Valley International Journals
Open Access Journal
International Journal Of Medical Science And Clinical Inventions
Volume 2 issue 10 2015 page no. 1362-1365 e-ISSN: 2348-991X p-ISSN: 2454-9576
Available Online At: http://valleyinternational.net/index.php/our-jou/ijmsci

Status Of High Level Gentamicin Resistant In An Enterococcus Species Isolated From RIMS, Hospital.

Damrolien Shan,[1] Kh Sulochana Devi,[2] Ksh Mamta Devi,[3] Gracy Laldintluangi,[4] Arup Roy,[4] Ellim Marak.[4]

1.Demonstrator, Microbiology Department, RIMS, Imphal.
2.Professor and Head of Microbiology Department, RIMS, Imphal.
3. Associate Professor, Microbiology Department, RIMS, Imphal.
4. PGT, Microbiology Department, RIMS, Imphal.

Corresponding Author: Dr. Shan Damrolien
Demonstrator, Microbiology Department, RIMS, Imphal Manipur, 795004
E-mail: damrolienshan@gmail.com

Abstract:

Background: Infections by enterococci have traditionally been treated by cell wall active agents in combination with an aminoglycoside. However emergence of high level aminoglycoside resistant (HLAR), beta lactam antibiotics and vancomycin resistant by some strains of enterococci led to failure of synergistic affects of combination therapy. The present study was undertaken to find out HLAR in RIMS, Hospital due to the fact that enterococci are causing 2nd leading cause of nosocomial infection and 3rd leading cause of bacteremia.

Methods:

Enterococci recovered from various specimens of RIMS, Hospital Imphal between November 2013 to May 2015 were identified and speciated by test scheme proposed by Facklam and Collin.

Antibiotic susceptibility testing was done by Kirby bauer disc diffusion method as per CLSI

Screening for high level gentamicin resistant(HLGR) was done by disc diffusion containing 120 mμ gentamicin and minimum inhibitory concentration of HLG was determined by E-test strip procured from Hi-media. E.faecalis ATCC 29212 were used for quality control strains.

Results: Of 5300 clinical samples, 54 isolates were identified as enterococcal strains of which 33 were E.faecalis, 18 were E.faecium and 3 were E.gallinarum. The E.faecalis strains shows resistant high level gentamicin 5/33 (15.15%). The E.faecium strains are resistant to high level gentamicin 12/18 (66.66%) and all the E.gallinarum strains are resistant high level gentamicin Minimum inhibitory concentration for high level gentamicin among the 54 enterococcal isolates were carried out, among them 33 isolates showed MIC of ≤ 4 μg/ml and 21 isolates showed MIC of >1024 μg/ml.

Key words: HLGR Enterococcus E-test.

I. INTRODUCTION

The name "enterocoque" was first used by Thiercelin in a paper from France published in 1899. In early 1930's, Enterococci were classified as group D Streptococcus based on the demonstration of Group D antigen but enterococci were differentiated from non-enterococcal group D Streptococci by distinctive biochemical characteristic.[1]

In late 1930's, the enterococci was significantly used for the streptococci that grows at 10ºC and 45ºC at pH 9.6 in presence of 6.5 % NaCl, survive at 60ºC for 30Minute and hydrolyse esculin.[2]

During the mid 1980's, studies involving fatty acid composition, nucleic acid hybridization and comparative oligonucleotide cataloguing of 16S RNA led to the acceptance that enterococci were significantly different from other streptococci to merit their own genus.[3]

Enterococci were traditionally regarded as a low grade pathogen but have emerged as an increasingly important cause of nosocomial infection. Prior to 1990's enterococci have been recognized as an important cause of bacterial endocarditis.[4] Infections by enterococci have traditionally been treated by cell wall active agents in combination with an aminoglycoside. However emergence of high level aminoglycoside (HLAR), beta lactam antibiotics and vancomycin by some strains of enterococci led to failure of synergistic affects of combination therapy.[1,5] The present study was undertaken to find out HLAR in RIMS, hospital due to the fact that enterococci are causing 2nd leading cause of nosocomial infection and 3rd leading cause of bacteremia.

