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Research Article

A STUDY ON HIGH LEVEL AMINOGLYCOSIDE RESISTANT ENTEROCOCCI ISOLATED FROM URINARY TRACT INFECTION

Reena Rajan[1]., Amirtha C[2]., Mohana Soundaram K.M[3] and Anandi V[4]

[1]Department of Microbiology, Penang International Dental College, Vinayaka Mission's University, Salem, Tamil Nadu
[2]Department of Medicine, Rajah Muthiah Medical College, Annamalai University, Chidambaram, Tamil Nadu
[3]Department of Microbiology, IRT Perundurai Medical College, Erode, Tamil Nadu
[4]Department of Microbiology, Vinayaka Mission's Medical College, Karaikkal, Tamil Nadu

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A B S T R A C T

Enterococci infection have been treated with cell wall active agents in combination with an aminoglycoside as the synergic effect overcomes the intrinsic resistance exhibited by Enterococci. High level aminoglycoside resistance leads to synergy resistance and treatment failure.The present study was conducted to determine the prevalence of high level aminoglycoside resistance among isolates of enterococci from urine sampleA total of 147 Enterococci isolates were included in this study. High Level Aminoglycoside Resistance (HLAR) was determined by agar dilution method and E test. Of the total isolates, 81.63% were Enterococcus faecalis, 14.29% were Enterococcus faecium and 4.08% were Enterococcus avium. 60(40.82%) were found to be resistant to Ampicillin, 76(51.70%) to High LeveGentamicin (HLG), 24(16.33%) to Nitrofurantoin by disk diffusion method. Out of 120 E.faecalis isolates, 39(32.50%) were resistant to Ampicillin and 57(47.50%) to High Level Gentamicin.Among the E.faecium isolates studied, 18(85.71%) were resistant to Ampicillin, 12(57.14%) to High Level Gentamicin. High level gentamicin resistance (MIC ≥2000µg/ml) was seen in 22.45 % isolates by agar dilution method. By E test 55 % E.faecalis isolates and 71.43% E.faecium were with a Minimum Inhibitory Concentration(MIC) of ≥1024µg/ml of Gentamicin.Monitoring of HLAR in Enterococci should be carried out by high content aminoglycoside disk and agar screen method.

INTRODUCTION

Enterococci are gram positive facultative anaerobes, which have become increasingly important as hospital acquired pathogens. Enterococci are second most common causative agent of nosocomial urinary tract infection. Enterococci are responsible for health care associated infections such as blood stream, intraabdominal and surgical site infection.There has been a world wide trend in increasing occurrence of Enterococcal infection and emergence of antimicrobial resistance among these isolates during the past decade.[1]

Enterococci are intrinsically resistant to a number of antimicrobial agents and display low level resistance to aminoglycosides and lincosamides and their ability to acquire resistant genes to currently available antibiotics results in the selection and spread of Multi Drug Resistant (MDR) strains. Multi Drug Resistant Enterococci display a wide repertoire of drug resistant mechanisms such as overexpression of efflux pumps, drug target modification, inactivation of therapeutic agents and sophisticated cell envelope adaptive response that promotes survival of these organism in the human host and nosocomial environment. [1,2] The standard treatment for deep seated enterococci infections has been a bactericidal and synergistic combination of a cell wall acting agent (Ampicillin or Penicillin G) with an aminoglycoside (streptomycin /gentamicin).[2] Enterococci have acquired aminoglycoside resistance genes that encodes various aminoglycoside modifying enzymes, which result in high level resistance to aminoglycosides. The high level resistance to aminoglycoside abolishes synergism in the combination with betalactams and glycopeptides that are important in the treatment of severe enterococcal infection.[3,4]

The first gentamicin resistant strains was detected in the United states in 1981. High level gentamicin resistance is associated with bifunctional enzyme possessing acetylase (6') and phosphotransferase activities conferring resistance to all aminoglycosides except streptomycin.[4] High level streptomycin resistance may be mediated ribosomally or due to transferable plasmid encoding two aminoglycoside modifying enzyme streptomycin adenyl transferase and neomycin phosphotransferase. Most E.faecium isolates

Corresponding author:* **Reena Rajan
Department of Microbiology, Penang International Dental College, Vinayaka Mission's University, Salem, Tamil Nadu

produce 6'acetylase transferase –acetylase that make them inherently resistant to amikacin, kanamycin, netilmycin and tobramycin there by nullifies the efficacy of combination therapy with these agents. [4,5]

Hence the present study was done to isolate, speciate and to determine the antimicrobial susceptibility profile of Enterococci isolates from urine sample and to detect the prevalence of high level aminoglycoside resistance in our clinical setting.

