Original article

Determination of High level aminoglycoside resistance of Enterococci among hopitalised patients

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Abstract:

Objective: This study was undertaken to isolate enterococci from various clinical samples, study the species distribution, antimicrobial susceptibility pattern and detection of High level aminoglycoside strains.

Methods: A prospective study was conducted in the Department of Microbiology, Coimbatore Medical College Hospital. Speciation of Enterococcal isolates was done and antimicrobial susceptibility pattern performed by disc diffusion method as per NCCLS guidelines. High level aminoglycoside resistant strains identified by disc diffusion using high level aminoglycoside discs and E - test done for MIC determination.

Results: A total of 1140 clinical samples comprising urine, blood, pus and body fluids were tested and 87 isolates of enterococci obtained. E.faecalis-78(89.6%);E.faecium-9(10.4%). All the isolates were 100% sensitive to Vancomycin, Teicoplanin, Linezolid and Nitrofurantoin(urinary isolates). 16 isolates showed high level aminoglycoside resistance. HLGR strains showed MIC > 512 µg/ ml and all the isolates had MIC < 4 µg/ ml for vancomycin.

Conclusion: Routine testing of the enterococcal isolates for high level aminoglycoside resistance need to be done for selection of appropriate drug and also constant surveillance of antimicrobial susceptibility to be done.

Keywords: *Enterococci-resistance, HLAR, antibiotic resistance, hopitalised patients*

1. Introduction:

Over the past two decades, enterococci have emerged as nosocomial pathogens due to inherent resistance to antibiotics, ability to adhere to indwelling medical devices, and ability to survive adverse environmental conditions.[17] There are 23 species of Enterococci with clinical significance of which Enterococcus faecalis accounts for 80 – 90% of isolates; Enterococcus faecium for 5 - 15% cases. The most frequent infections caused by these organisms are urinary tract infections. The second most frequent infections are Intra - abdominal or post surgery wound infections. The third most frequent infections are blood stream infections.

Enterococcus species are intrinsically resistant to many antimicrobial agents including β-lactams and low level aminoglycoside resistance.[20] They have acquired resistance to other antimicrobial agents including high-level resistance to aminoglycosides and glycopeptides.[20]High-level gentamicin resistance is most often associated with high-level resistance to all alternative aminoglycosides.[19] Gentamicin resistance is a good predictor of resistance to other aminoglycosides. Enterococcal resistance to gentamicin and streptomycin occurs by different mechanisms. Hence it is important to test susceptibility to both agents.[19]

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The incidence of other species of Enterococci from clinical sources shows an alarming increase with the properties of intrinsic resistance to several antibiotics including betalactams, highlevel gentamicin resistance and glycopeptides. Hence proper identification to species level is essential for proper management and prevention of this bacterial infection in any health care institution.

Serious infections due to Enterococci are often refractory to treatment and mortality is high. Serious Enterococcal infections are treated with a combination of a cell wall active agent (penicillin, ampicillin or vancomycin) and an aminoglycoside.The high level resistance to either aminoglycosides or penicillin makes this combination ineffective. It results in treatment failure and spreading of resistant strains in the health care institutions. This highlights the significance for their identification from clinical specimens and study the antimicrobial susceptibility pattern for initiating appropriate therapeutic regimen.Hence the present study is conducted to know the species prevalence and the high level aminoglycoside resistance of enterococcal isolates.

2.Materials and Methods

2.1 Study population and clinical samples:

Enterococcal isolates were obtained from various samples like urine, blood, pus and body fluids. The inclusion criteria were urinary catheterization, prolonged hospitalization, surgical and non-surgical wound infection, burns wound, suspected septicemia, abdominal drain fluid from post- operative patients. Commensal enterococcal isolates from gastrointestinal tract, oral cavity were excluded.