DOI: 10.18535/ijmsci/v2i10.01

2. AIMS AND OBJECTS

i. To speciate the enterococcal isolates of RIMS, Hospital.
ii. To study the status of high level gentamicin resistant in RIMS, Hospital.

3. Material and Methods

A total of 5300 clinical samples were collected in appropriate sterile containers from the out patients as well as in patients and transported to the Microbiology Laboratory, RIMS from November 2013 to May 2015.

The samples were plated on to Mac Conkeys Agar and Blood Agar and incubated at 37ºC overnight and if no growth is observed, the plates will be further incubated for next 24 hours. Direct gram stain was done for the clinical samples like pus, swab, sputum.

Genus level identification of the Enterococcus is based on the growth character in Mac Conkey Agar, Blood Agar, CLED agar, Gram staining and Catalase reactions. Further genus level identification was done by Growth in 6.5% NaCl, Heat tolerance Test at 60ºC for 30 minutes, Bile aesculin hydrolysis test and PYR Test .

Identification of Isolates upto the species level was done by standard biochemical test according to the conventional scheme of Facklam and Collins [6] and Manual for Identification of Medical bacteria, Cowan and Steel's 3rd Edition.[7]

Carbohydrate fermentation tests was performed in the Brain Heart Infusion Broth base with 1% mannitol, sorbitol, inulin, arabinose, sucrose, raffinose and trehalose. L-Arginine dehydrolase and Motility testing was also be done for species level identification. Antibiotic susceptibility test of the enterococcal isolates along with the quality control strain was done by Kirby Bauer's disc diffusion method either in Muller Hinton Agar in according to the latest CLSI guideline.[8] (Figure 1)

The following antibiotic disc were used – penicillin (10units), gentamicin high level(120µg), ciprofloxacin (5µg), nitrofurantoin for urine (300µg), linezolid (30µg) and vancomycin (30µg).

Screening for high-level aminoglycoside resistance and vancomycin resistance in enterococcus species was done by using 120mg HLG and the MIC of high level gentamicin was determined by E test in Mueller Hinton agar.(Figure 2)

Fig 1:Antibiotic susceptibility testing

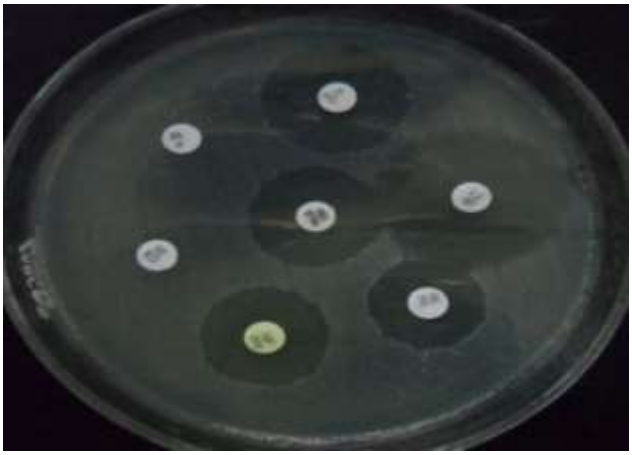


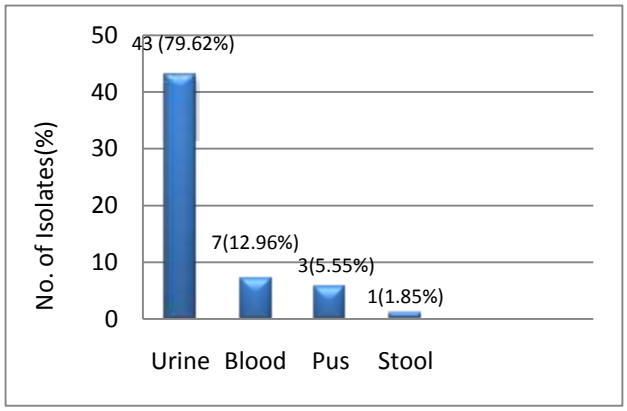
Fig 2: MIC determination by E-test

4. Results

In this studies 54 isolates were identified as Enterococcus strains from clinical samples of urine 43/54 (79.7%), blood 7/54(12.9 %), Pus 3/54(5.5%) and stool 1/54(1.8%). (Figure 3)

The species identities of the enterococcal isolates include *E. faecalis* 33/54 (61.12%), *E. faecium* 18/54(33.33%) & *E.gallinarum* 3/54(5.55%). (Figure 4)

Figure3: Clinical sample from which enterococci were isolates.



