Research Article

Prevalence and comparison of high-level aminoglycoside resistance in vancomycin-sensitive and vancomycin resistant *Enterococcus* at a tertiary care hospital in Rohillkhand region

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Abstract

Introduction: Enterococci originally commensals of gastrointestinal tract,have now become important nosocomial pathogens. The emergence of VRE(Vancomycin Resistant *Enterococcus*) strains also resistant to ampicillin and HLAR has made the treatment further difficult. The spread of such strains in a health care environment has to be constrained and so the present study was carried out to highlight the occurrence of HLAR strains and VRE strains in our hospital .The frequency of association of HLAR with VSE (Vancomycin sensitive *Enterococci)* and VRE(Vancomycin resistant *Enterococci)*and any significant difference between the two was also studied.**Methods:** A total of 100 *Enterococci* were isolated from 6272 clinical specimen during a study period of 1 year. These strains were speciated on the basis of biochemical tests. Antimicrobial susceptibility test was performed by Kirby baeur disc diffusion method. High level Aminoglycoside resistance was tested by two methods 1) 150µg gentamicin and 200 µg streptomycin disc 2) Agarscreening method. Similarly, strains resistant to Vancomycin by disc diffusion method were subjected to confirmatory test by determining their MIC by Agar dilution. **Results**: A total of 100 *Enterococci* were isolated during a study period of one year. The prevalence of HLAR was found to be 49%. The prevalence of VRE was found to be 12%. VRE strains also possessing resistance to High strength Gentamycin and or streptomycin was 8%.The frequency of association of HLARin VRE was 1.5 times higher as compared to VSE. **Conclusion:** The prevalence of High level gentamicin and high level streptomycin resistance amongst Enterococcal isolates in our institute was high. More enterococcal strains were found to be resistant to both gentamicin andstreptomycinthan to gentamicin or streptomycinalone. The HLAR rate in VRE was 1.5 times higher than in VSE.

Key words: high level aminoglycoside resistance**,** Vancomycin sensitive *Enterococci***,** Vancomycin Resistant *Enterococcus*

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Introduction

Enterococci , although are commensals of gastrointestinal tract, they have emerged as important nosocomial pathogen in the last decade. Their emergence in past two decade is due to their resistance to commonly used antibiotics namely Aminoglycosides, cephalosporins, aztreonam and semisynthetic penicillin.

Enterococci are also known to acquire and transfer resistant genes easily which is a responsible for emergence of HLAR strains,VRE strains,and beta lactamase production [1].

Enterococci are intrinsically resistant to aminoglycoside antibiotic which do not cross the cell wall efficiently.The addition of β-lactam antibiotic however sufficiently disorganizes the cell wall and then aminoglycosides gain access to the ribosomal target [2]. But *Enterococci* have responded by developing several mechanisms for resistance to the synergistic combination eg.binding protein alterations and beta

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lactamase production for the penicillin and high level resistance to aminoglycoside by enzymatic degradation or ribosomal alteration leading to loss of synergism [3].

Enterococci resistant to vancomycin have emerged as a significant problem in the healthcare system. Glycopeptide antibiotics exert their effect by inhibiting bacterial cell-wall synthesis; VRE strains on the other hand manufacture cell-wall precursors with decreased affinity for the glycopeptide, which prevents the antibiotic from blocking cell-wall synthesis [1]. *Enterococci* strainswhich are resistant to multiple antibiotics including ampicillin and aminoglycosides (including high-level resistance), are frequently responsible fortherapeutic problems.

This study was designed to study the prevalence of VRE and HLAR strains of *Enterococci*, and whether there was any correlation between HLAR and Vancomycin-Sensitive *Enterococci* (VSE) and Vancomycin-Resistant *Enterococci* (VRE).

Material and Methods

100 Enterococcal strains were isolated during a study period of one year from December 2012 to December 2013 from Rohillkhand Medical College and Hospital, a tertiary care hospital of northern India in the state of Uttar Pradesh.

Gram staining, catalase reaction, bile aesculin, growth in 6.5 per cent NaCl and Sugar fermentation tests were performed to identify and confirm *Enterococci*.Antimicrobial susceptibility test was performed by Kirby baeur disc diffusion method [4].

High level Aminoglycoside resistance was detected by following methods:

Two methods were used for detection of HLAR strains
1. **High content disc:** Disc of 120 µg gentamicin and 300 µg of streptomycin were used for disc diffusion testing.Interpretation was as follows:A zone ofless than or equal to 6 mm = Resistant;7–9 mm = Inconclusive; ≥ 10 mm = Susceptible.Resistant: is not synergistic with cell wall–active agent (eg, ampicillin, penicillin, and vancomycin). Susceptible: is synergistic with cell wall–active agent (e g, ampicillin, penicillin, and vancomycin). If disk diffusion result was inconclusive an agar screen test was performed.

