

High Level Aminoglycoside Resistant Pattern of *Enterococci* Isolated from Urine at Tertiary Care Hospital

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Original Research Article

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Article History

Received: 02.06.2018

Accepted: 12.06.2018

Published: 30.06.2018

DOI:

10.36347/sjams.2018.v06i06.023



Abstract: UTIs constitute the most common type of disease produced by *Enterococcus* spp. *Enterococci* with high level resistance to aminoglycosides (HLAR), beta lactamase production & glycopeptide resistance including vancomycin resistance are posing a great therapeutic challenge, not only for clinicians but also for healthcare institutions. Therefore we conducted the study to find out prevalence of drug resistance in Enterococcal isolates with regards to HLAR (HLSR & HLGR) and Vancomycin resistance in our set up. *Enterococcus* isolated from all urine samples received in bacteriology section, from various wards was identified and speciated. Antibiotic susceptibility testing of isolated strains of *enterococcus* was done by Kirby bauer disk diffusion method, CLSI 2015. HLAR detection in enterococcus was done by disk diffusion method and Vancomycin MIC testing was done by E-test method. In our study, species isolated were *E. faecalis* and *E. faecium*, *E. faecalis* was predominant species (96%) followed by *E. faecium* (4%). High level gentamicin resistance (HLGR) were seen in 50% of all enterococcal isolates and High level streptomycin resistance (HLSR) was seen in 46% of isolates. HLGR was seen in 100% *E. faecium* isolates and in 47.9% of *E. faecalis* isolates. HLSR was seen in 50% *E. faecium* and 45.8% *E. faecalis*. High level of resistance was observed against ciprofloxacin and penicillin. 100% Vancomycin sensitivity was seen as tested by E-strip MIC test method. The synergistic effects obtained by combination of aminoglycoside with penicillin or vancomycin disappear in strains that show HLR to former. Therefore it is important to identify enterococcal species and HLR pattern correctly.

Keywords: High level aminoglycoside resistance (HLAR), *Enterococci*, Urine.

INTRODUCTION

Enterococci are members of the healthy human intestinal flora, but are also leading cause of highly antibiotic resistant, hospital-acquired infection. There is growing evidence that these bacteria frequently possess several specific traits that enable them to survive in the hospital environment, colonize patients, and cause infections such as bacteraemia, peritonitis, endocarditis and urinary tract, wound, and device-related infections [1,2]. UTIs constitute the most common type of disease produced by *Enterococcus* spp. Enterococcal UTIs are commonly acquired in hospital or long-term care settings, and thus, are more likely to be resistant to many antibiotics [3,4]. Among members of the genus *Enterococci*, *Enterococcus faecalis* and *Enterococcus faecium* are the most common species isolated from human infections [4]. Infections by *Enterococci* have

traditionally been treated with cell wall active agents in combination with an aminoglycosides however emergence of high level resistance to aminoglycosides, β lactam antibiotics and to vancomycin by some strains together with association of HLAR with multidrug resistance has led to failure of synergistic effects of combination therapy [3, 5, 6]. *Enterococci* with high level resistance to aminoglycosides (HLAR), beta lactamase production & glycopeptide resistance including vancomycin resistance are posing a great therapeutic challenge, not only for clinicians but also for healthcare institutions [4]. Multidrug resistance complicates treatment of enterococcal infections and the therapeutic spectrum of these cases is limited. Careful review of in vitro susceptibility data is required to treat infections caused by *enterococci*. Empiric therapy of enterococcal infections should be guided by local patterns of drug resistance. Nowadays emergence

of MDR *enterococci* is thought to be due to antibiotic selective pressure. This organism is considered as second leading cause of hospital acquired infections. Therefore we conducted the study to find out prevalence of drug resistance in Enterococcal isolates with regards to HLAR (HLSR & HLGR) and Vancomycin resistance in our set up[1].