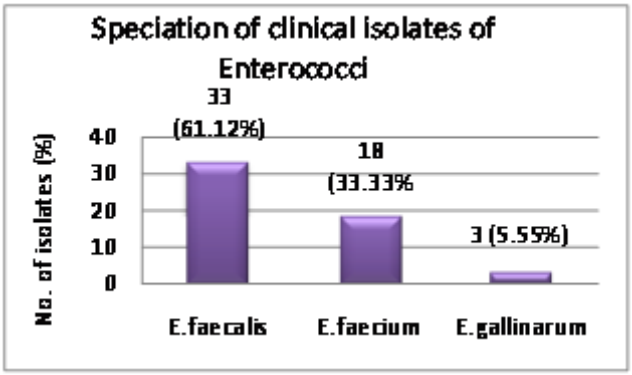


Figure 4: Speciation of enterococcal isolates.

Antimicrobial susceptibility testing

Table 1 shows the results antimicrobial resistance profile of enterococcal isolates. Of the 54 isolates, penicillin resistance were 50/54 (92.59%), ciprofloxacin 44/54(81.48%) and high level gentamicin resistance were 21/54(38.88%). Vancomycin and linezolid were found sensitive to all the isolates. Five isolates of *E.faecalis* strains shows resistant to high level gentamicin 5/33(15.15%), 12/18 (66.66%) isolates *E.faecium* were resistant to high level gentamicin and all *E.gallinarum* were high level gentamicin resistance.

Table 1: antimicrobial resistance profile of enterococcal isolates.

Antibiotic	Total no. of isolates (n=54)		E. faecalis (33)		E. faecium (18)		E.gallinarum (3)	
	R	%	R	%	R	%	R	%
Penicillin	50	92.59	29	87.87	18	100	3	100
Ciprofloxacin	44	81.48	24	72.72	17	94.44	3	100
High level gentamicin	21	38.80	5	15.15	12	66.66	3	100
Linezolid	0	0	0	0	0	0	0	0
Vancomycin	0	0	0	0	0	0	0	0

Determination of MIC's by E-test. (Table 2)

Minimum inhibitory concentration for high level gentamicin among the 54 enterococcal isolates were carried out, among them 33 isolates showed MIC of ≤ 4 µg/ml and 21 isolates showed MIC of >1024 µg/ml. Twenty seven isolates of *E.faecalis* showed MIC of ≤ 4 µg/ml and 6 isolates were MIC of >1024 µg/ml. Twelve isolates of *E.faecium* showed MIC of >1024 µg/ml and 6 isolates showed MIC of ≤ 4 µg/ml and all the 3 isolates of *E.gallinarum* were MIC of >1024 µg/ml.

Table 2: MIC determination by E-tests

Species	*E. faecalis* (n=33)	*E. facium* (n=18)	*E. gallinarum* (n-3)
MIC in µg/ml	No. of isolates	No. of isolates	No. of isolates
≤4	27	6	0
>1024	6	12	3

5. Discussion

Enterococcal infection is one of the important emerging nosocomial pathogens because of their widespread resistance to commonly used antibiotics. In this study, we determined the species distribution and antimicrobial susceptibility profile of clinical enterococcal isolates in RIMS Hospital, Imphal.

In this study, *E.faecalis* (48.4%) were the predominant isolates followed by *E.faecium* (40.7%) and *E.gallinarum* (11.7%). Traditionally, E.faecalis outnumber the E.faecium in the ratio of 10:1. but some workers reported a change in the ratio of *E.faecalis* to *E.faecium* from 3.7:1 in 1996 to 1.9:1 in 1999 in tertiary care hospital.[9] The ratio observed in this study is 1.8:1 thus consistent with the report of the other studies. The emergence of *E.faecium* infection is probably due to greater survival advantage and antimicrobial resistant than those of *E. faecalis*.

