

## Detection of High Level Aminoglycosides Resistant Enterococci In A Tertiary Care Hospital.

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### Abstract:

**BACKGROUND:** Emergence of high-level aminoglycoside and glycopeptide resistance has significantly contributed to the mortality, particularly in serious enterococcal infections. High Level Aminoglycoside Resistance (HLAR) is related to the slow uptake or permeability of these agents. **AIM:** The present study was undertaken to determine HLAR pattern of enterococci in our hospital. **MATERIALS AND METHODS:** This study was done in the Department of Microbiology, Meenakshi medical college, during the period of February 2012- February 2013. A total of 52 enterococcal isolates were collected from various clinical samples and speciation was by a series of biochemical reactions as per standard protocol. Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method and microbroth dilution method according to CLSI guidelines 2012. **RESULT:** In the present study majority of the Enterococcal isolates in our study were isolated from urine sample. Among the 52 isolates of enterococci *E.faecalis* was the predominant species. The highest percentage of antibiotic resistance was seen in Erythromycin followed by Ciprofloxacin, Tetracycline, Ampicillin and Vancomycin. The High level Gentamycin Resistance (HLGR) was 48.7% in *E.faecalis* and 54.6% in *E.faecium* & High Level Streptomycin Resistance (HLSR) was 34.1% in *E.faecalis* and 54.6% in *E.faecium*. 23 strains (17 *E.faecalis* and 6 *E.faecium*) showed resistance to High Level Gentamycin (HLG) in the concentration range of >500µg/ml. 17 strains (12 *E.faecalis* and 5 *E.faecium*) showed resistance to High Level streptomycin (HLS) at the range of >1000 µg/ml. **CONCLUSION:** This study emphasizes the need to screen for HLAR in patients suffering from enterococcal infections. Routine screening for high level aminoglycoside resistance is important to limit the spread of resistance and to have a surveillance programme.

**Keywords:** HLAR, *E.faecalis*, *E.faecium*, Antibiotic susceptibility testing.

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### Abbreviation:

HLAR- High Level Aminoglycoside Resistance  
HLGR- High level Gentamycin Resistance  
HLSR- High Level Streptomycin Resistance  
MIC - Minimum Inhibitory Concentration  
CLSI- Clinical and Laboratory Standards Institute

### I. Introduction:

Enterococci have now become the second most common cause of nosocomial infections. (Desai *et al.*, 2001). The most common nosocomial infections caused by enterococci are Urinary tract infections, followed by intra abdominal and pelvic infections. *E.faecalis* (80% to 90%) and *E.faecium* (5% to 10%) are the most common species causing human infections. Enterococci have acquired resistance to several class of antibiotics either by mutation or through transfer of genetic material through plasmids and transposons (Murray *et al.*, 1990). Along with Vancomycin resistance enterococci in addition to increasing incidence of HLAR is a major obstacle for treatment (Purva *et al.*, 1999).

Enterococci have become increasingly important not only because of their ability to cause serious infections but also because of their increasing resistance to many antimicrobial agents. The emergence of high level resistance to aminoglycosides (HLAR),  $\beta$  lactam antibiotics and to vancomycin by some strains, together with multi drug resistance has led to failure of synergistic effects of combination therapy.

It also exhibits a low to moderate level resistance to aminoglycosides, corresponding to minimum inhibitory concentration (MIC) of 62–500 µg/ml. This resistance is related to the slow uptake or permeability of these agents. Aminoglycoside uptake is enhanced by exposing enterococci to a beta-lactam. HLAR (MIC > 2000mg/ml) has emerged recently, which is either ribosomally mediated or due to the production of inactivated enzymes (Sarika Jain *et al.*, 2011). The limited choice of efficient therapy in serious enterococcal infections has been complicated by emergence of resistance to ampicillin, high-level aminoglycoside and glycopeptides.

The present study was undertaken for determining HLAR in enterococci, especially from our hospital

## II. Materials And Methods:

This study was done in the Department of Microbiology Meenakshi medical college, during the period of February 2012- February 2013. A total of 52 enterococcal isolates were collected from various clinical samples (urine, pus, sputum, blood & fluid) and identified by a series of biochemical reactions as per standard protocol according to CLSI guidelines 2012.

Antibiotic susceptibility testing done by Kirby Bauer disc diffusion method. The following antibiotic discs were used, Ampicillin(10µg), Erythromycin (15µg), Ciprofloxacin (5µg), Tetracycline(30µg), Vancomycin(30µg). HLAR resistance was tested against Gentamicin (120 µg) and Streptomycin (300 µg).

The MIC was done by microbroth dilution for the following antibiotics: high level gentamicin, high level streptomycin (Ranbaxy pharma ltd.) by microbroth dilution method according to CLSI guidelines 2012. MIC was visually read after 24 hrs of incubation at 37°C. MIC was defined as the lowest drug concentration resulting in 90% reduction in turbidity when compared to the drug free control. The concentrations of the antibiotics ranged from 2000-1.95µg for HLG and HLS, Interpretive criteria for resistance to HLG and HLS according to CLSI guidelines 2012 were as follows: High level gentamicin (>500) & High level streptomycin (>1000).

