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RESEARCH ARTICLE OPEN ACCESS

Prevalence of High Level Gentamicin Resistance among the Clinical Isolates of *Enterococci* Species

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Abstract

***Enterococci* infections, have drawn attention of clinicians due to rapid increase in high level aminoglycoside resistance (HLAR). This resistance predicts failure of β-lactam antibiotic and aminoglycoside combination, which is the current treatment of choice for serious *enterococci* infections. This led us to investigate about the prevalence of HLAR *enterococci* in our hospital setting in Shimoga, Karnataka. All the clinical samples received at Microbiology laboratory , McGan hospital, Shivamogga between June 2016 and December 2016 was considered for the study. *Enterococci* were identified and further speciated by following conventional method. Antibiotic susceptibility pattern of all the isolates was determined according to CLSI guidelines. High level gentamicin resistance was detected by disc diffusion method using 120µg gentamicin disc. A total of 64 *Enterococci* species were isolated from the clinical specimens. On speciation, *Enterococcus faecalis* (*E. faecalis*) accounted for 72% (46) of the total isolates, 24%(16) of the isolates were *Enterococcus faecium* (*E. faecium*)and 4% (2) isolates were *Enterococcus durans* (*E. durans*). High level gentamicin resistance was noted in 42% (27) among the total isolates. *E. faecium* was found to be more resistant compared to *E. faecalis. E. durans* was a sensitive strain. Our study reconfirms the high prevalence of HLAR in the clinical setting. The study stresses on the need for regular screening of HLAR in all *Enterococci* infections and revise the battery of drugs for its treatment according to the report.**

Keywords: *Enterococcus, Enterococcus faecalis, Enterococcus faecium,* high level aminoglycoside resistance, Gentamicin

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INTRODUCTION

Enterococci, previously classified as group D *Streptococci* is now placed under a new family; Enterococcaceae. These Gram positive cocci reside in human intestine and are considered as normal bowel flora. Though they are considered to be less virulent than *Streptococcus pyogenes*, they are one of the most feared nosocomial pathogens isolated. Common nosocomial infections caused by this genus are urinary tract infections, intra-abdominal infections, peritonitis, bacteremia and endocarditis.[1,2]

Compared to other clinically important Gram positive cocci, this genus is intrinsically resistant to the antimicrobial agents commonly used in hospitals. In addition, Antibiotic resistance has been acquired, and disseminated via horizontal transfer of mobile genetic elements mediated mainly by conjugative plasmids. Though all acquired resistance of this organism is not significant clinically, high level aminoglycoside resistance greatly affects *Enteroccoci* therapy. This is because; severe enterococci infections are treated with combination of cell wall acting agent like vancomycin and an aminoglycoside. And high level aminoglycoside resistance predicts resistance to this combination therapy.[1,3,4]

This resistance pattern seen in genus *Enterococci* has become an issue of concern since decades but remains so even now. Hence this study was conducted to investigate the same so as to design the guidelines regarding measures to prevent spread of the resistant strains in the hospital and also to prevent the emergence of vancomycin resistance which is the treatment of choice for HLAR *Enterococci.*

MATERIALS AND METHODS

This cross-sectional study was carried out in the Microbiology laboratory, McGan hospital, Shivamogga, between June 2016 and December 2016. The study was approved by the Institutional Ethical Committee of Shimoga Institute of Medical Sciences, Shivamogga.

All the pus, urine, blood and sterile body fluid samples received in our laboratory during the study period was included for the study. Faecal samples, and samples which were leaked were excluded .

The pathogen isolated by growth on blood agar after 24 hour aerobic incubation at 37°C was identified as enterococci species by Gram's staining, catalase test and bile esculin hydrolysis test. However, for blood samples, subculture for 7 days was done before reporting negative. Further, speciation was done by arginine decarboxylation, pyruvate utilization, and fermentation of arabinose, raffinose, mannitol and ribose tests.[5,6]

Susceptibility pattern of the isolated enterococci species were carried out according to CLSI guidelines using disc diffusion method. Detection of High level gentamicin resistance was performed using120µg gentamicin antibiotic disc (Himedia).[7]

In HLGR detection by disc diffusion method, zone of inhibition of 6 mm was considered as resistant, 7-9 mm as inconclusive and ≥ 10 mm as Susceptible.[7]

RESULTS

A total of 64 *enterococci* species were isolated during our seven month study period. Out of these 64 isolates, 27 were from pus, 21 from urine and 16 from blood samples.