MATERIALS AND METHODS

A total of 147 isolates from clean catch mid stream urine were included in this study. The samples were cultured on Blood agar and Mac Conkey agar and incubated at 37^0 C for 18-24 hours. Any significant growth obtained were identified by colony morphology, gram staining, catalase test, growth in 6.5% sodium chloride, aesculin hydrolysis in the presence of 40% bile and by biochemical reactions using conventional test scheme (Facklam and Collins). Further identification to species level was done by using 1 % carbohydrate solution of glucose, lactose, raffinose, arabinose, sorbose, sorbitol, sucrose, pyruvate utilisation test, arginine dihydrolase, motility and pigment production.[6]

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was done on Muller Hinton agar by Kirby Bauer disk diffusion method using the following antibiotics: viz Ampicillin (10µg), Amikacin (30µg), Ciprofloxacin (5µg), Teicoplanin (30µg), Nitrofurantoin (300µg), Kanamycin (200µg) and results were interpreted as per Clinical Laboratory Standard Institute (CLSI) guidelines.[7] Enterococcus faecalis 29212 was used as quality control strain. High level resistance aminoglycoside was determined by disc diffusion method using high level gentamicin disc (120µg) and streptomycin disc (300µg).A zone diameter of ≤ 6mm was considered as resistant, 7-9 mm as intermediate and ≥10mm as sensitive for kanamycin, gentamicin and streptomycin.[5,8]

Screening for aminoglycoside resistance was performed by using Brain heart infusion agar with 500 µg/ml for Gentamicin and 2000 µg/ml for Streptomycin.10µl of bacterial suspension (equivalent to 0.5 McFarland standard) was inoculated by spotting and the plates were incubated at 37^0 C for 24 hours. Presence of more than one colony or haze of growth was read as resistance. E.faecalis 51299 was used as positive control and E.faecalis 29212 was used as negative control [5]

Minimum Inhibitory concentration of Gentamicin was determined by E test and results were interpreted according to CLSI guidelines. MIC of ≥ 500µg/ml for Gentamicin was considered as high level resistance. [9]

RESULTS

A total 147 Enterococci isolates obtained from urine sample were included in this study. Of the total isolates, 81.63 % were Enterococcus faecalis, 14.29 % were Enterococcus faecium and 4.08 % were Enterococcus avium. Among the Enterococci studied, 60 (40.82%) were found to be resistant to Ampicillin, 76(51.70%) to High Level Gentamicin(HLG), 21(14.29%) to Teicoplanin, 99 (67.35%) to Ciprofloxacin, 24(16.33%) to Nitrofurantoin by disk diffusion method**.**

Table 1 Overall resistance pattern of Enterococci by disk diffusion method

Antibiotics	Sensitive	Intermediate	Resistant
Ampicillin	84 (57.14%)	3 (2.04%)	60 (40.82%)
High Level Streptomycin	110 (74.83%)	0 (0.00%)	37 (25.17%)
Ciprofloxacin	42 (28.57%)	6 (4.08%)	99 (67.35%)
Teicoplanin	126 (85.71%)	0 (0.00%)	21 (14.29%)
Amikacin	27 (18.37%)	0 (0.00%)	120 (81.63%)
Nitrofurantoin	111 (75.51%)	12 (8.16%)	24 (16.33%)
High Level Gentamicin	71 (48.30%)	0 (0.00%)	76 (51.70%)
Kanamycin	24(16.33%)	0 (0.00%)	123(83.67%)

Out of 120 E.faecalis isolates, 39(32.50%) were resistant to Ampicillin, 57(47.50%) to High Level Gentamicin, 18(15.00%) to Nitrofurantoin, 102(85.00%) to Kanamycin by Kirby Bauer disk diffusion method.

