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# HIGH LEVEL AMINOGLYCOSIDE RESISTANT ENTEROCOCCUS SPECIES : A STUDY

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## ABSTRACT

**Background and Objectives:** The emergence of *Enterococcus* species as a causative agent of Health care Associated Infections, poses therapeutic challenge to clinicians. *Enterococci* are well equipped with intrinsic resistance to multiple antibiotics. Acquired resistance to commonly used antibiotics such as Penicillin, Aminoglycoside and Vancomycin have made the situation even worse. Detection of High Level Aminoglycoside Resistance (HLAR) in *Enterococcus* species can predict the loss of synergy between cell wall active antimicrobial agents and Aminoglycosides. Hence, the present study was undertaken to detect the incidence of High level Aminoglycoside Resistant (HLAR) *Enterococcus* species isolated in a rural hospital and to study their antibiotic susceptibility profile. .

**Method:** HLAR in *Enterococcus* species was detected by disk diffusion test using High level Streptomycin (HLS - 300 µg) disk and High level Gentamicin (HLG - 120 µg) disk and Agar dilution method as per CLSI guidelines. High Level Gentamicin Resistance (HLGR) was also confirmed by HiMedia Ezy MIC Strip. Antibiotic sensitivity test was done by Kirby Bauer Disc diffusion method as per CLSI guidelines.

**Result:** Out of total 190 *Enterococcus* species isolated from different clinical samples, High Level Aminoglycoside Resistance was detected in 115 (60.5%) *Enterococcus* strains. Amongst 115 HLAR *Enterococcus* strains, 59 (51.4%) were *E. faecalis* and 56 (48.6%) were *E. faecium.*

**Conclusion:** We hereby conclude that *Enterococcus* strains, isolated from different clinical specimens must be screened routinely for HLAR (High Level Aminoglycoside Resistance) by all Clinical Microbiology Laboratories to improve the therapeutic outcome.

**Keywords:** *Enterococci*, High Level Aminoglycoside Resistant (HLAR), Multi-drug resistant.

## INTRODUCTION

*Enterococci* are Gram positive bacteria that normally inhabit the gastrointestinal tract of many animals including humans. However, when they colonize the sites where they are not normally found, they can become pathogens[1]. Enterococci are now the second most common cause of nosocomial urinary tract and wound infections and third most common cause of nosocomial bacteremias[2]. Serious enterococcal infections (e.g., bacteremia and endocarditis) require treatment with a bactericidal combination of antibiotics that should include penicillin group of drugs (e.g., Ampicillin or Penicillin G) to which the *Enterococcus* isolate is susceptible and an Aminoglycoside (e.g., Gentamicin or Streptomycin) to which the *Enterococcus* isolate does not exhibit high-level resistance[3]. High-level Aminoglycoside resistance (HLAR) among *Enterococci* is increasingly being reported world-wide. The presence of HLAR is predictive of the loss of synergy between a cell-wall-active agent (e.g. Penicillin, Ampicillin or Vancomycin) and an

Aminoglycoside, which makes the treatment of serious enterococcal infections difficult[4].

Hence, the present study was undertaken to detect the incidence of High-level aminoglycoside resistant (HLAR) Enterococcus species isolated from different clinical samples and their antibiotic susceptibility profile.

## MATERIAL AND METHODS

A total number of 190 *Enterococci* were isolated from various specimens like urine, blood, pus and wound swab, body fluids, etc from July 2011 to August 2013. The specimens were collected from patients attending Indoor and Outdoor Patients Department of our Hospital and sent to Department of Microbiology. Our hospital is a tertiary care hospital in a rural set up. All *Enterococcus* species were identified by conventional methods[2]. The speciation of *Enterococcus* species was done by scheme proposed by Facklam and Collins[5]. Antibiotic susceptibility test was done for all 190 strains by Kirby- Bauer disk diffusion method, according to CLSI guidelines[6]. Antibiotics like Ampicillin(10µg), Linezolid (15µg),Vancomycin (30µg), Erythromycin (15µg), Tetracycline (30µg), Quinupristin-dalfopristin (15µg) were used. Additionally, for urine samples only, Nitrofurantoin disc (300µg) was used. HLAR (High Level Aminoglycoside Resistance) was detected by disk diffusion test using High level Streptomycin (HLS - 300 µg) disk and High level Gentamicin (HLG - 120 µg) disk and Agar dilution method as per CLSI guidelines[6].

