom were admitted in the intensive care unit. Pneumonia, an infection rarely caused by Enterococci, appears to occur more frequently in hospitalised patients receiving antimicrobials lacking anti-enterococcal activity. [4] Though some of these isolates may represent colonization rather than infection, treatment directed at Enterococci may be advisable considering that such patients are usually at high risk for developing subsequent bacteremia.

E. faecalis constitutes 84.9% and E. faecium15.1% of the total Enterococcal isolates in this study. Among the HLAR

isolates, 69.4% were E. faecalis and 30.6%E.faecium. Proportion of resistant isolates was seen to be higher among E. faecium: 88.6% of these isolates were HLAR in comparison to 35.5% among E. faecalis.

This study finds concordance between the results of high content disc screening and MIC determination by agar dilution. Only four isolates showed a low MIC for gentamicin; three had MIC 8µg/mL and one showed a MIC of 32µg/mL. Some studies have found the agar screening method superior for detection of HLAR. [13] This method was not evaluated by this study. However, seeing the concordance between MIC results and high content disc screening, the latter method can be reliably and relatively easily used to find out high level aminoglycoside resistance in clinical isolates of Enterococci.

The Enterococcal isolates in our study were also screened for vancomycin resistance by the vancomycin screening agar. Nine percent of the total isolates showed resistance to vancomycin by this method. However, among the HLAR isolates, the proportion of vancomycin resistant Enterococci (VRE) stood higher at 19.2%.

CONCLUSION

Incorporation of HLAR screening into routine bacteriology reporting will save the clinician valuable time in deciding appropriate therapy for an enterococcal infection. This is especially warranted in view of the high HLAR prevalence seen in most studies. Our study also points towards the need for increased vigilance for VRE among such resistant isolates. High content disc screening appears to be an easy and reliable method to detect HLAR in the routine laboratory.

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