### Statistical analysis:

Statistical analysis was done using SPSS software version 22.0. The test of proportions was used for analysing the resistance patterns between Kirby Bauer disc diffusion method and MIC by microbroth dilution method for different enterococcal species.

## III. RESULTS:

### 3.1. SAMPLE DISTRIBUTION IN ENTEROCOCCAL ISOLATES

In the present study majority of the Enterococcal isolates in our study were isolated from Urine sample (55%) followed by Pus (34.6%), Blood (5.7%) and Sputum (3.9%). (TABLE-1)

TABLE-1 SHOWING SAMPLE DISTRIBUTION

SAMPLES	NO. OF SAMPLES	PERCENTAGE
Urine	29	55.8%
Pus	18	34.6%
Sputum	2	3.9%
Blood	3	5.7%
<b>Total</b>	<b>52</b>	<b>100%</b>

### 3.2. DISTRIBUTION OF AGE & SEX IN ENTEROCOCCAL ISOLATES

Highest prevalence was seen in female (65.4%) followed by males (34.6%). The maximum percentage of isolation was seen among the age group 40-60 years.

### 3.3. DISTRIBUTION OF ENTEROCOCCAL SPECIES

Among the 52 isolates of enterococci 41 isolates (78.8%) were *E.faecalis* and 11 isolates (21.2%) were *E.faecium*. (TABLE-2)

TABLE-2 SHOWING SPECIES DISTRIBUTION

ENTEROCOCCUS SPECIES	NO.OF ISOLATES	PERCENTAGE
<i>E.faecalis</i>	41	78.8%
<i>E.faecium</i>	11	21.2%
<b>Total</b>	<b>52</b>	<b>100%</b>

### 3.4. ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ENTEROCOCCAL SPECIES

Antibiotic Susceptibility Pattern of different species of Enterococcal Isolates By Kirby Bauer disc diffusion Method (Table 3)

**TABLE-3 SHOWING ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ENTEROCOCCAL ISOLATES BY KIRBY BAUER DISC DIFFUSION METHOD**

Antibiotic	<i>E.faecalis</i> (N=41)				<i>E.faecium</i> (N=11)			
	Sensitive		Resistant		Sensitive		Resistant	
	No	%	No	%	No	%	No	%
Erythromycin (15 µg)	6	14.6	35	85.3	1	9.1	10	90.9
Ciprofloxacin (5µg)	11	26.8	30	73.1	2	18.2	9	81.8
Tetracycline (30 µg)	11	26.8	30	73.1	6	54.6	5	45.4
Ampicillin (10µg)	31	75.6	10	24.3	3	27.3	8	72.7

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High level gentamycin (120µg)	21	51.2	20	48.7	5	45.4	6	54.6
High level streptomycin (300µg)	27	65.8	14	34.1	5	45.4	6	54.6
Vancomycin (30µg)	37	90.2	4	9.3	9	81.8	2	18.2

Highest percentage of resistance to Erythromycin (85.3% in *E.faecalis* and 90.9% in *E.faecium*) followed by Ciprofloxacin (73.1% in *E.faecalis* and 81.8% in *E.faecium*), Tetracycline (73.1% in *E.faecalis* and 45.4% in *E.faecium*) and Ampicillin (24.3% in *E.faecalis* and 72.7% in *E.faecium*).

Vancomycin resistance was 7.3% in *E.faecalis* and 18.2% in *E.faecium*.

The HLGR was 48.7% in *E.faecalis* and 54.6% in *E.faecium* & HLSR was 34.1% in *E.faecalis* and 54.6% in *E.faecium*.

### 3.5. MIC range of High Level Gentamycin & High Level Streptomycin of enterococcal species (Table 4)

**TABLE-4 : SHOWING MIC RANGE OF HIGH LEVEL GENTAMYCIN & HIGH LEVEL STREPTOMYCIN**

Antibiotic drugs	Species	MIC		CLSI break points		
		Resistant isolates	Resistant %	S	I	R
High level gentamycin (n=26)	<i>E.faecalis</i> (n=20)	17	41.5			
	<i>E.faecium</i> (n=6)	6	54.5	≤500	-	>500
High level streptomycin (n=20)	<i>E.faecalis</i> (n=14)	12	29.2			
	<i>E.faecium</i> (n=6)	5	45.4	≤500	-	>1000

23 strains (17 *E.faecalis* and 6 *E.faecium*) showed resistance to HLG in the concentration range of >500 µg/ml. 17 strains (12 *E.faecalis* and 5 *E.faecium*) showed resistance to HLS at the range of >1000 µg/ml.

### 3.6. Comparison of antibiotic resistance by Kirby Bauer disc diffusion method & MIC of enterococcal species (Table 5)

**TABLE 5: SHOWING COMPARISON OF ANTIBIOTIC RESISTANCE BY KIRBY BAUER DISC DIFFUSION METHOD & MIC**

Antibiotic	<i>E.faecalis</i> (N=41) % of Resistant isolates by		<i>E.faecium</i> (N=11) % of Resistant isolates by	
	Disc diffusion method	MIC	Disc diffusion method	MIC
High level gentamycin	48.7	41.5	54.6	54.5
High level streptomycin	34.1	29.2	54.6	45.4

Comparison of antibiotic resistance by Kirby Bauer disc diffusion method & MIC difference are not significant in our study and it was analysed statistically.