Among the E.faecium isolates studied, 18(85.71%) were resistant to Ampicillin, 12(57.14%) to HLG, 6 (28.57%) to Nitrofurantoin and 18(85.71%) to Kanamycin Out of 6 E.avium isolates, 3 were resistant to Ampicillin and Kanamycin and 4 isolates to HLG. None of these isolates were resistant to Nitrofurantoin**. [Table 2]**

Table 2 Antibiotic resistant pattern of Enterococcus species by disk diffusion method

Antibiotics	E.faecalis (120)	E.faecium (21)	E.avium (6)
Ampicillin	39 (32.50%)	18 (85.71%)	3 (50.00%)
High Level Streptomycin	30 (25.00%)	3 (14.29%)	4 (66.67%)
Ciprofloxacin	78 (65.00%)	18 (85.71%)	3 (50.00%)
Teicoplanin	18 (15.00%)	3 (14.29%)	0 (0.00%)
Amikacin	99 (82.50%)	18 (85.71%)	3 (50.00%)
Nitrofurantoin	18 (15.00%)	6 (28.57%)	0 (0.00%)
High Level Gentamicin	57 (47.50%)	12 (57.14%)	4 (66.67%)
Kanamycin	102(85.00%)	18(85.71%)	3(50.00%)

By agar dilution 103 (70.07%) isolates showed high level gentamicin resistance with MIC ≥ 500µg/ml and 49(33.33%) were resistant to high level streptomycin (HLS) with MIC ≥2000µg/ml**.[Table 3]**

Table 3 Determination of HLGR among Enterococci by Agar dilution method

Isolate	HLGR		
	500 µg/ml	1000 µg/ml	2000 µg/ml
E.faecalis (120)	84(70.00%)	66(55.00%)	21(17.50%)
E.faecium (21)	15(71.43%)	15(71.43%)	9(42.86%)
E.avium (6)	4(66.67%)	4(66.67%)	3(50.00%)
Total (147)	103(70.07%)	85(57.82%)	33(22.45%)

HLGR: High Level Gentamicin Resistance

By E test 66 (55.00%) E.faecalis isolates were resistant to High level Gentamicin (≥1024 µg/ml), HLGR was found to be 71.43% and 66.67 % among E.faecium and E.avium isolates respectively.

DISCUSSION

Eventhough Enterococci are not regarded as highly pathogenic organisms, they are most commonly encountered in nosocomial bacteremia, endocarditis and urinary tract infection.[1] Enterococci with high level resistance to gentamicin are increasing in prevalence and are generally resistant to all aminoglycosides including amikacin, kanamycin, netilmycin and tobramycin with the occasional

exception of streptomycin.[5] Kanamycin disc proves to be an accurate and reliable substitute to predict Amikacin-Penicillin synergy than does Amikacin. [4]

As routine disc diffusion does not detect High Level Aminoglycoside resistance among enterococci, MIC determination is definitive for high level resistance and resistance to synergism. Hence alternative methods such as agar screening, high content disc and broth dilution method have been proposed to detect HLAR among these strains. [2] In the present study, out of 147 isolates from urine sample, 81.63% were Enterococcus faecalis, 14.29% were Enterococcus faecium and 4.08% were Enterococcus avium. A study conducted by Chandrim *et al* [10] have reported an isolation rate of 42.86 % E.faecalis, 36.51% E.faecium and 19.05 % E.avium from urine sample. Out of these, 34 % E.faecalis, 29% E.faecium and 20% E.avium were resistant to High Level Gentamicin by disk diffusion method.

In another Indian study Revati *et al* [11] have shown 56.86% E.faecalis and 33.33% E.faecium were isolated from urine. In contrast to present study, Maradia *et al* [12] have reported E.faecium (67.95%) as the common species isolated from urine, followed by E.faecalis (32.05%)

Among the Enterococci isolates studied, 60(40.82%) were found to be resistant to Ampicillin, 76(51.70%) to High Level Gentamicin (HLG), 99 (67.35%) to Ciprofloxacin, 24(16.33%) to Nitrofurantoin by disk diffusion method.In a study among urinary isolates, Maradia *et al* [12] have reported 91.67% Enterococci resistant to Ampicillin, 29.49% to HLG and 76.28% resistant to Ciprofloxacin. HLGR(32%) was reported in a study among clinical isolates of Enterococci by Archana *et al*. [13] A study among clinical isolates of Enterococci, by Bhatt *et al* [14] 53% isolates were with high level resistance to gentamicin and 38% showed high level resistance to streptomycin by disc diffusion method.

