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HIGH LEVEL AMINOGLYCOSIDE RESISTANCE AMONG CLINICAL ISOLATES OF ENTEROCOCCUS SPECIES

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ABSTRACT

Background: *Enterococci,* initially considered as normal commensal of intestinal tract have recently emerged as a medically important pathogen. Emergence of high-level aminoglycoside and glycopeptide resistance has significantly contributed to the mortality, particularly in serious *Enterococcal* infections. **Aim:** The aim of the study was to know the high level aminoglycoside resistance among *Enterococci* in a tertiary care hospital. The study was carried out over a period of Nov 2020 to Mar 2022. **Method:** *Enterococci* strains were isolated from various clinical samples by culture and biochemical methods and its antibiotic susceptibility testing was seen by Kirby Bauer method as per CLSI guidelines. HLAR was determined by disc diffusion method using high level Gentamicin disc (120 µg) and high level streptomycin (300 µg). HLGR was confirm by Himedia E strip test. **Result:** In 200 clinical isolates of *Enterococcus,* 170 were *Enterococcus faecalis* and 30 species were *Enterococcus faecium*. Out of 200 *Enterococcus* isolates .HLGR shows in 62.5% sample and HLSR shows in 19%. **Conclusion:** This study illustrates the high prevalence of HLAR in *Enterococci* in our region, which emphasizes the need to predict synergy between beta-lactams and aminoglycosides for management of *Enterococcal* infections.

KEYWORDS : Enterococcus, High level aminoglycoside resistant, High level sterptomycin resistant

INTRODUCTION

Enterococci have emerged as an important source of hospital-acquired infections, including those related to the surgical site, respiratory tract, urinary tract, skin and soft tissue infections, and bacteremia. Control and treatment of *Enterococcal* infections are problematic due to their intrinsic resistance to various antimicrobials, their capabilities to develop new resistance and to survive in the external environment for a long time [1, 2]. *Enterococci* acquire resistance to a wider range of antimicrobial agents particularly, aminoglycosides, glycopeptides, and beta-lactams. This poses a therapeutic challenge to clinicians as they are left with very few treatment options [3, 4]. A common regimen for the treatment of serious *Enterococcal* infections is the synergistic combination of cell wall inhibitors as vancomycin with aminoglycosides [5]. Although *Enterococci* are intrinsically resistant to low levels of aminoglycosides, high-level resistance to aminoglycosides (HLAR) is mediated by acquisition of genes encoding aminoglycoside-modifying enzymes (AME). High-level gentamicin resistance (HLGR) in *Enterococci* is predominantly mediated by aac (6)-Ie-aph(2)-Ia gene, which encodes the bifunctional aminoglycoside modifying enzyme AAC (6)-APH (2). The action of such enzyme in *Enterococci* eliminates the synergistic activity of gentamicin when combined with a cell wall active agent, such as ampicillin or vancomycin. Other AME genes conferring gentamicin resistance such as aph (2)-Ib, aph (2)-Ic, and aph (2)-Id have been also detected in enterococci. Furthermore, highlevel streptomycin and kanamycin resistance in enterococci are mediated by aph (3)-IIIa [6].

Aim: The current study was conducted to investigate the rate of HLAR.

Methods:

Study design:

A prospective study was conducted from November 2020 to may2022 from RNT medical college and MB hospitals. A total number of 200 non repetitive *Enterococcal* isolates were collected from different clinical samples from outpatient clinic and hospitalized patients.

Inclusion criteria:

- Pure isolates of Enterococcus from various clinical samples were taken in the study.
- Well labeled samples were considered.

Exclusion criteria:

- Samples with mixed growth were excluded.
- Samples inadequately labelled or insufficient amount were not taken in consideration.

Bacterial isolates and species identification

A prospective study was conducted from November 2020 to may2022 from RNT medical college and MB hospitals. A total number of 200 non repetitive *Enterococcal* isolates were collected from different clinical samples from outpatient clinic and hospitalized patients. The clinical specimens were initially cultured on blood agar, MacConkey agar (HiMedia, India) and chromagar at 37°C for 24hours. Plates of *Enterococci* were identified by Gram staining, colony morphology, catalase test, and Bile Esculin test,growth at 6.5% NaCl, growth at 37°C and 45°C . All isolates were identified to species level on the basis of biochemical tests.[7] Antibiotic susceptibility testing for ampicillin and vancomycin were seen by Kirby Bauer method as per CLSI guidelines.[8]

Antibiotic susceptibility testing

Antibiotic susceptibility testing was carried on Mueller- hinton agar by Kirby-Bauer disc diffusion method. High level aminoglycoside resistance (HLAR) method was detected by following methods.

Disc diffusion method

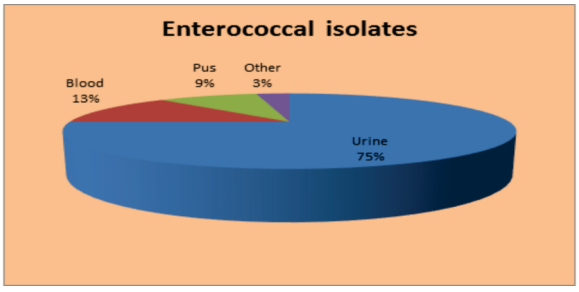
Colonies of *Enetrococcus* was inoculated into peptone water and incubated at 37°C for 4 hours. Growth was indicated by the appearance of turbidity in the medium. Turbidity of the medium was compared with 0.5 McFarland tube. Lawn culture

was performed on MHA agar plate with the help of sterilized swab, Gentamicin (120 µg) and Streptomycin (300 µg) drugs was inoculated with a sterile - forceps and then incubated. Results were interpreted according to the CLSI guidelines.