2.2 Species identification:

Species identification done by standard biochemical tests. Enterococci were identified by gram staining, colony morphology, catalase reaction, growth on bile esculin agar and in 6.5% NaCl broth, and presence of pyrrolidonyl arylamidase. Species-level identification was performed by formation of acid in mannitol, sorbitol, sucrose,arabinose, raffinose, pyruvate and sorbose broth, pigmentation, motility, growth on tellurite agar, and arginine hydrolysis.

2.3 Antimicrobial sensitivity testing:

Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method as per CLSI guidelines for the following antibiotics:Ampicillin, Penicillin,Ciprofloxacin, ofloxacin, Linezolid, Nitrofurantoin, Vancomycin, Teicoplanin, High level gentamicin 120 µg; High level streptomycin 300 µg.

2.4 High level aminoglycoside resistance by disc diffusion method:

High level aminoglycoside resistance was determined by Kirby-Bauer disc diffusion method agar using discs with 120µg/mL of gentamicin and 300µg/mL of streptomycin. A 0.5 McFarland standard suspension was streaked onto Mueller-Hinton agar, and disks containing 120 µg of gentamicin and 300 µg of streptomycin were applied. Plates were incubated at 35°C in ambient air for 18 h. A zone size of 6 mm in diameter indicated high-level resistance, and ≥10 mm indicated susceptibility. Zone sizes of 7 to 10 mm in diameter were considered intermediate.

2.5 High level aminoglycoside resistance by E-test:

E- test was performed for determination of Minimum inhibitory concentration. Gentamicin high level E strips: Part A (1024 - 8 µg); and Part B (8.192 – 0.064 µg) were used.

Place one strip Part A (1024 - 8 µg) on Mueller-Hinton agar plate with its higher concentration facing the edge of the plate and the markings on the strip facing upwards. Press gently on the handle of the strip and assure that all discs are in full contact with

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the medium. Close the plate and invert to check whether all the discs are in full contact with the medium. Place the other strip (part B) in another plate in a similar manner. Incubate at 35^0C for 18 - 24 hours.

MIC value would be the value at which the zone convenes the comb - like projections of the strips and not the handle. If there is no zone of inhibition observed, report the MIC as greater than the highest concentration of the strip. If the zone of inhibition is lower then report the MIC as less than the lowest concentration. If MIC > 500µg, then reported as HLAR.

2.6 MIC for vancomycin:

MIC for vancomycin was determined by E-strips containing vancomycin in the following concentration, Part A: 256 - 2 µg; Part B: 2.048 - 0.016 µg .The results are interpreted as Sensitive:4 µg; Intermediate:8- 16 µg ; Resistant:32 µg

3.Results

A total of 1140 samples were tested and 87 enterococcal isolates of obtained. Study population included all age group and both gender. Urine, blood, pus from surgical and Non surgical wounds, and body fluids from inpatients analysed. Maximum isolates were from urine followed by surgical, non - surgical wounds and blood. Among the Enterococcal isolates, E.faecalis was the predominant species isolated 78/87(89.6%) followed by E.faecium 9/87(10.3%). No other species were isolated.

Fig - 1: Enterococcus species isolated

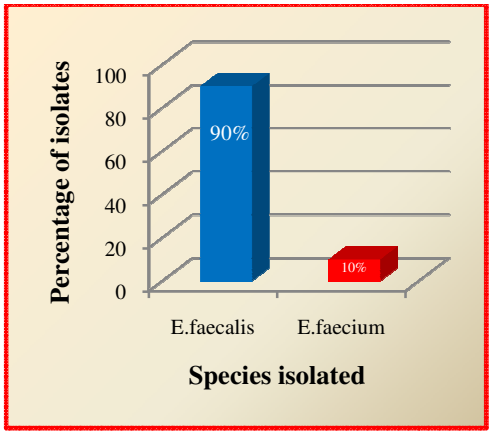
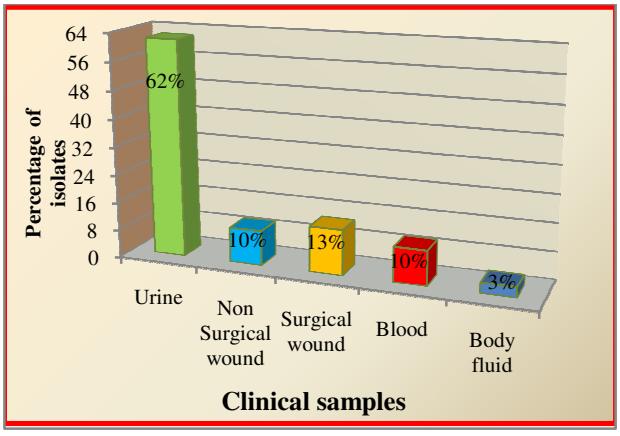


