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Original article

High Level Gentamycin Resistance among *Enterococcus Species* Isolated From Clinical Samples in a Tertiary Care Hospital

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ABSTRACT

Background: Enterococcus, which comprises of gram positive organism acts as opportunistic pathogens which has their natural habitat in genital tract, gut and oral cavity. Resistance to wide variety of antibiotics has made enterococcus one of the important cause for nosocomial infection. Emergence of aminoglycoside resistance has made it a feared pathogen. **Objective:** Objective was to isolate, speciate and determination of high level gentamycin resistance among Enterococcus species. **Material and Methods:** Clinical samples like pus, urine, blood, CSF etc. were collected and processed. Speciation was done through sugar fermentation test, followed by antibiotic sensitivity test. **Results:** Of the 81 isolates processed for species identification and antibiotic susceptibility testing, 38 were identified as *E. faecalis*, 27 as *E. faecium*, 15 *E.avium* and One *E.durans*. A total of 24 isolates showed resistance to high level gentamicin by disc diffusion method. Thus there were about 30[37.03%] Multi Drug Resistance [MDR] isolates among the 81 Enterococcus species. **Conclusion:** Increased incidence of High Level Aminoglycoside Resistance**[**HLAR]and MDR among the enterococcal species in the study, indicates indiscriminate use of broad spectrum antibiotics. Antibiotic stewardship, proper hygiene and rational use of antibiotics have to be implicated to prevent resistance among the enterococcus species.

KEYWORDS: *Enterococcus spp.*, High Level Gentamycin Resistance, High Level Aminoglycoside Resistance, Vancomycin Resistant Enterococcus.

INTRODUCTION

In the 1900's, considered as nosocomial pathogens, enterococcus has gradually entered the mainstream of pathogens along with its ability to exhibit antimicrobial resistance. Comprising of gram positive organism, enterococcus acts as opportunistic pathogen having their natural habitat in genital tract, gut and oral cavity. Of the two most common *Enterococcus* species, *E. faecalis*has been found to be responsible for 80-90 per cent and *E. faecium*for the remaining human enterococcal infections [1].The most frequent infections caused by these organisms include urinary tract infections followed by intra-abdominal or intrapelvic abscesses. Blood stream infections are the third most common infections caused by this organisms [1].Being able to survive in hospital environment and their resistance towards variety of antibiotics has lead enterococcus to be one of the major causes in nosocomial infection.

The use of cell wall active agents such as penicillin or vancomycin with aminoglycosides results in synergistic bactericidal activity, but with increase in HLAR [high level aminoglycosidic resistant] strains, caused by production of

aminoglycoside modifying enzymes [AMEs], makes standard therapy with aminoglycosides and β-lactam antibiotics difficult [2]. Extensive administration and irrational use of antimicrobial agents lead to the emergence of such resistant species leading to difficulties in treatment of severe enterococcal infection making it feared opponents[3].

The purpose of the study was to speciate the enterococcal species and frequency of occurrence of HLAR [High level aminoglycoside resistance] and VRE [Vancomycin resistant enterococcus] among the clinical isolates.

MATERIALS AND METHODS

The present study was a cross-sectional study conducted from January 2011 to May 2012. Clinical specimens including urine, pus, blood and various were collected for which prior ethical clearance was taken from Institutional Ethics Committee. Relevant clinical data and demographic variables were recorded in a preformed questionnaire.

Clinical samples were collected in sufficient amount in their respective containers and swabs. Direct smears were prepared from the pus and various exudates and examined microscopically after gram staining. Pus and various swabs were inoculated onto 5% sheep blood agar and MacConkey agar whereas urine samples were inoculated on Cysteine Lysine Electrolyte Deficient [CLED] agar. Blood samples were collected in brain-heart infusion [BHI] broth and incubated overnight at 37^{o}C aerobically. After incubation subculture was done daily on 5% blood agar and MacConkey agar for 7 days. After inoculation the culture media were incubated aerobically at 37^{o}C for 16-18 hours.

Identification of Enterococcus species

Identification of *Enterococcus spp.* was done as per standard protocol. Gram-stained smear from the growth obtained on culture media were examined microscopically for detection of gram-positive cocci in pairs of approximately 0.5-1 µm in diameter. Further identification was done on the basis of catalase test, bile esculin hydrolysis test, motility test on mannitol motility medium [MMM], growth on 6.5% NaCl, heat tolerance test, sugar fermentation test using arabinose, mannitol, pyruvate, raffinose and sorbitol, arginine/lysine decarboxylation test and growth on potassium tellurite agar [PTA]. *E. faecium*ATCC 19434and *E. faecalis* ATCC 29212 were used as quality control strains.

Antibiotic susceptibility test of Enterococcus species

Peptone water suspension of clinical isolates equivalent to 0.5 McFarlands standard was prepared. A sterile swab was dipped into the suspension and inoculated [lawn culture] on the Mueller Hinton Agar plate in three directions rotating the plate at every 60^{0} to ensure even application of the inoculum. Plate surface was allowed to dry and antibiotic discs of ampicillin, ciprofloxacin, norfloxacin, vancomycin, high level gentamicin [120µg], erythromycin, penicillin and tetracycline were dispensed onto the surface of the inoculated agar plate. Antibiotic Plates were incubated at 37^{0} for18-24 hours.