MATERIALS AND METHODS

The study was conducted in department of microbiology over a period of one year from January 2016 to December 2016. *Enterococcus* isolated from all urine samples received in bacteriology section in our hospital from various wards clinically diagnosed with urinary tract infection was identified and speciated by test scheme proposed by Facklam and Collins[7]. Antibiotic susceptibility testing of isolated strains of *enterococcus* was done by Kirby bauer disk diffusion method on Muller Hinton Agar according to CLSI 2015[5]. HLAR detection in *enterococcus* was done by disk diffusion method. Antibiotic tested were Penicillin(10units), Tetracyclin(30µg), Levofloxacin(5 µg), Norfloxacin(10 µg), Nitrofurantoin (30 µg), High level gentamicin(120µg), High level streptomycin(300 µg), Linezolid(30 µg), Teicoplanin(30ug) and Vancomycin(30 µg). MIC of Vancomycin was determined by E-test method. These antibiotics were obtained as commercial discs from HIMEDIA LABORATORY, Mumbai. For quality control, ATCC

strains were used: *S.aureus* 25923 and *E.faecalis* 29212.

RESULTS

A total of 50 urine samples obtained from patients presenting with urinary tract infections, yielding growth of *enterococcus* was studied during 1 yr period. Among these, 74% of the samples were received from In patient department (IPD) and 26% were from Outpatient department (OPD). 64% of patients belonged to age group 20-40yrs. Mostly *enterococcus* was isolated from female patients (82%) followed by male patients (18%). In our study, species isolated were *E. faecalis* and *E.faecium*, *E. faecalis* being most common species (96%) followed by *E.faecium* (4%). *E.feacalis* showed resistance in high percentage as compared to *E.faecium*. High level gentamicin resistance (HLGR) were seen in 50% of isolates and High level streptomycin resistance (HLSR) was seen in 46% of isolates. HLGR was seen in all the *E.faecium* isolates (100%) and in 47.9% of *E.faecalis* isolates. HLSR was seen in 50% of *E.faecium* and 45.8% *E. faecalis*. Combined HLAR was observed in 30% of all enterococcal isolates. Of which 50% was seen in *E.faecium* and 29% *E.faecalis* showed Combined HLAR. (Table1)

All isolates were sensitive to vancomycin by MIC E-strip method. Antibiotic susceptibility pattern of enterococcus is shown in Table 2

Table-1: HLAR pattern among *Enterococcus* species

	HLGR	HLSR	HLAR
Species			
<i>E.faecalis</i> (48) 96%	23(47.9%)	22(45.8%)	14(29%)
<i>E.faecium</i> (2)4%	2(100%)	1(50%)	1(50%)
Total (50) 100%	25(50%)	23(46%)	15(30%)

Table-2: Antibiotic resistant pattern of *Enterococcus* species

Antibiotics Species	Penicillin	Tetracycline	Ciprofloxacin	Levofloxacin	Norfloxacin	Nitofurantoin	HLS	HLG
<i>E.faecalis</i> N=48(96%)	58%	45.8%	66.6%	50%	58.3%	10.4%	45.8%	47.9%
<i>E.faecium</i> N=2(4%)	50%	50%	100%	100%	50%	0%	50%	100%

DISCUSSION

Recently interest in *Enterococci* has increased not only because of their ability to cause serious nosocomial infections but also due to its multidrug resistant pattern. *Enterococci* species are found to be intrinsically resistant to cephalosporins and aminoglycoside. Even though bacteria were found to be sensitive to these drugs invitro experiments, unsatisfactory efficacy was found in clinical practice [9]. Correct speciation is very important since there is

variation in resistance to antibiotics by particular enterococcal species[10]. Low prevalence of resistance to linezolid, vancomycin and teicoplanin detected in both *E.faecium* and *E.faecalis*. Therefore, these drugs are widely used drugs for the effective treatment of enterococcal infections. Since antibiotic resistance varies in *enterococci* species, there is great need to identify enterococcal strains to the species level, which would facilitate the appropriate selection of antibiotics [11]. The high prevalence of MDR enterococcal

infection in tertiary care setup is due to excessive and indiscriminate use of broad spectrum antibiotics and high rate of patient transfer from peripheral centers [12].

HLAR *enterococci* first reported in France in 1979 and since then they have been isolated from all the continents. There is little need to test aminoglycosides other than streptomycin and gentamycin, as these are the agents with most clinical data. Till date, all strains with HLR to gentamicin have also shown resistance to synergism and/or to tobramycin, kanamycin, and amikacin by virtue of enzyme 2'-APH-6'-ACC. This enzyme is not active against streptomycin and thus gentamicin resistance strains are not necessarily resistance to streptomycin; in other words, variable percentage of strains will have HLR to gentamicin while lacking HLR to streptomycin [3]. The HLAR have become serious problem as incidence of HLAR has been disseminated in many enterococcal species. Therefore, its identification up to species level is essential for an appropriate management of the infection.