Fig - 2:Enterococcal isolates among various clinical isolates



Females were affected more than males and the gender ratio was 1.4:1. Maximum percentage of isolation was in the age group > 50 years. Next common age group was 0 - 10 years.

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Fig -3:Isolates in relation to Patient's sex

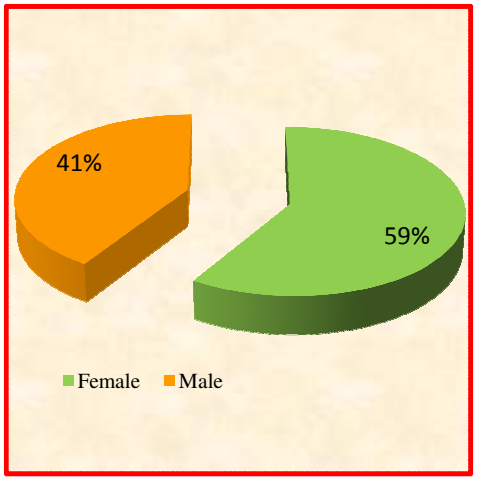
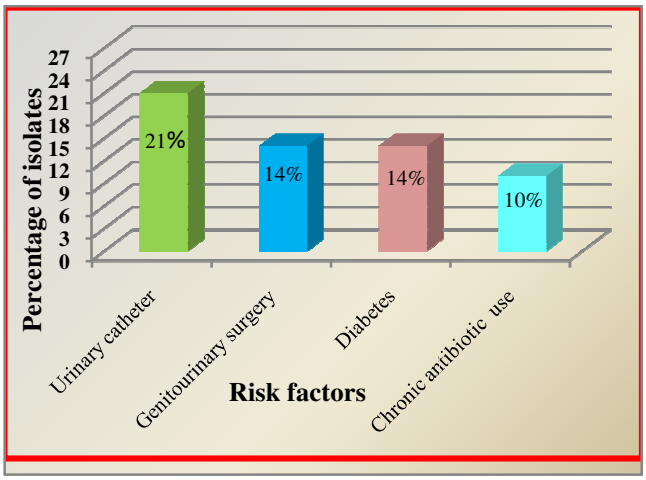


Fig - 4: Predisposing risk factors



Many risk factors were identified in the study viz., Long hospital stay, urinary catheterization, Genitourinary surgery, Diabetes and Chronic antibiotic use. Urinary catheterization accounted for 21% of the total isolates; patients following instrumentation and surgery of genitourinary tract had enterococcal infection(13.7%).

Antimicrobial sensitivity testing done for all the isolates by Kirby Bauer disc diffusion method. Both E.faecalis and E.faecium showed 100% sensitivity for Vancomycin, Linezolid and Teicoplanin and Nitrofurantoin (urinary isolates). E.faecalis showed more than 50% sensitivity for ampicillin and > 60% sensitivity for penicillin. More than 50% strains were resistant to ciprofloxacin and ofloxacin. E.faecium exhibited more than 60% resistance for Ampicillin, Penicillin, Ciprofloxacin and ofloxacin.