2.Agar screen Method: Agar screen method was used to detect high level aminoglycoside resistance amongst the Enterococcal isolates. Brain heart infusion Agar containing 500 g of gentamycin and 1000 g of Streptomycin were prepared.The plates were divided into small sectors and on each sector standard innoculum of each bacterial isolate was innocculated.5-6 colonies of the isolate were suspended in brain heart infusion broth and concentration was adjusted to 0.5 McFarland standard (1.5×10^8cfu/ml). 10 µl of the suspension was spot inoculated on to Brain heart infusion Agar containing gentamycin 500µg/ml and streptomycin 1000µg/ml. The plates were incubated at 37°C for 24-48 hrs. Any growth on the plate indicated resistant strain of *Enterococci* means no synergism with cell wall active agent.

Vancomycin resistance: Strains resistant to vancomycin disc(30µg) were screened for Vancomycin resistance by usingVancomycin screen agar(VSA) containing Vancomycin at concentration of 6 µg/ml.Growth of >1 colony was interpreted as Resistant. Confirmation of VRE was done by determining their MIC by Agar dilution method. Control strains used were *E.faecalis* ATCC-29212(susceptible) and *E.faecalis* ATCC-51299(resistant).MICinterpretative standards according to CLSI guidelines is susceptible ≤ 4, 8–16 intermediate and ≥ 32 resistant or VRE [5].

Results were evaluated according to the Clinical laboratory Standards Institute (2011) [5]and were analysed statistically using the $\chi 2$ test.

Result

100 *Enterococcus* strains were isolated during a study period of one year from 6572 different clinical specimen. Of these 56 strains were isolated from urine, 33 from pus, 04 from vaginal swabs, 05 from blood cultures and 02 from body fluids (ascitic fluid). No *Enterococci* were isolated from catheter tip, CSF and pleural fluid.

75 isolates were identified as *E. faecalis*, 20 isolates as *E. faecium*, 2 were identified as *E. avium*, 1 each of *E.dispar*, *E. durans* and *E. raffinosus*by biochemical tests .

Resistance to the High Strength Gentamicin 120µg disc were shown by 21 isolates ,05 were inconclusive or intermediate sensitiveand 74 were sensitive.Similarly,out of 100 isolates, 34 were resistant to High Strength streptomycin200µg disc, 62 were sensitive and 04 isolates remained inconclusive.

Out of 75 isolated *E. faecalis* species, 15 were resistant to high-level gentamicin and 24 were resistant to high-level streptomycin by disc diffusion method.

Table 1: High Level Aminoglycosides Resistance (HLAR) Amongst Enterococci Isolate

Species	Total No. of Isolates	Resistant to Gentamicin		Resistant to Streptomycin	
		Disk Diffusion	Screen Agar	Disk Diffusion	Screen Agar
E. faecalis	75	15	17	24	26
E. faecium	20	4	6	8	8
Other spp.	5	2	2	2	2
Total	100	21	25	34	36

Table 2: High-level aminoglycoside resistance rate of Vancomycin-sensitive *Enterococci* andVancomycin-resistant *Enterococci* using standard agar screening

	Vancomycin Resistant Enterococci(n=12)	Vancomycin Sensitive Enterococci(n=88)	P value
High level gentamycin resistance(HLGR)	6/12(50%)	19/88(21.5%)	< .01
High level Streptomycin resistance(HLSR)	8/12(66.6%)	28/88(31.8%)	<.01
High level Amino glycoside resistance(HLGR+HLSR)	8/12(66.6%)	41/88(46.4%)	>.05

Table 3: Distribution of HLAR among vancomycin resistant and vancomycin sensitive Enterococcal strains:

Enterococcal strains	HLSR	HLGR
VRE Faecalis(n=6)	4	3
VRE faecium(n=4)	3	2
VRE avium(n=1)	1	1
VRE durans (n=1)	0	0
Vre dispar(n=0)	0	0
VRE raffinosus(n=0)	0	0
VSE faecalis(n=69)	22	14
VSE faecium(n=16)	4	4
VSE dispar(n=1)	0	0
VSE raffinosus(n=1)	1	0

Of the 20 *E. faecium*isolates, 04 were resistant to high-level gentamicin and 08 were resistant to high-level Streptomycin by disc diffusion method.(table 1)

Of the other 05 isolates including *E. avium, E. dispar, E. durans andE.raffinosus* species, high-level resistance to gentamycin was shown by 02 isolates and high-level streptomycin resistance by 02 isolates. *Enterococcus* isolates which were resistant to one aminoglycoside, not necessarily be resistant to another aminoglycosides.