In the present study, out of 120 E.faecalis isolates, 39(32.50%) were resistant to Ampicillin, 57(47.50%) to High Level Gentamicin and 30(25.00%) to High Level Streptomycin by Kirby Bauer disk diffusion method. Among the E.faecium isolates studied, 18(85.71%) were resistant to Ampicillin, 12(57.14%) to HLG and 3(14.29%) to HLS. A high percentage of High Level Aminoglycoside Resistance (HLAR) was found in E.faecium than in E.faecalis. In a study among urine sample by Amit *et al* [15], 33 % of E. faecalis were HLGR and 31 % were HLSR, where as 96.67 % of E.faecium were reported to be HLGR and 90% as HLSR. A similar finding was also reported by N.Ganguide *et al*. [8] A study by Seema *et al* [16] have shown high level gentamicin resistance more common among enterococci isolated from urine sample (41.50%) compared to other clinical samples.In another study, Sivasankari *et al* [17] have reported 48.70% E.faecalis and 54.60 % E.faecium isolates resistant to High level gentamicin.

A study by Mendritta *et al* [18] have reported high level aminoglycoside resistance significantly higher among Enterococcus faecium than in E.faecalis with 46% isolates showed high level resistance to gentamicin and streptomycin by agar dilution method. In our study 103 (70.07%) isolates showed high level gentamicin resistance with MIC ≥ 500µg/ml and 49(33.33%) were resistant to high level streptomycin with MIC ≥2000µg/ml.

By E test 66 (55.00%) E.faecalis isolates were resistant to High level Gentamicin (1024 µg/ml), HLGR was found to be 71.43% and 66.67 % among E.faecium and E.avium isolates respectively. There was correlation between MIC for Gentamicin produced by agar dilution method and E-test. In the present study, 55 % E.faecalis isolates and 71.43% E.faecium were with a Minimum Inhibitory Concentration of 1024µg/ml for Gentamicin by E test. In a similar study K.Suresh *et al* [19] reported 48.27 % E.faecalis and 6.90% E.faecium had MIC >512 µg to gentamicin by E test.

In a study among urinary isolates of Enterococci, Jyothi *et al* [20] showed 49% of isolates with high level resistance to gentamicin by Enz MIC strip method. In a South Indian study by Elango Padmini *et al* [21] among clinical isolates of enterococci 42.7% were HLGR (MIC≥512µg/ml) by E test. In a study from North India by Jain *et al* [22] have reported high prevalence of HLAR among enterococci isolates from patients with bacteremia. 54% of the isolates were found to be HLAR and disc diffusion and agar screen method results for HLAR were concordant.

Enterococci are becoming increasingly resistant to traditional antimicrobial therapy. To treat complicated enterococcal infection, a bactericidal combination of penicillin (ampicillin or penicillin G with aminoglycoside (gentamicin or streptomycin) is required.However enterococcal resistance to high level aminoglycoside, ampicillin and vancomycin has resulted in limited therapeutic options. No treatment regime currently available is likely to produce reliable bactericidal effect on Enterococci with high level resistance to both gentamicin and streptomycin.

CONCLUSION

High Level Aminoglycoside Resistance (Gentamicin ≥500µg/ml) among Enterococci were found to be 70.07 % in our study. The percentage resistance to high level aminoglycosides were higher among Enterococcus faecium than in Enterococcus faecalis. Results for HLAR detection by agar screen method and E test were concordant.Hence screening for enterococci for HLAR should be carried out by high content disk and agar screen method in clinical settings.

References

1. Barbara E.Murray 1990. The Life and Times of the Enterococcus. *Clinical Microbiology Review*, 3(1):46-65
2. David J. Herman, Dalen. Gerding 1991. Antimicrobial Resistance among Enterococci Antimicrobial agents and Chemotherapy, 35(1) :1-4
3. A.H. C. Uttley, R. C. George, J. Naidoo, N. Woodford,A. P. Johnson, C. H. Collins, D. Morrison, A. J. Gilfillan L. E. Fitch, J.Heptonstall 1989 . High-level vancomycin-resistant enterococci causing hospital Infections. *Epidemiology and Infection,* 103(1).:173-181
4. Joseph.W.Chow. Aminoglycoside resistance in Enterococci 2000. *Clinical Infectious Diseases*, 31:586-9
5. Seema Sood, Meenakshi Malhotra, B K Das, Arti Kapil 2008. Enterococcal infections and antimicrobial resistance. *Indian Journal of Medical Research*, 128(2):111-121