Table - 1: Antibiotic susceptibility pattern of E. faecalis

Antibiotic	Sensitive		Resistant	
	Number of isolates	%	Number of isolates	%
Ampicillin (10µg)	45	57.7	33	42.3
Penicillin (10 U)	51	65.4	27	34.6
Ciprofloxacin (5µg)	36	46.2	42	53.8
Ofloxacin (5µg)	36	46.2	42	53.8

Table - 2 :Antibiotic susceptibility pattern of E. faecium

Antibiotic	Sensitive		Resistant	
	Number of isolates	%	Number of isolates	%
Ampicillin (10µg)	3	33.3	6	66.6
Penicillin (10 U)	3	33.3	6	66.6
Ciprofloxacin (5µg)	3	33.3	6	66.6
Ofloxacin (5µg)	3	33.3	6	66.6

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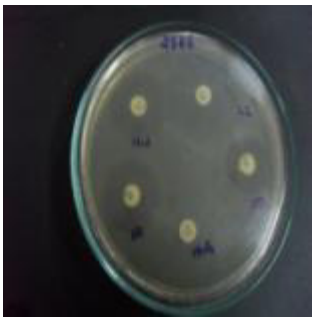
Nitrofurantoin (300 µg)	51	100	-	-
Vancomycin (30µg)	78	100	-	-
Linezolid (30µg)	78	100	-	-
Teicoplanin (30µg)	78	100	-	-
Gentamicin (120 µg)	36	46.2	14	53.8
Streptomycin (300 µg)	54	69.2	8	30.7

Nitrofurantoin (300 µg)	9	100	-	-
Vancomycin (30µg)	9	100	-	-
Linezolid (30µg)	9	100	-	-
Teicoplanin (30µg)	9	100	-	-
Gentamicin (120 µg)	3	33.3	6	66.6
Streptomycin (300 µg)	6	66.6	3	33.3

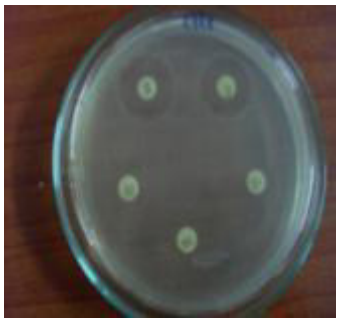
HLAR pattern studied by Kirby Bauer disc diffusion method. E.faecium was more resistant to gentamicin than E.faecalis. 42 strains of E.faecalis exhibited HLGR(53.8%); 12strains showed both HLGR and HLSR(15.3%). 30 strains showed only HLGR and was sensitive to streptomycin (38.4%).The remaining 12 isolates of E.faecalis were sensitive to gentamicin(46%). High level streptomycin disc (300µg) detected totally 24 HLSR strains (30.76%); 12 strains were both HLGR and HLSR; 12 showed only HLSR(15.3%).

HLAR detection by high content gentamicin and streptomycin disc

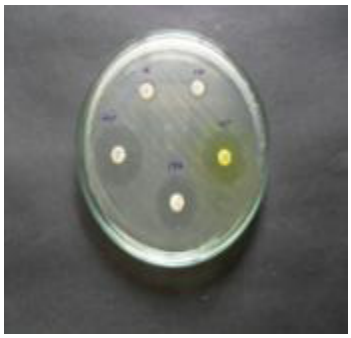
Fig - 5 **Fig - 6** **Fig - 7**



HLS



-**S**;



HLG - **R**; Vancomycin -**S**; Linezolid - **S**; HLG -**R**;HLS -**R**;Vancomycin-**S**;Teicoplanin -**S**; Ampicillin - **S**; Penicillin - **S**;

Teicoplanin - **S** , Linezolid - **S** , Nitrofurantoin - **S**; Amikacin - **R**;

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MIC for Gentamicin determined by E - test. HLGR strains showed MIC more than 512 µg/ ml. 42 isolates of E.faecalis and 6 isolates of E.faecium had MIC > 512 µg(resistant). All strains were sensitive to vancomycin with MIC < 4 µg/ ml.