As per CLSI guidelines, for detection of High Level Gentamicin Resistance by Agar dilution method, Gentamicin concentration was taken as 500 µg/ml. 10 µL of 0.5 Mc Farland suspension of test strain was spotted onto BHI (Brain Heart Infusion) agar surface containing Genatamicin 500 µg/ml[6]. High Level Gentamicin Resistance (HLGR) was also confirmed by putting the Himedia Ezy MIC strip. A lawn culture of the test strain (turbidity adjusted to 0.5 Mc Farland) was done on Mueller Hinton (MH) agar plate and Himedia Ezy MIC strip was put and MIC (Minimum Inhibitory Concentration) was detected after incubation at $37^0$C for 18 hours. MIC range of the strip was from 0.064-1024 µg/ml. *E.faecalis* ATCC 29212 was used as quality control for all the tests.

## RESULTS

Out of 190 Enterococcus strains isolated, High level aminoglycoside resistance (HLAR) was detected in 115(60.5%) strains. HLAR strains were detected by disc diffusion test, agar dilution method and HLGR by Gentamicin Ezy MIC strip (photo1,2 & 3). Amongst 115 HLAR strains, 59 (51.4%) strains were *E.faecalis* and 56 (48.6%) were *E.faecium* (fig1). Only High level Gentamicin resistance (HLGR), only High level Streptomycin resistance (HLSR) and both HLGR and HLSR producing *E.faecalis* strains were detected as 23(38.9%), 7 (11.9%) and 29 (49.2%) respectively out of total 59 HLAR positive *E.faecalis* strains (fig 2). Similarly, out of total 56 HLAR producing *E.faecium* strains, 34(60.7%) produced only High level Gentamicin resistance (HLGR), 5(8.9%) produced only High level Streptomycin resistance (HLSR) and 17 (30.4%) produced both High level Gentamicin resistance (HLGR) and High level Streptomycin resistance (HLSR) (fig 3). Out of 93 HLAR *Enterococcus* strains isolated from urine samples, 49 (52.7%) were *E.faecalis* and 44 (47.3%) were *E.faecium*. 11(68.8%) HLAR strains were isolated from total 16 pus and wound swab samples. Out of 11 HLAR strains, *E. faecalis* and *E. faecium* were 7 and 4 strains respectively. Out of total 25 blood samples, 9(36%) strains produced HLAR and *E. faecium* strains were shown to exhibit more HLAR than *E. faecalis*. Amongst 9 HLAR *Enterococcus* strains, only 3(33.3%) strains were *E. faecalis* whereas 6(66.7%) were *E. faecium*. Only 2 HLAR strains were detected from other specimens, which were identified to be *E. faecium* (fig 4). Maximum 36 (31.3%) HLAR strains were isolated from Surgery

ward followed by Pediatrics (22), Medicine (16) and Gynaecology ward (10) (fig 5). Out of 36 HLAR strains isolated from Surgery ward, 23 (63.9%) were *E. faecalis* and rest 13 were *E. faecium*. Only 7 (6.1%) HLAR strains were isolated from different ICUs (MICU-3, NICU-3 and PICU-1). No HLAR strain was isolated from OT ICU .

Out of 115 HLAR strains, 90 *Enterococcus* strains were MDR (Multi-drug Resistant). MDR *Enterococcus* strains were detected on the basis of resistance (acquired) to Erythromycin, Tetracyline and High level aminoglycosides. All (100%) HLAR Enteococcus strains were sensitive to Vancomycin and Linezolid. Out of 115 HLAR strains, only 7 (6.1%) and 23 (20%) were sensitive to Quinupristin-dalfopristin and Ampicillin respectively. Nitrofurantoin sensitivity was tested for 93 HLAR *Enterococcus* strains isolated from urine sample only and 85(91.4%) HLAR strains were sensitive to Nitrofurantoin.

## DISCUSSION

A common regime for treatment of serious enterococcal infections is the combination of cell-wall inhibitors, such as penicillin, ampicillin or vancomycin; with aminoglycosides, such as streptomycin or gentamicin. The addition of cell-wall inhibitor agent helps in the penetration of the aminoglycoside into the bacterial cytoplasm, making the intrinsically resistant organism aminoglycoside sensitive. The presence of HLAR in *Enterococci* makes the synergism of cell-wall inhibitor and aminoglycoside ineffective[7].