In the detection of HLAR using high potency disc all the intermediate sensitive or inconclusive strains

whentested by HLAR screen agar,the number of HSG resistant isolates changed to 25and HSS resistant isolates to 36.

In total 49% of *Enterococci* showed HLAR in our set up out of which 04% were resistant to gentamicin only, 14% isolates were resistant to streptomycin only and 31 isolates (31%) were resistant to both antibiotics.

But,there were no significant differences between the two methods used to determine the aminoglycoside resistance rates in the enterococcal isolates.

Of the 100 isolates, 12 (12%) were resistant to vancomycin with MIC≥ 32μg/ml. Although High-level aminoglycoside resistance was 1.5 times more in VRE isolates than HLAR in VSE isolates ,this difference was statistically not significant.(p value> .05). High-level aminoglycoside resistance to both streptomycin and gentamicin was more common in *E. faecium*than in *E. faecalis*strains

Discussion

With the emergence of high level amino glycosides resistance (HLAR), β-lactamase producing *Enterococci*and glycopeptides resistance including Vancomycin Resistant *Enterococci* (VRE), a study about their spectrum of infections and antibiotic sensitivity pattern becomes important. The difficulty of treating infections caused by Vancomycin resistant *Enterococci* (VRE) strains which might be resistant to all antimicrobial agents used for the treatment of systemic infections, also emphasizes the need of detection of such strains accurately [6].

High levels aminoglycoside resistant Enterococci were first reported in France in 1979 and since then have been isolated from all the continents. High level aminoglycoside resistant *Enterococci* often have plasmids which carry determinants encoding resistance to other antibiotics, besides limiting the option of using a combination of cell wall active antibiotics and aminoglycosides[7].

The emergence of vancomycin resistance*Enterococci* in addition to the increasing incidence of high-level enterococcal resistance to penicillin and aminoglycosides presents a serious challengefor physicians treating patients with infections due to these micro-organisms.

The prevalence of HLAR was high in our set up constituted to be 49%while the prevalence of VRE was 12%. Latika shah in 2012 has also reported high HLAR 53% for gentamycin and 40 % for streptomycin with 8% VRE at their institute [8]. Nearly 50 per cent of the isolates showing high level aminoglycoside resistance (HLAR) and a total of 13 (8.6%) isolates showingvancomycinresistance have also been reported by Sanal C Fernandes and B Dhanashree in 2012[9] Luna adhikari in 2010 [10] and E. padmasini et al [11] in 2013 have also reported high prevalence of HLAR at their setting.Thus a high prevalence of HLAR along with increasing VRE has been reported from all parts of India. Such strains may disseminate in a health care setting and therefore routine screening of all enterococcal isolates with High strength Gentamycin is important.

In the present study,VRE strains also resistant to high level gentamycinor streptomycin was 8%. The clinical importance of HLAR in VRE isolates, however, is much greater than in other strains because of the lack of synergy among glycopeptide antibiotics and aminoglycosides.

Also, HLAR was found to be one and a half (1.5 times) higher in VRE isolates than in isolates of VSE (66.6% in VRE and 46.4% in VSE. When statistically analysed this association was however clinically not significant. (p value>.05)

Furthermore, such resistance in association with resistance to other antimicrobial drugs may spread to other Grampositive bacteria including *Staphylococcus aureus*, which may theoretically result in groups of organisms for which there is no effective antimicrobial treatment. Considering this possible risk, all clinically significant isolates of *Enterococci*should be examined for their antibiotic sensitivity pattern, including HLAR, before the administration of a β-lactam or glycopeptide antibiotic in combination with an aminoglycoside.

Also, in the present study, Linezolid was found to be 100% sensitive in all Enterococcal isolates by Kirby baeur disk diffusion method of antibiotic sensitivity testing. As, Linezolid is also available in oral form and rapidly completely absorbed after oral administration with a mean bioavailability of approximately 100%, Linezolid can be safely used in severe Enterococcal infection and seems to be an appropriate therapeutic option[12].

Conclusion

The prevalence of High level gentamicin and high level streptomycin resistance amongst Enterococcalisolates in our institute was high. More enterococcal strains were found to be resistant to both gentamicin and streptomycinthan to gentamicin or streptomycinalone. The HLAR rate in VRE was one and a half times higher than in VSE.

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