Fig - 8: HLAR of E.faecalis by Kirby Bauer disc

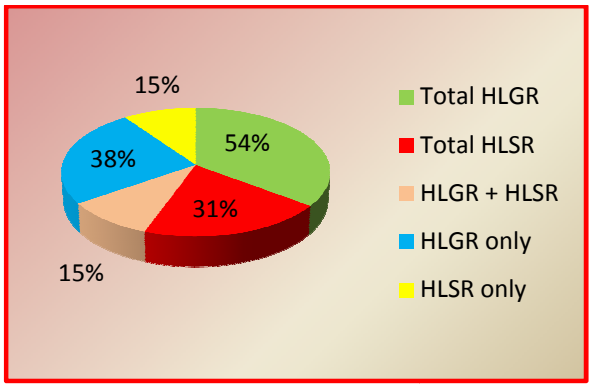
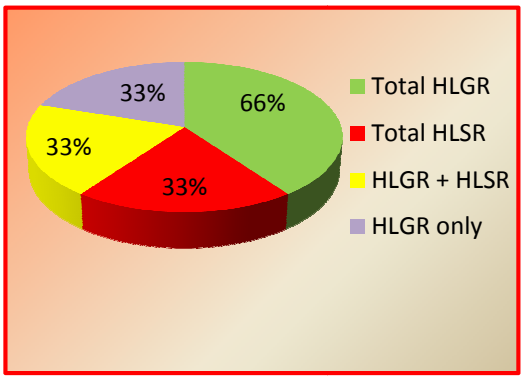
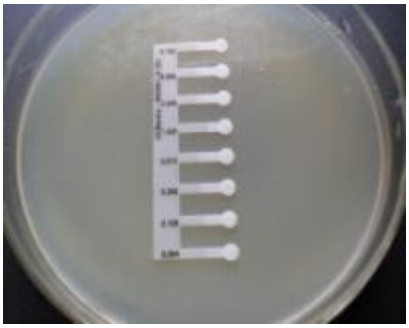


Fig - 9: HLAR of E.faecium by Kirby Bauer disc diffusion method



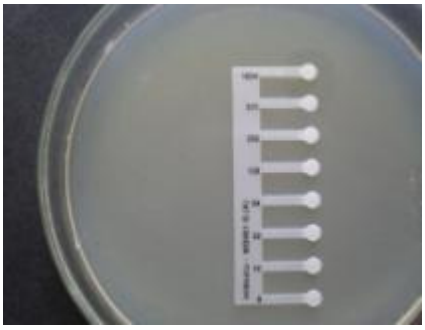
MIC of Gentamicin by E – test

Fig - 10



No zone of intersection upto 8 µg / ml

Figs - 11



MIC > 512 µg / ml – HLGR

4. Discussion:

Enterococci are commensals of the gastrointestinal tract of human beings. Over the past 2 decades, enterococci have become important nosocomial pathogens, probably due to inherent resistance to antibiotics (such as cephalosporins), ability to adhere to indwelling medical devices, and ability to survive adverse environmental conditions.[3]

Enterococci are not generally regarded as highly virulent bacterial pathogens, however, resistance to many antimicrobial drugs complicates the treatment of enterococcal infections. Acquired resistance to high concentrations of ampicillin, aminoglycoside, and glycopeptide antibiotics, specifically vancomycin, has exacerbated this problem[19]

In this study E.faecalis is the predominant species isolated (89.6%) followed by E.faecium (10.4%).

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This is in accordance with other studies by Gary cotter et al[3]; Simonsen et al[4]; P J Desai et al[18]; Keryn J Christiansen et al[12].Similar report was observed in other studies also.[13,18,21,22,26,30] Several species of enterococci are currently recognized, but 85-95% of enterococcal infections are caused by *E.faecalis*, and 5-10% are caused by E.faecium.[4,18] Although a few studies [14,22] have documented an increase in the prevalence of *E. faecium,* in this study the prevalence of this species was considerable low. This may be due to the fact that high prevalence of E.faecium is usually related to increase resistance to vancomycin which was not the case in this study[18].