In our study, 60.5% HLAR producing Enterococcus strains were isolated. Our study correlated well with Deshpande et al who had reported 58.8% HLAR producing *Enterococcus* strains[8]. Mendiratta et al in 2008 reported 46% HLAR producing *Enterococcus* strains[9], whereas Vinod kumar et al in 2008 reported 65.6 % HLAR producing *Enterococcus* strains[10]. The study conducted by Mendiratta et al also reported that 69.6 % of their HLAR strains were *E.faecalis* and 30.4 % were *E. faecium*[9], whereas in the present study 51.4 % strains were *E. faecalis* and 48.6 % were *E. faecium*. As per $\chi^2$ test, when only HLGR producing *E. faecalis* (23) and only HLGR producing *E. faecium* (34) are considered, we conclude, that isolation of only HLGR producing *E.faecium* is significantly higher than only HLGR producing *E. faecalis*[11]. In our study, out of 115 HLAR strains, 90 (78.3%) were MDR which is quite higher than reported by Deshpande et al (57%) in 2008[8]. MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories[12]. Nitrofurantoin sensitivity was tested for 93 HLAR *Enterococcus* strains and it showed good activity (total 91.4% sensitivity) against both *E.faecalis* and *E.faecium*. Probably, the resistance could not be acquired by *Enterococcal* strains as Nitrofurantoin is not used commonly nowadays.

HLAR (High level aminoglycoside resistance) i.e., Streptomycin MICs >2000 µg/ml or Gentamicin MICs > 500 µg/ml is an acquired resistance[2]. HLAR occurs due to the presence of AME ( Aminoglycoside modifying enzymes). The most frequently encountered enzyme include dual function 2´phosphotransferase and 6´acetyl transferase conferring HLR to all available Aminoglycoside (Kanamycin, Gentamicin, Amikacin, Netilmicin, Tobramycin) except streptomycin[13]. Hence, gentamicin resistance is a good predictor of resistance to other aminoglycosides except streptomycin[14]. 6´adenyl transferase is another AME which is responsible for HLR to streptomycin but does not inactivate other useful aminoglycosides[13].

## CONCLUSION

We hereby conclude, that as High level Aminoglycoside resistant (HLAR) *Enterococcus species c*an predict the loss of synergy between cell-wall active antimicrobial agents and Aminoglycosides, all *Enterococcus* strains must be screened routinely for HLAR by all Clinical Microbiology Laboratories to get an effective

therapeutic outcome. Administration of Penicillin –Aminoglycoside combination without knowing HLAR status of the causative *Enterococcus species* can be fatal in life threatening Enterococcal infections.

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**Fig 1: Distribution of High level aminoglycoside resistance (HLAR) among *E.faecalis* & *E.faecium*. (Total HLAR= 115)**