On speciation, *Enterococcus faecalis* accounted for 72% (46) of the total isolates, 24% (16) of the isolates were *Enterococcus faecium* and 4% (2) isolates were *Enterococcus durans.* Distribution of these three enterococci species in various clinical samples is documented in Table 1.

The antibiotic sensitivity pattern of the isolates is documented in Table 2. Species wise resistance pattern to various antibiotics is depicted in Fig. 1. For urinary isolates, apart from the antibiotics listed in the table, nitrofurantoin (300µg) and norfloxacin (10µg) antibiotic discs were used. And out of 21 urinary isolates, 9 (43%)

Table 1. Sample wise distribution of different *Enterococci* species

Sample	*E. faecalis*	*E. faecium*	*E. durans*	Total
Pus	16	09	02	27
Urine	18	03	00	21
Blood	12	04	00	16
Total	46	16	02	64

were resistant to nitrofurantoin and 13 (62%) were resistant to norfloxacin.

High level gentamicin resistance was noted in 27 (42%) isolates. Number of HLGR noted in different enterococci species is depicted in Fig. 2.

Table 2. Antibiotic sensitivity pattern of *Enterococci* isolates

Antibiotics	Sensitivity (n=64)
Ampicillin	20 (31%)
Ciprofloxacin	33(52%)
Levofloxacin	31(48%)
Vancomycin	64(100%)
Linezolid	64(100%)
High level gentamicin	37(58%)

DISCUSSION

Genus *Enterococci,* once neglected group; considering them as commensals have gained great attention these days due to high rate of severe nosocomial infections caused by them. Two most common species isolated from clinical samples are *E. faecalis* and *E. faecium.* These are the organisms of low pathogenicity but with a property of inborn resistance to various antibiotics like relative resistance to most of the β-lactam antibiotics because of low affinity penicillin binding proteins (PBP). *Enterococci* also shows tolerance to penicillin i.e., penicillin acts only as bacteriostatic. *Enterococci* also show intrinsic low level resistance to aminoglycosides due to lack of oxidative uptake of the drug.[8,9]

However, serious enterococci infections like bacterial endocarditis are treated with

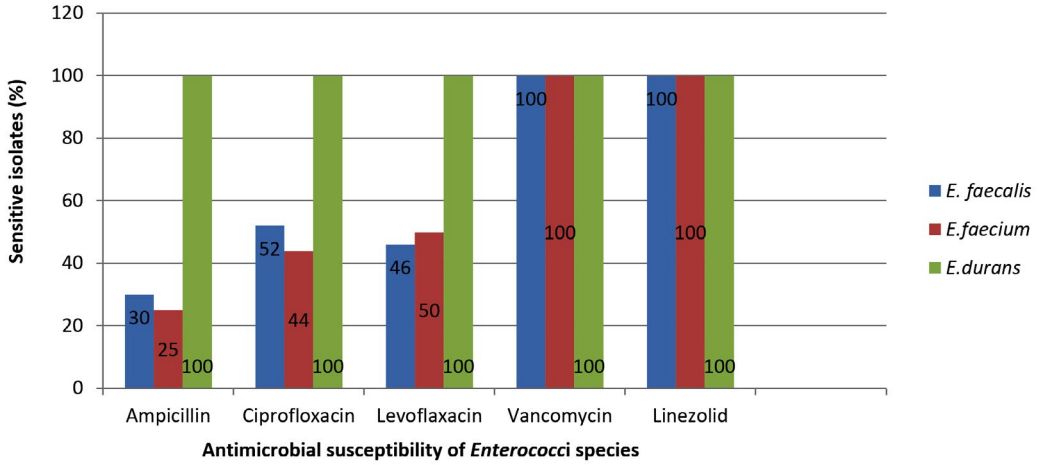

Fig. 1. Antibiotic sensitivity pattern of different *Enterococci* species.

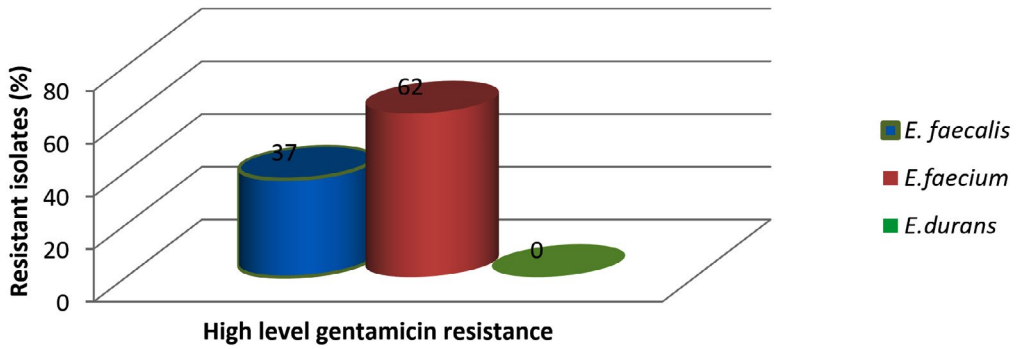

Fig. 2. High level gentamicin resistance in different *Enterococci* species.