This study showed a female preponderance.This is consistent with those reported in other studies.[1,2,11,12]

In our study, highest prevalence was seen >50 years comprising 38%. The mean age of the patient with enterococcal infection was around 60 years.[12,13,14]This study revealed a strong association between urinary catheterization and enterococcal infection. This correlates with other studies.[4,10]

In this study, majority of the isolates were from urine. This is in accordance with other studies.[2,10,17] This study revealed a strong association between urinary catheterization and enterococcal infection. This correlates with other studies.[4,10]In this study,genitourinary surgery and instrumentation of the urinary tract were identified as risk factors.P J Desai [18] has found instrumentation to be the major cause of urinary tract infection. Maria Bitsori et al[34] reports a higher rate of anatomical abnormalities and corrective surgery for enterococcal infection[34]. Similar risk factors were observed in other studies.[9,10,45]

Diabetic patients and patients who had prolonged stay in the hospital had a higher risk than other patients,similar to other studies.[8,9,10,12,13,14,15,16,31] Chronic antibiotic use predisposed to enterococcal infection. This is in accordance to other studies.[4,8,10,12,14,15,16,35] Our study revealed E.faecium to be more resistant to antimicrobials than E.faecalis.Similar findings have been reported by other studies also.[5]

This study showed E.faecium to be more resistant to ampicillin than E.faecalis. This correlates with the study by Jyotsna Agarwal et al[28] and Steven Gordon et al[17] This high susceptibility rate to ampicillin is similar to rates from other studies.[18]

This study showed 100% sensitivity to vancomycin. Similar results observed in other studies.[7,22,29,31,35,46] Similarly some other studies also report a very low level of resistance.[18]

This study showed 100% sensitivity to Teicoplanin and Linezolid. This is in accordance to other studies.[6,7], [38,10,19,32] Similarly Agrawal et al has reported 100% sensitivity to linezolid.[25,31]Very low level resistance seen in some studies.[31]

Both E.faecalis and E.faecium exhibited >50% resistance for ciprofloxacin in this study. High level of ciprofloxacin resistance reported in some studies. This study showed 100% sensitivity to nitrofurantoin. Similar results observed in other studies. In this study, high level gentamicin resistance was seen in both E.faecalis and E.faecium. This is consistent with other studies.E. faecium strains were observed to be more resistant to the tested antimicrobials similar to studies from India and outside.[5,7,27,29]

In this study, MIC for gentamicin by E –test for E.faecalis showed 12 isolates ≤ 128 – 512 µg/ ml(sensitive);12 isolates > 512 - 1024 µg/ ml(resistant) and 2 isolates > 1024 µg/ ml (resistant). For E.faecium, 1 isolate had MIC ≤ 128 – 512 µg/ ml(sensitive); 1 isolate > 512 - 1024 µg/ ml(resistant); and 1 isolate > 1024 µg/ ml (resistant).

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There is 100% agreement between results of disc diffusion and E test.

John E Schulz et al[33] reports that no false high level aminoglycoside resistance occurred and no false gentamicin susceptibility was noted by E - test[33] He has also compared agar screen and E test for detection of HLAR and found no false susceptibility. Martha L Sanchez et al[36]have reported that E test was able to detect all HLAR strains shown by agar dilution method and reported that the E test results compared with the agar dilution method demonstrated complete concordance in the detection of HLGR.

Conclusion:

- There is limited information on the presence of HLAR enterococci in a tertiary care set up**.**
- To conclude, the present study highlighted the importance of occurrence of high level aminoglycoside resistant enterococci. This would necessitate routine testing of the isolates for high level aminoglycoside resistance.
- Alternative treatment regimes need to be sought if HLAR is detected.
- Rationale use of antibiotics to be practised.
- Risk factors viz., prolonged hospital stay and chronic antibiotic usage and prolonged catheterization may be avoided.
- Constant surveillance of antimicrobial susceptibility need to be done.

Acknowledgement*:*

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