

Original Research Article

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Determination of High Level Aminoglycoside Resistance in Enterococcal Isolates in a Tertiary Care Hospital

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ABSTRACT

Keywords

Enterococci, High level Aminoglycoside resistance, Gentamicin, Streptomycin, Agar dilution, E-test, Disc diffusion

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Enterococci gained great significance as a nosocomial pathogen in the recent past mainly due to its intrinsic to many antimicrobials including low level aminoglycoside resistance. Detection of the acquired high level aminoglycoside resistance (HLAR) had become essential as clinical failures reported especially with endocarditis. Total of 60 enterococcal isolates were tested (30 from clinical cases as cases and 30 from stool samples as controls) for high level aminoglycoside resistance for gentamicin and streptomycin by three methods -agar dilution, E-test and disc diffusion method. High level gentamicin resistance (HLGR) detected by agar dilution (70%, 3.3%), E-test (63%, 10%) and disc diffusion (46%, 33%) were higher in cases than control samples. HLSR detected by agar dilution (16%, 33%) and disc diffusion (30%, 33%) was also higher in cases than controls. When compared to all three methods, agar dilution and E-test gave nearly same results than disc diffusion method. The present study showed significantly higher rates of HLAR in clinical isolates when compared to isolates from stool samples. This could be due to the fact that clinical isolates are from patients who would have got exposed to various antibiotics while the stool isolates are from healthy people without exposure to antibiotics. Hence routine testing of HLAR is required for all enterococcal isolates especially from serious enterococcal infections.

Introduction

Enterococci are gram positive, facultative anaerobes that can grow under extreme conditions. They are predominant habitat is gastrointestinal tracts of animals and humans (Murray, 1990).

Many of the isolates belong to the species *Enterococcus faecalis*, and *Enterococcus faecium*. These organisms are well known to

cause urinary tract infection (UTI), wound infections, bacteremia, infective endocarditis, meningitis etc.

Naturally enterococci have low virulence factors but over past two decades it gained great significance as nosocomial pathogens. It is ranked 2nd among nosocomial bacteremial infection in the United States of America (Moellering, 1992). This is mainly due to its intrinsic resistance to some anti-microbial

agents like β -lactams, cephalosporins, lincosamides and aminoglycosides at low level. *Enterococci* exhibits acquired resistance to penicillins, high level aminoglycoside resistance (HLAR), chloramphenicol, erythromycin, linezolid, fluoroquinolones. The standard treatment of such drug resistant enterococci is with vancomycin but it acquired resistance even to vancomycin and other glycopeptides. Eg: Vancomycin resistant enterococci (VRE) (Murray, 2000). Enterococci with HLAR are being isolated with increased frequency which resulted in clinical failures and relapses after therapy in patients especially with endocarditis (Eliopoulos, 1990) (Eliopoulos, 1993). Added to this problem, standard susceptibility testing methods which will not predict resistance to penicillin – aminoglycoside synergism. So, it is recommended to test for HLAR in all enterococcal isolates especially from cases of meningitis and infective endocarditis to determine whether a synergistic aminoglycoside containing combination should be used therapeutically. It is useful to test high level streptomycin (HLS) resistance along with high level gentamicin (HLG) resistance because of different aminoglycoside modifying enzymes (Lee, 1993).

Clinical and laboratory standards institute recommends testing synergy between ampicillin, penicillin or vancomycin and an aminoglycoside, preferably by determination of MIC which can predict HLAR (CLSI Guidelines). This study is done to access the HLAR among enterococcal isolates from various clinical specimens and compared with isolates from stool samples of healthy individuals.

Materials and Methods

A total of 60 enterococcal isolates were collected; 30 from various clinical samples

like blood, urine, pus, wound swabs and 30 from stool specimens of asymptomatic persons. This is a case control study carried out in a tertiary care hospital after getting approval from the Ethical Committee.

Out of 30 isolates collected from clinical samples 83% [25 out of 30] were from urine specimens followed by 6.6% from blood specimens. Among 30 clinical isolates of enterococci, 36% (11 out of 30) were collected from intensive care unit specimens, followed by specimens from medical units that is 20% (6 out of 30).