6. R.R.Facklam and M.D Collins 1989.Identification of Enterococcus species isolated from human infections by a conventional test scheme. *Journal of Clinical microbiology*, 27(4):731-745.
7. Clinical and Laboratory Standard Institute (CLSI). Performance standards for antimicrobial susceptibility testing.18th ed.Wayne USA .CLSI, 2008.
8. Nita Gangurde, Manisha Mane, Sunita Phatale 2014.Prevalence of Multidrug Resistant Enterococci in a Tertiary Care Hospital in India :A Growing Threat. *Open Journal of Microbiology*, 4(1):11-15
9. Martha .L. Sanchez, Mary S Barrett, Ronald N Jones 1992 .Use of E test to Predict High Level Resistance to Aminoglycosides Among Enterococci. *Journal of Clinical Microbiology*, 30(11):3030-3032
10. Chandrim Sengupta, Anusha Venkatesan, Sangamitra.V, Subha .S, Radha Madhavan 2015. High Level Gentamicin Resistance among Enterococcus species Isolated from Clinical Samples in a Tertiary care Hospital. *International Journal of Medical and Health Sciences*,4(4):437-440
11. Revati Sharma, Chitra Pai 2013. Prevalence of Various Enterococcal infections and Its Antibiotic susceptibility with special reference to vancomycin and High Level Gentamicin resistance in a tertiary care centre in Navi Mumbai .International. *Journal Of .Pharmaceutical .Science* .Review and Research, 23(2):122-125
12. Mitali R Maradia, Kanksha Mehta, Khyati Prajapati, Minesh Vadsmiya, Pranay Shah, Mahendra Vegad 2017. Prevalence of multidrug –resistant Enterococcus species isolated from urine samples in a tertiary care hospital, Western India. *International Journal of Medical Science and Public Health*, 6(4):1-5
13. Archana Rao .K, Deepa S, Venkatesha D 2014. Enterococci –A journey of a successful pathogen. *International Archives of Integrated Medicine*, 1(4):49-57.
14. Bhatt P, Shete V, Sahni A K, Grover N, Chaudhari C N, Dudhat V L, Pandit P 2014. Prevalence of High level Aminoglycoside resistance in Enterococci at a Tertiary Care Centre. *International Journal of Recent Scientific Research*,5 (8) : 1515-1517
15. Amit Singh, Balram Ji Omar, R .C.Pande, Ramkant Yadhav, Amita Dohrae 2013. Speciation and Antimicrobial susceptibility pattern of Enterococcui from a rural tertiary health care center-A two year study. *International Journal Of Science and Technology*,3(1):1-9
16. Seema Mittal, Pooja Singla, Antariksha Deep, Kiran Bala, Rama Sikka, Meenu Garg, and Uma Chaudhary 2016. Vancomycin and High Level Aminoglycoside Resistance in Enterococcus spp in a Tertiary Health Care Centre: A Therapeutic Concern. *Journal of Pathogens*, 1-5.
17. Sivasankari S, Somasunder V.M, Senthamarai S, Anitha C, Kumudhavathi M.S, Suneel Kumar Kumar Reddy A, Amshavathani S K, Venugopal 2013. Detection of High Level Aminoglycosides Resistant Enterococci In A Tertiary Care Hospital. *International Organisation of Scientific Research Journal Of Pharmacy and Biological Sciences* ,8(5) :53-57
18. DK Mendritta, H Kaur, V Deotale, DC Thamke, R Narang, P Narang 2008. Status of High level Aminoglycoside resistant Enterococcus faecium and Enterococcus faecalis in a rural hospital of Central India. *Indian Journal of Medical Microbiology*, 26(4):369-71
19. K.Suresh, B.Saipriya, G. Viswanath 2013. Isolation, Speciation and Determination of High level Aminoglycoside resistance of Enterococci among hospitalised patients in Davangere. *National Journal of Laboratory Medicin*e, 2(1):12-15
20. Jyothi P, Metri B C, Peerapur B V 2014. High Level Resistance to Aminoglycosides in Urinary Isolates of Enterococci. *Annals of Medical and Health Sciences Research* ,4 (1):58
21. Elango Padmasini, R. Padmaraj, and S. Srivani Ramesh 2014. High Level Aminoglycoside Resistance and Distribution of Aminoglycoside Resistant Genes among Clinical Isolates of *Enterococcus* Species in Chennai, India. *The ScientificWorld Journal* ,1-5
22. Sarika Jain, Ashwani Kumar, Bineeta Kashyap, Iqbal R Kaur 2011. Clinico-epidemiological profile and high-level aminoglycoside resistance in enterococcal septicemia from a tertiary care hospital in east Delhi. *International Journal of Applied and Basic Medical Research*,1(2): 80-83

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