In our study, only two species were isolated *E.faecalis* and *E.faecium* which were in concordance with the findings of other studies [13,14]. The predominant species isolated was *E. faecalis*(96%) followed by *E. faecium*(4%). Similar to the findings reported by Srivastava.P *et al.* and Bose *et al* [13, 14, 15]. In contrary, Baragudi Mahesh *et al* [16] reported *E.faecium* as predominant species [16]. The incidence of other species of enterococci is underestimated in present study because of frequent misidentification. Hence proper identification to species level is essential for proper management and prevention of this bacterial infection in any health care setup [10]. Although, *E.faecalis* was predominant species isolated in our study, *E.faecium* was more multidrug resistance species compared to *E.faecalis*. It was not clear whether it was statistically significant as *E.faecium* number was very less [3, 17, 18].

In present study, enterococcal infection was observed among females (82%) followed by males (18%). Similar findings were reported by other studies [13,19]. Predominance of infection in females could be due to proximity of perianal area with urethra.

The present study showed that all *enterococcus* isolates have high resistance to ciprofloxacin and penicillin, which was in concordance with the findings of other studies [20,21]. 58% of penicillin resistance was seen in our study which was similar to the findings of other studies [5,22]. Many other studies have demonstrated resistance to penicillin ranging from 16-100% [21]. Penicillin resistance is seen due to resistance mechanism involving low affinity penicillin binding proteins or production of beta-lactamases. Penicillin along with aminoglycosides

considered treatment of choice; therefore, resistance to these antibiotics plays an important role in clinical setting.

In our study, 80% of enterococcal isolates were multi drug resistant, out which both the *E.faecium* isolates were MDR. The high prevalence of MDR enterococcal infection in tertiary care setup is due to excessive and indiscriminate use of broad spectrum antibiotics and high rate of patient transfer from peripheral centres[12].

In present study, linezolid and teicoplanin was found to be 100% sensitive in enterococcal isolates. Similar findings were reported by previous studies [5,23]. Srivastava P *et al.* and Naik T B *et al.* also reported 100% sensitivity against linezolid. As, linezolid is available in oral form and rapidly completely absorbed after oral administration. Linezolid can safely be used in severe enterococcal infection and seems to be appropriate therapeutic concern [24].

In present study, 50% isolates were HLGR and 46% isolates were HLSR. While combined HLAR was seen in 30% of isolates. Such high level resistance has been shown by Jain S *et al.* (60% and 55%) [20]. The previous studies indicated HLGR to be more common in all isolates than HLSR, similar findings was seen in our study. This is of great concern as it eliminates the synergy of aminoglycosides with beta-lactam antibiotics which is the treatment of choice for enterococcal infection and thus limiting the therapeutic options. While among enterococcal species, HLGR was high in *E.faecium* 100% as compared to 47.9% *E.faecalis*. Similarly, HLSR was high among *E.faecium* 50% than the *E.faecalis* 45.8%. Adhikari L *et al.* reported similar findings, with high HLGR(41.18%) and HLSR(41.18%) in *E. faecium*[25].

In India, prevalence of VRE has been reported between 0-30% [26]. The emergence of VRE has seriously affected the treatment of infections caused by enterococcus, limiting the choice of antibiotic for treatment. In our study, vancomycin resistance was seen in 4% isolates as tested by disc diffusion method which on testing by E-test method 100% vancomycin sensitivity was observed. The inaccuracy of the disk diffusion method has resulted in an unwarranted utilization of this drug treatment regime. Therefore routine MIC monitoring of important antibiotic like vancomycin has to be done before reporting resistance or intermediate sensitive [22]. Absence of VRE in our study indicated that vancomycin retains its therapeutic efficacy against majority *enterococci* isolated from patients in our hospital.

CONCLUSION

Detecting HLAR is important and it should be adopted as a part of routine microbiological testing in

order to prevent rise of HLAR enterococcus as nosocomial pathogen. In our study we observed high prevalence of HLAR and nil resistance against glycopeptides, but presence of glycopeptide together with HLAR calls for regular surveillance of antibacterial susceptibilities to detect emerging resistance and prevent the establishment and spread of antibacterial resistance strains. Also the synergistic effects obtained by combination of aminoglycoside with penicillin or vancomycin disappear in strains that show HLR to former. Therefore it is important to identify enterococcal species and HLR pattern correctly.

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