Genus identification of the isolates was done by Heat Test (Barrow 1993) and 6.5 % NaCl tolerance test. Specification was done based on fermentation of arabinose sugar which differentiate *E.faecalis* and *E.faecium*. *E.faecalis* ATCC 29215 (Susceptible) and *E.faecalis* ATCC 51299 (Resistant) were used as controls (Collee, 1996; Facklam, 1989).

Antibiotic susceptibility testing for high level resistance for gentamicin and streptomycin was done by Kirby Bauer disc diffusion method using gentamicin 10 μ g and 120 μ g discs and streptomycin 30 μ g and 300 μ g disc (Himedia laboratories) ATCC strains as controls.

Minimum inhibitory concentration (MIC) to gentamicin and streptomycin was done for all 60 strains by agar dilution technique and interpreted according to CLSI guidelines. The break points recommended are >2000 μ g/ml for streptomycin and >500 μ g/ml for gentamicin (CLSI Guidelines). The same criteria was followed in our study and strains that showed MIC of >2000 μ g/ml for streptomycin and >500 μ g/ml for gentamicin were recorded as having high level resistance.

Epsilometre test (E-test) was performed on all isolates for Gentamicin by using E-strips from

Himedia laboratories. MIC was read where the eclipse intersects the MIC scale on the strip and concentrations as mentioned above are taken as break points according to CLSI guidelines which were recorded as HLSR and HLGR (CLSI Guidelines).

Results and Discussion

Total 60 isolates tested in the study using 10 µg and 120µg gentamicin discs. HLGR using 120µg gentamicin disc, where 46% (14 out of 30) of clinical isolates and 3.3% (1 out of 30) of control strains were resistant.

With 10 µg gentamicin disc, 80% (24 out of 30) of clinical isolates and 13% (4 out of 30) of control strains were found to be resistant.

HLGR using 120µg gentamicin disc, where 46% (14 out of 30) of clinical isolates and 3.3% (1 out of 30) of control strains were found to be resistant.

When compared to all these 3 methods, agar dilution and E-test gave nearly same results (23 out of 60 and 2 out of 60) than disc diffusion method (15 out of 60).

Using 30 µg streptomycin disc 63% (19 out of 30) clinical isolates and 6.6% (2 out of 30) of control strains were streptomycin resistant.

HLSR was detected by using 300 µg disc where 16% (7 out of 30) of clinical isolates and 3.3% (1 out of 30) of control strains were resistant.

According to CLSI recommendations, enterococcal isolates with MIC >500 µg/ml are considered as HLGR. In the present study 70% (21 out of 30) of clinical isolates and 3.3% (1 out of 30) of control strains were found to be resistant to gentamicin after antibiotic susceptibility testing done by agar dilution method.

According to CLSI recommendations, enterococcal isolates with MIC of > 2000 µg/ml are considered as HLSR. In our study 30% (9 out of 30) clinical isolates and 3.3 % (1 out of 30) of control strains were resistant to streptomycin. Enterococci not only show intrinsic resistance to all aminoglycosides at low levels, they also exhibit acquired resistance to high levels of aminoglycosides due to genetic exchange.

The recommended therapy for serious enterococcal infections is combination therapy with a cell wall acting agent like penicillin or vancomycin along with aminoglycoside like gentamicin or streptomycin. They have synergistic action which can overcome intrinsic resistance of organism and makes them susceptible to aminoglycoside activity.

However treatment with this combination in infections by HLAR enterococci may not show any benefit leading to increased morbidity and mortality.

Hence it is important to know whether the clinical isolates of enterococci are susceptible to HLA or not, especially in serious infections like endocarditis and meningitis where bactericidal concentrations of drug is needed. It is recommended to detect HLGR and HLSR separately, so that appropriate aminoglycoside can be added to the regime.

Speciation was done with arabinose fermentations test. The strains which fermented arabinose were taken as *E.faecium* and which cannot ferment was taken as *E.faecalis*. *E.faecalis* was found to be predominant species (85%) followed by *E. faecium* (15%). The present study showed significantly higher rates of HLAR in clinical isolates when compared to isolates from stool samples (Table 1–4).