Speciation:

Speciation was done through sugar fermentation test using arabinose, mannitol, pyruvate, raffinose, and sorbitol.**[Table 1]**

Table 1: Sugar Fermentation Test – Species of *Enterococcus*

Acid Produced	*E.feacalis*	*E.faecium*	*E.durans*	*E.avium*
Arabinose	-	+	-	+/-
Mannitol	+	+	-	+
Pyruvate	+	-	-	-
Raffinose	+	+/-	-	+/-
Sorbitol	+	+/-	-	-

RESULTS

Of the 81 isolates processed for species identification and antibiotic susceptibility testing, 38[46.9 %] were identified as *E. faecalis*, 27 [33.3%] as *E. faecium*, 15 *E.avium*[18.5%]and One*E.durans*[1.23%] which were isolated primarily from urine[71.05%*E.faecalis*, [85.18%*E.faecium*, 80% *E.avium* and only One*E.durans* isolate] blood[21.05% *E.faecalis*, 14.8%*E.faecium*,20% *E.durans*] followed by other exudates [7.89% *E.faecalis*]**[Table 2].**

A total of 24 [29.62%] isolates showed resistance to high level gentamicin by disc diffusion method. HLAR among *E. faecium*isolates [29.62%] was a little lower than*E. faecalis*[34.21%] whereas it was low in *E.avium*[12.5%]. The isolates such as *E.avium, E.durans, E. faecalis* and *E. faecium* showed multi drug resistance, it was five, one, 16 and eight in numbers respectively. Thus there were about 30[37.03%] MDR isolates among the 81 Enterococcus species. Concomitant resistance HLGR strains to b lactam antibiotics were high in both the species. This we compared with AST disc diffusion considering ampicillin and penicillin susceptibility pattern. Overall it was around 80%-90% with penicillin and ampicillin.

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Table 2: Source and Specimen of Isolate

Specimen	E.faecalis	*E.faecium*	*E.avium*	*E.durans*
Urine	27[71.05%]	23[85.18%]	12[80.0%]	1[100%]
Blood	8[21.05%]	4[14.81%]	3[20.0%]	0
Others	3[7.89%]	0	0	0
Total	38[100%]	27[100%]	15[100%]	1[100%]

Table 3: Antibiotic Resistivity Pattern in the Isolates

Antibiotics	*E.avium* [N=15]	*E.durans* [N=1]	*E.faecalis* [N=38]	*E.faecium* [N=27]
Ampicillin	6 [40%]	1[100%]	21 [55%]	7 [25%]
Ciprofloxacin	7 [46%]	1 [100%]	28 [73%]	23 [85%]
Norfloxacin	9 [60%]	1 [100%]	28 [73%]	18 [66%]
Vancomycin	3 [20%]	0 [--]	4 [10%]	3 [11%]
HLG	3 [20%]	0 [--]	13 [34%]	8 [29%]
Erythromycin	9 [60%]	0 [--]	31 [81%]	4 [14%]
Penicillin	9 [60%]	0 [--]	35 [92%]	18 [66%]
Tetracycline	0 [60%]	0 [--]	18 [47%]	9 [33%]

DISCUSSION

In the recent years, there has been a scenario of enhanced in enterococci because of their ability to cause serious infections as well as increasing resistance to many antimicrobial agents[1,5, 6]. In the present study four species *E. faecalis* 38[46.9%] and *E. faecium* 27[33.3%],*E. durans*One [1.23%], *E.avium* 15[18.5%] were recovered in contrary to more by others from India[7, 8].Our isolation rate was different from that of Nagpur [9][*E. faecalis* 86% and *E. faecium* 14%] in Central India and Coimbatore[88% *E. faecalis*][10]. However, report of higher isolation of *E. faecium*[80.7%] over *E. faecalis*[19.2%] has beenthere from Mumbai[11].

In the present study, 29.62% of the enterococci showed HLAR. HLAR among *E. faecium* isolates [29.62%]was alittle lowerthan E. *faecalis*[34.21%] whereas it was low in *E.avium*[12.5%]in contrast to the study my M K Mandiratta et al[3], where HLAR was significantly [$p<0.05$] higher in *E. faecium*[59.1%] than *E. faecalis*[7.8%], as also reported by Gordon *et al* [12]. High HLGR in *E. faecalis* has been observed in studies by Bhat KG et al and Agarwal VA et al [7,9].

We, in this rural set up, found the prevalence of HLGR *E. faecalis* to be lower while that of HLGR E. *faecium* to be slightly higher than that reported from urban hospitals [7,9,11,13]. The reason could be the prevalence of a particular species in an area along with the use of broad spectrum antibiotics.

HLGR has also been linked to β lactamase production, resistance to ciprofloxacin and chloramphenicol[1, 14]. In fact Schouten *et al*reported that as the prevalence of HLGR increases, β lactamase production in Enterococci may also increase [14]. Both *E. faecium* and *E. faecalis* showed concomitant resistance to ciprofloxacin, where ciprofloxacin resistance among the enterococcal isolates were 69.62% [55/81]. In addition, resistance through β lactamase production intrinsically has increased concern regarding enterococcus.This study revealed the prevalence of

multidrug resistant HLAR strains of *E. faecalis* and *E. faecium* in this rural hospital.

CONCLUSION

Our study reveals the problem of multiple drug resistant and HLAR among enterococci. Increase in MDR among the enterococcal species indicates indiscriminate use of broad spectrum antibiotics or a result of nosocomial infection. Rationale use of antibiotic, implication of antibiotic stewardship and proper hygiene especially among inpatients will have a positive impact.

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