Minimum Inhibitory Concentration (MIC) method

Minimum inhibitory concentration of Gentamicin was determined by E-test. The strains which were resistant by disc diffusion method were checked by MIC. The colonies were inoculated in Brain Heart Infusion (BHI) broth. Growth was indicated by the appearance of turbidity which was compared with 0.5 McFarland tube. Lawn culture was done on MHA agar plate with a sterile swab and E- strip was inoculated on MHA plate and incubated. All the results were interpreted according to CLSI guidelines. MIC≥ 500µg for Gentamicin was considered as high level resistance.[8]

RESULTS



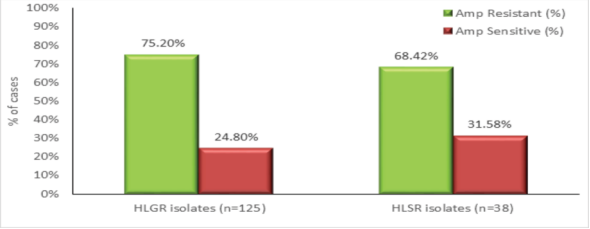
Picture no.1 Shows Prevalence of Enterococcal isolates among various specimens (n=200)

Table 1: HLGR, HLSR, HLAR) in Enterococcal Isolates by disc diffusion method

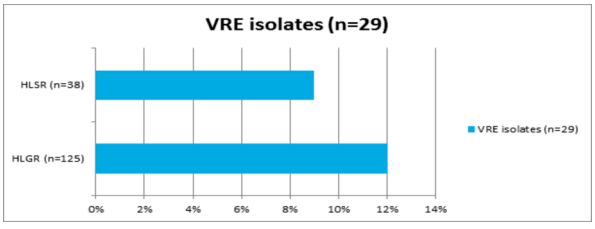
Entero. Sp.	No. of Isolates	Number (%) of Resistant Isolates		
		HLGR	HLSR	HLAR(BOTH)
E. Faecalis	170 (85%)	105(61.76%)	18(10.58 %)	17(10%)
E. Faecium	30 (15%)	20(66.67%)	20(66.67 %)	18(60%)
Total	200 (100%)	125(62.5%)	38(19%)	35(17%)

TABLE 2 : showing high level aminoglycoside resistance in enterococcus species by MIC method (n=200)

ANTIBIOTIC	HLAR (MIC ≥ 500µg)	PERCENTAGE
HIGH LEVEL GENTAMICIN (0.064-1024mcg/ml)	125	62.5



Picture 2: shows Distribution of HLGR and HLSR with Ampicillin Resistant



Picture 3: shows Distribution of HLGR and HLSR with Vancomycin Resistant *Enterococci*

DISCUSSION:

Aminoglycosides are considered efficient in treating serious infections caused by both Gram-positive and Gram-negative organisms. However, the acquisition of extrinsic resistance to high-level aminoglycoside antibiotics in *Enterococci* renders these strains a serious challenge in clinical settings [9].

In our study highest isolates found to be from urine samples. In present study table 1 depicts Out of 200 *Enterococcal* isolates125(62.5%), [*E.faecalis* 61.76% and *E.faecium* 66.67% respectively] were found to be HLGR and HLSR were found to be 38(19%)[*E.faecalis* 10.58% and *E.faecium* 66.67%] and HLAR (HLGR And HLSR both) found in 17%[*E.faecalis* 10% and 60% in *E.faecium*] These results were in concurrence with the results of the study by **Jain S et al** (HLGR 60%,HLSR 55%,HLAR,54%),**Rana D et al** (HLGR 40%,HLSR 34%,HLAR 15%). Study conducted by **Paterson D et al** revealed lower resistance(HLGR 16%,HLSR 10% HLAR 3.6%) as compare to presnt study. Some authors detected only HLGR in their studies as **Irfan I et al** reported HLGR 28% (*E.faecalis* 27.6% and *E. faecium* 33.3%), Moussa **A A et al** reported 48% HLGR in *E.faecalis* and 46% in *E.faecium*. On the contrary, a study by **Jayavarthini et al**) reported HLGR of 4.7%. Recent studies also indicated HLGR to be more common in all species of *Enterococci* than HLSR. Similarly, we had observed HLGR to be more predominant than HLSR in our study isolates.

Prevalence of HLAR in cell wall active agents like Ampicillin and Vancomycin was also studied (picture no 3and 4). In the present study, out of 125 (100%) HLGR *Enterococci* isolates, resistance to Ampicillin and Vancomycin was shown by 94(75.20)%, 24(12%) of HLGR *Enterococci* isolates respectively. Also, out of 38 (100%) HLSR *Enterococci* isolates, 26 (68.42%) of isolates showed resistance to Ampicillin and only 18 (9%) of isolates showed resistance to Vancomycin. Which is accordance to the ***Rana D et al.*** Hence, resistance to the cell wall active agents was quite high in both the species.

In HLGR and VRE both had the maximum isolates were from urine 20 (83.33%), followed blood 4 (16.66%). And in HLSR and VRE both had 66.66% isolates from urine by followed by blood 4 (22.22%,) pus 2 (11.11%) .

CONCLUSION:

In conclusion, the present study illustrates the high prevalence of HLAR in *Enterococci* from patients with UTI and bacteremia in our region. Resistance to multiple antibiotics and inactivity to the synergistic killing of combination therapy of penicillin and aminoglycosides have given an excellent opportunity to *Enterococci* to survive and become secondary invaders in hospital infection. Hence, this study emphasizes the need to screen for HLAR in *Enterococcus* strains from patients with UTI and septicemia for predicting synergy between beta-lactams and aminoglycosides for *Enterococci*.

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