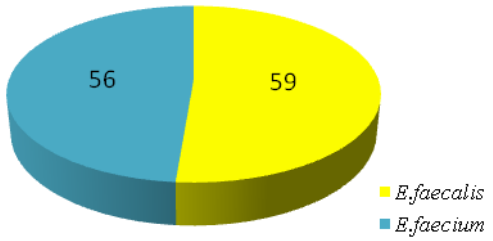



**Fig 2: Incidence of HLAR *E.faecalis* strains (n=59)**

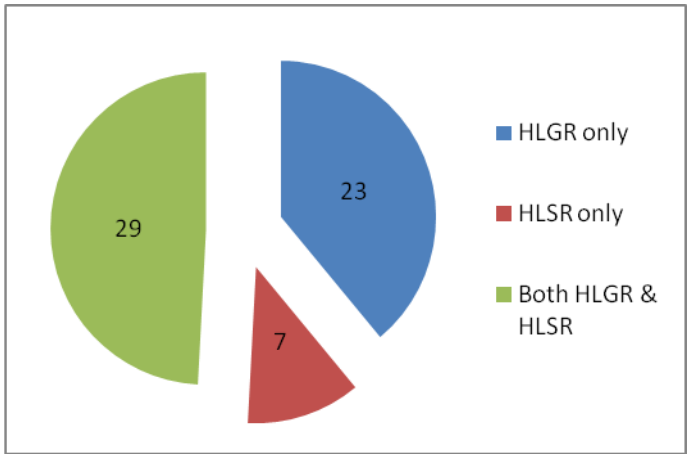



**Fig 3: Incidence of HLAR *E.faecium* strains (n=56)**

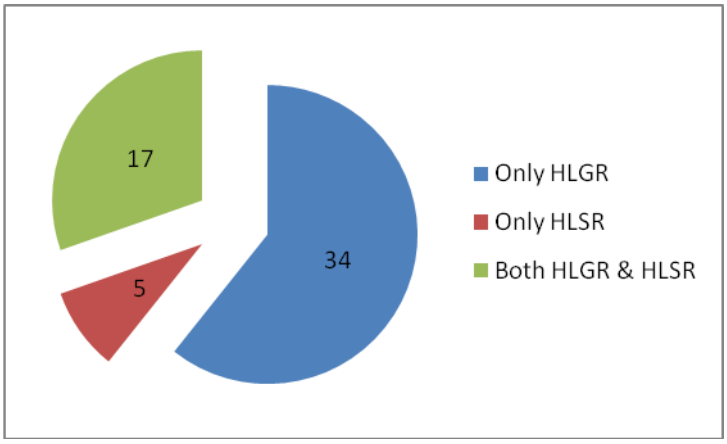
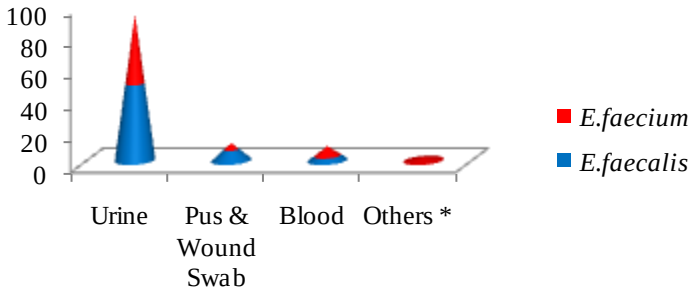
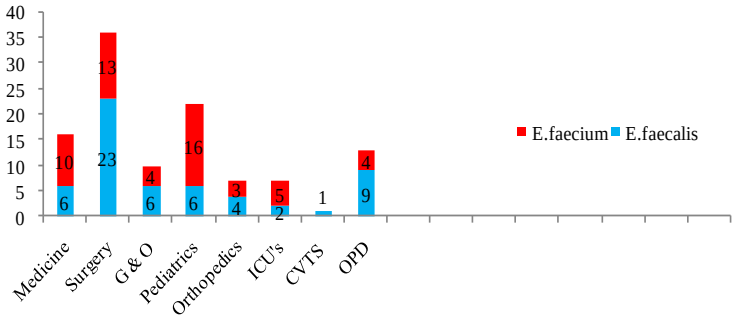


**Fig 4: Isolation of HLAR strains from different clinical samples (n=115)**




*** Others include Body fluids [CSF (n=2), Peritoneal fluid (n=1), Ascitic fluid (n=1)], Catheter tip(n=1), Drain fluid (n=2), Granulation tissue(n=1)].**

**Fig 5. Isolation of HLAR Enterococcus strains from different Clinical Specialities (n=115)**




**Photographs**

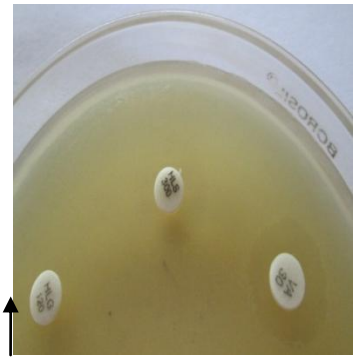


**Photo 1**
**Disk Diffusion Test for HLAR**

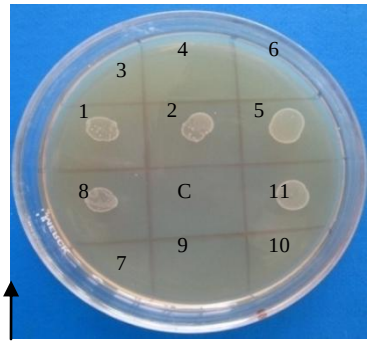



**Photo 2**
**Agar dilution method for HLAR Gentamicin concentration: 500 µg/ml (HLAR strains - No. 1, 2, 5, 8, 11, Sensitive strains- No. 3, 4, 6, 7, 9, 10, C - Control)**

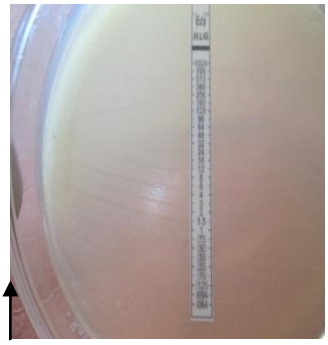


**Photo 3**
**Gentamicin Ezy MIC Strip (HLG) test for HLAR (MIC> 1024 µg/ml)**