In our study, the antibiogram of the enterococcal isolates by disc diffusion method shows penicillin and ciprofloxacin resistance was 92.60% and 81.40 % and 100%. Vancomycin and linezolid were sensitive to all the enterococci isolates which is consistent with the reported by other Indian studies.[10] In this study, 38.88 % of enterococcal isolates shows high level gentamicin resistance in which *E. gallinarum* showed 100% HLGR, *E. faecium* 66.66% and *E.faecalis* 15.15% respectively, which is comparable with the study of other Indian studies in tertiary care teaching hospital. [11]

Resistance to penicillin is usually intrinsic and is primarily due to low penicillin binding protein and it result in loss of synergistic effect between β lactam and aminoglycosides leading to the treatment failures.

6. Conclusion

Though enterococci have long been recognized as low virulence bacteria occurring as commensal in human intestine, recently their role in causing infection is better defined.

In this study, enterococci cause urinary tract infection, bacteremia, wound infection and acute gastroenteritis with multiple drugs resistance due to exposure to broad spectrum antibiotic. Multidrugs resistance are more observe among the E.faecium and E.gallinarum. Thus it is important to speciate the enterococci isolates. The ratio of E.faecalis to E.faecium is decreasing as reported in the literature which is evident in our study.

Antibiotic resistance in Enterococcus is either intrinsic or acquired. Intrinsic traits expressed by enterococci include resistance to semisynthetic penicillinase resistant penicillins, cephalosporins, low level aminoglycosides and

low level clindamycin. Whereas acquired resistance includes resistance to chloramphenicol, erythromycin, high level of clindamycin, tetracycline, high level of aminoglycosides, penicillin, flouroquinolone and vancomycin. HLGR is due to release of various aminoglycoside modifying enzyme.

Routine antibiotic sensitivity testing by disc diffusion method may not able to identify many of the strains with intermediate sensitivity to high level gentamicin which can result in the therapeutic failure. However MIC determination by E-test to high level gentamicin are useful alternative for early and accurate identification of HLGR. Thus it is important to co-relate the results of disc diffusion with that of E-test.

7. References:

1. Murray BE. The life and times of the enterococcus. Clin Microbiol Rev 1990;3:45-65.
2. Facklam RR, Teixeira LM. Enterococcus. In: Lollier L, Balows A, Sussman M, editors. Topley & Wilson's microbiology and microbial infections. 9th ed. New York: Oxford University Press; 1998; p. 669-82.
3. Facklam RR. Recognition of Group D Streptococcal Species of Human Origin by Biochemical and Physiological Tests. Applied microbiology, June 1972;2:1131-39.
4. Sherman JM. The streptococci. Bacteriol Rev 1937;1: 3-97.
5. Patterson JE, Zervos M. High level gentamicin resistant in Enterococcus: Microbiology basis and epidemiology. Rev Infect Dis 1990;644-51.
6. Facklam RR, Collins MD. Identification of Enterococcus Species Isolated from Human Infections by a Conventional Test Scheme. Journal of Clinical Microbiology, April 1989;27:731-34.
7. Schleifer & Kilpper-Balz. Streptococcus. In:GI Barrow, RA Feltham, editors. Cowan and Steel's Manual for Identification of Medical bacteria, 3rd Edition.Great Britain:Cambridge University press;1993.p.59-67.
8. Clinical and Laboratory Standard Institute. Perfomance standards for antimicrobial susceptibility testing, Wayne,PA.17 the informational supplement:January 2007; M100-S17.
9. Mohanty S, Jose S, Singhal R, Sood S, Dhawan B, Das BK, et al. Species prevalence and antimicrobial susceptibility of enterococci isolated in a tertiary care hospital of north India. Southeast Asian J Trop Med Public Health 2005;36:962-5.
10. Jada SK, Karthika JK.Prevalence of Enterococcus species from various clinical specimens in Shri Sathya Sai Medical College and Research Institute with special reference to speciation and their resistance to vancomycin. Int J Med Clin Res 2012;3(4):154-160.
11. Mendiratta DK, Kaur H, Deotale V, Thamke DC, Narang R, Narang P. Status of High level Aminoglycoside Resistance Enterococcus faecium and Enterococcus faecalis in rural hospital of central India. Indian J Med Microbiol 2008;26(4):369-7.