### Statistical analysis:

The statistical analysis was taken up, to find if any difference in resistance level between Kirby Bauer disc diffusion method and MIC by microbroth dilution method. The differences are not significant. Hence the resistance levels by both the methods are same in our study.

## IV. Discussion:

In our present study, we have isolated 78.8% of *E.faecalis* and 21.2% of *E.faecium*. Only 2 species were recovered in contrast to more species by others from India (Desai *et al.*, 2001, Bhat *et al.*, 1997). Our isolation rate is close to Vinod Kumar *et al* 2011 who have isolated 81.03% of *E.faecalis* and 18.7% of *E.faecium*.

In this present study maximum number of enterococci were isolated from urine (55.8%) followed by pus (34.6%). This is slightly lower than Ruoff *et al.*, 1990 who also isolated maximum number of enterococci from urine (68.2%). Talebi *et al* 2007 also reported maximum number of enterococcal isolates from urine sample (85%) followed by pus (15.5%). Karmarkar *et al.*, 2004 isolated 47.13% of enterococci from urine sample and described that urinary tract as commonest site of isolation of enterococci. The maximal enterococcal urine isolation could be due to structural abnormalities in the urinary tract, indwelling catheter or following any instrumentation.

Antibiotics resistance among Enterococci is a global problem. Antibiotic resistance in enterococci is either intrinsic or acquired. In our study the highest resistance is seen against Erythromycin 85.3% to *E.faecalis* and 90.9% to *E.faecium*, Sanal C. Fernandes *et al.*, 2013, also have reported highest resistance to

Erythromycin 81% to *E.faecalis* and 90.1% to *E.faecium* and also Sarika jain *et al.*, 2011 also have reported a similar finding that highest resistance was seen in erythromycin 77.7% to *E.faecalis* and 89.6% to *E.faecium*.

In this study 24.3% of *E. faecalis* and 72.7% of *E.faecium* were resistant to ampicillin which was concordant with a study conducted by Salem-Bekhit *et al.*, 2012, who also reported ampicillin resistance of 15.7% in *E.faecalis* and 70.4% in *E.faecium*.

In our study 73.1% of *E.faecalis* are resistant to ciprofloxacin and 81.8% of *E.faecium* are resistant to ciprofloxacin. Similar findings were also reported by Sarika Jain *et al* 2011 where 75% of *E.faecalis* and 84.4% of *E.faecium* were resistant to ciprofloxacin. In our study we have isolated 18.1% *E.faecium* and 9.3% *E.faecalis* showing higher resistance of *E.faecium*. Similar findings were also reported by Karmarkar *et al.*, 2004) who also reported greater resistance among *E.faecium*.

In our study 48.7% of *E.faecalis* and 54.6% of *E.faecium* were resistant to High level gentamycin. Similar findings were reported by Sanal C. Fernandes *et al.*, 2013 (53.5% of *E.faecalis* and 53% of *E.faecium* were resistant to High level gentamycin). The presence of HLGR is predictive of the loss of synergy between gentamicin and a cell-wall-active agent such as ampicillin or Vancomycin (Murray *et al.*, 1998).

In our study 34.1% of *E.faecalis* and 54.6% of *E.faecium* were resistant to HLS. Similar findings were reported by Sanal C. Fernandes *et al.*, 2013 who have reported 48.8% of *E.faecalis* and 58.8% of *E.faecium* were resistant to HLS.

In our study resistance to High level gentamycin was seen higher in *E.faecium* than in *E.faecalis*, similar findings were observed by Gordon *et al* 1992 who also reported significantly higher resistance to HLG and HLS by *E.faecium* than in *E.faecalis*. Similar result was also observed by Mendiratta *et al.*, 2008. High HLGR in *E.faecalis* and HLSR in *E.faecium* observed has also been reported (Bhat *et al.*, 1997, Agarwal *et al.*, 1999) as also vice versa (Karmarkar *et al.*, 2004). In our study HLG and HLS is slightly lower than other studies done all over India. The reason could be due to that our hospital is in a rural setup and usage of antibiotics is restricted. The reason for increased prevalence could be due to chronic cases.

All the enterococcal isolates resistant to HLG & HLS by Kirby Bauer disc diffusion method were subjected to MIC by Micro broth dilution method. In our study MIC was in the range >31.2-2000 µg/ml for HLG and MIC range of >125-2000 µg/ml for HLS which was high compared to Ajay Kumar oli *et al.*, 2012. In our study HLAR was high in *E. faecium* than in *E.faecalis*

## V. Conclusion:

This study emphasizes the need to screen for HLAR in patients suffering from enterococcal infections as a routine screening for to detect HLGR and HLSR as this will help to limit the spread of resistance and have a surveillance pattern.

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