Table.1 Shows antibiotic susceptibility testing and comparison of disc diffusion, agar dilution and E-test methods for gentamicin

	Disc diffusion						Agar dilution (µg/ml)					E-test				
	Disc content(10 µg)			120 µg			<125	250	500	1000	>1000	<125	250	500	1000	≥1000
	S	I	R	S	I	R										
Cases	6 (20%)	0	24 (80%)	14 (46%)	2 (8%)	14 (46%)	4 (13%)	1 (3.3%)	4 (13%)	5 (16%)	16 (53%)	10 (33%)	0	0	0	19 (63%)
Controls	26 (86%)	0	4 (13%)	29 (96%)	0	1 (3.3%)	27 (90%)	1 (3.3%)	0	1 (3.3%)	1 (3.3%)	27 (90%)	0	0	0	3 (10%)

S-sensitive, I-Intermediate, R- resistance

Table.2 Shows antibiotic susceptibility testing by disc diffusion and agar dilution method for streptomycin

	Disc diffusion						Agar dilution (µg/ml)					
	30 µg			300 µg			<500	1000	2000	3000	>3000	
	S	I	R	S	I	R						
Cases	5 (16%)	6 (20%)	19 (63%)	22 (73%)	3 (10%)	5 (16%)	9 (30%)	9 (30%)	3 (10%)	4 (13%)	5 (16%)	
Controls	27 (90%)	1 (3.3%)	2 (6.6%)	29 (96%)	0	1 (3.3%)	27 (90%)	2 (6.6%)	0	0	1 (3.3%)	

S-sensitive, I-Intermediate, R- resistance

Table.3 Shows results of antibiotic susceptibility test by agar dilution Method for gentamicin and streptomycin

	Agar dilution method			
	Gentamicin		Streptomycin	
	S	R	S	R
Cases	9 (30%)	21 (70%)	21 (70%)	9 (30%)
Controls	29 (96%)	1 (3.3%)	29 (96%)	1 (3.3%)

S-sensitive, I-Intermediate, R- resistance

Table.4 Comparison of disc diffusion and agar dilution methods for streptomycin resistance detection

	Disc diffusion						Agar dilution (µg/ml)				
	30 µg			300 µg			<500	1000	2000	3000	>3000
	S	I	R	S	I	R					
Cases	5 (16%)	6 (20%)	19 (63%)	22 (73%)	3 (10%)	5 (16%)	9 (30%)	9 (30%)	3 (10%)	4 (13%)	5 (16%)
Controls	27 (90%)	1 (3.3%)	2 (6.6%)	29 (96%)	0	1 (3.3%)	27 (90%)	2 (6.6%)	0	0	1 (3.3%)

S-sensitive, I-Intermediate, R- resistance

This could be due to the fact that clinical isolates are from patients who would have got exposed to various antibiotic while the stool

isolates are from healthy people without exposure to antibiotics.

Our study correlates with the observations of Suresh *et al.*, which showed *E.faecalis* were 89.61%, followed by *E.faecium* 10.4% (Suresh, 2013) and Simonson *et al.*, showed *E.faecalis* were 82.5% and *E.faecium* were 16% (Simonson, 2003)

The present study correlates with observations of Swathi *et al.*, who reported HLGR – 75%, HLSR – 95%, HLAR – 73% which was done by semi-automated system mini API method (Swati, 2014)

In a study by Deshpande *et al.*, resistance to HLG was 73.5%, HLS was 70.8% and combined resistance to HLG and HLS was found in 58.8% of all isolates (Deshpande, 2013)

This study was performed by broth dilution method.

Dadfarma *et al.*, in a study from Iran reported 43.7% HLGR phenotype which was done by using disc diffusion method and MIC by both microdilution method (Dadfarma, 2013)

Enterococci are intrinsically resistant to low level aminoglycosides and further acquire resistance to High Level Aminoglycosides.

Acquired resistance may be due to previous antibiotic exposure. The treatment of serious infections like infective endocarditis and meningitis require bactericidal treatment in order to overcome intrinsic resistance of enterococci by a combination of High Level Aminoglycoside and β – lactum agent.

Increased frequency of clinical failures and relapses after therapy was encountered due to failure of synergy because of High Level Aminoglycoside Resistance.

The present study concluded that there is high prevalence of HLAR enterococci in our set up and hence routine testing of HLAR is required

for all enterococcal isolates especially from serious enterococcal infections.

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