combination of β-lactam antibiotic and an aminoglycoside. To a bacteriostatic beta lactam, the addition of aminoglycoside results in synergistic, bactericidal effect and beta lactam antibiotic indeed enhances the uptake of aminoglycoside resulting in synergistic killing of the organism. Aminoglycoside warrantied for usage in this combination are only gentamicin and streptomycin.

But the current detriment for the effective treatment is emergence of High level aminoglycoside resistance. It is defined by growth at concentration of 2000mg/L and 500 mg/L of streptomycin and gentamicin respectively on brain heart infusion agar. Presence of this high level resistance abolishes the synergistic effect of these agents. HLR to gentamicin is mainly due to aminoglycoside modifying enzyme and that to streptomycin is because of mutation in 30S ribosomal subunit and presence of streptomycin adenyltransferase.[8,9]

In our study, *E. faecalis* was recovered the most, followed by *E. faecium.* Both these species have shown highest resistance to ampicillin (69%) followed by levofloxacin (52%).Many studies in India have reported resistance to penicillin in the range of 40-80 per cent.[10-12]

It is glad to note that no vancomycin resistant enterococci (VRE) were isolated in our setting. And similarly, all the isolates were sensitive to linezolid which was consistent with other studies from India.[13-15] However, the prevalence of vancomycin resistant *Enterococci* (VRE) in India is reported to be between 0% and 30%.[16-18]

High level gentamicin resistance was noted in 42% of the isolates and *E. faecium* was found to be more resistant (62% of the total *E. faecium* isolated) compared to *E. faecalis* (37% of the total *E. faecalis* isolated). *E. durans* was found to be a sensitive strain.

Our study report is almost in line with that of various studies carried out in different parts of our country like study by Dadfarna N et al.,[19] who have reported 43.7% HLAR, Barman et al.,[20] who have reported 56.7% HLAR, Jain S et al.,[15] and Fernandes et al.,[18] who have noted 54% and 53% HLAR in their studies respectively.

Alternative treatment options for serious HLAR Enterococci infections

Vancomycin is the drug of choice in these cases of HLAR *Enterococci* infections. But however resistance to vancomycin has also been noticed since 1986. The best available evidence suggests that the emergence and spread of these pathogens are promoted by poor infection control techniques and by antibiotic selective pressure. Antibiotic selective pressure favouring the emergence and spread of Vancomycin Resistant Enterococci(VRE) may involve more than simply the extent of vancomycin use. Specifically, extended-spectrum cephalosporins and similarly active beta-lactams and drugs with potent activity against anaerobes appear to predispose to Vancomycin resistant *Enterococci* colonization and infection. Hence this should be taken care of by following strict antibiogram policy in all medical setup.[21]

Daptomycin is the drug of choice in VRE. Daptomycin, a cyclic lipopeptide has a unique mechanism of action that results in destruction of the membrane potential.[22] Daptomycin – Ceftarolin combination is opted in daptomycin non susceptible strains as ceftarolin is proved to restore daptomycin activity by promoting its surface binding.[23]

Quinupristin-Dalfopristin, a protein synthesis inhibitor is the drug of choice in HLAR, VR *E. faecium* infections. However, it has no action in *E. faecalis* infections due to intrinsic resistance.[21] Linezolid, an oxazolidinone antibiotic with broad spectrum activity against Gram-positive bacteria,that inhibits protein synthesis is not used in serious HLAR Enterococcal infections like endocarditis inspite of its low resistance rate due to its bacteriostatic nature.[21]

CONCLUSION

Increased prevalence of HLAR has become a problem of reality. Combination of β-lactam antibiotics and an aminoglycoside, which stood first among the choice for the treatment of serious enterococci infection, can no more help in killing these pathogens. This is a grave development and the fact that enterococci have great potential for the dissemination of acquired resistant gene is a matter to be considered seriously. A must screening for HLAR using gentamicin and/or streptomycin by disk diffusion/ agar dilution /broth microdilution should be practiced for all serious enterococci infections. Judicious use of Vancomycin, the drug of choice for HLAR enterococci infections is also advisable.

Along with these, strict containment measures, adherence to hospital infection control policy to prevent their dissemination should be taken up in all clinical settings.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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