Original Article

DOI: NJLM/2013/5240:0005

Microbiology Section

Isolation, Speciation and Determination of High Level Aminoglycoside Resistance of *Enterococci* Among Hospitalised Patients in Davangere

K. SURESH, B. SAIPRIYA, G. VISWANATH

ABSTRACT

Background: *Enterococcus*, considered a normal commensal of intestinal tract is one of the fast emerging pathogen causing serious and life threatening hospital borne infections. This is attributed to acquisition of multi-drug resistance.

Aims and Objectives: The present study was undertaken to isolate and characterize *Enterococci* from clinical specimens and determine the anti-microbial susceptibility pattern of these isolates.

Methods: A total of 58 *Enterococcal* isolates from various clinical samples were speciated as per the scheme of Facklam and Collins. Anti-bacterial susceptibility pattern was determined by Kirby Bauer disc diffusion method with recommended drugs including high level aminoglycoside resistance. MIC for Vancomycin and Gentamicin was determined by E – test method.

Results: Of the total 58 *Enterococcal* isolates, 52 were *E. faecalis* and 6 *E. faecium*. Antibiotic susceptibility tests showed high level resistance to penicillin, Ampicillin, Gentamicin and Streptomycin. All strains were sensitive to Vancomycin, Linezolid, Teicoplanin, *E. faecium* was more resistant than *E. faecalis* to the tested antibiotics. MIC for vancomycin was in range 1-4µg/ml.

Conclusions: We conclude that *Enterococcal* strains with high rate of resistance to penicillin and aminoglycosides are prevalent in our nosocomial setting. Therefore, there is an urgent need for more rational and restricted use of antimicrobials in order to minimize the selection and spread of such strains.

Key Words: *Enterococci*, High level aminoglycoside resistance (HLAR), MIC

INTRODUCTION

Enterococci, an indigenous flora of the intestinal tract, oral cavity and the genito urinary tract of the humans and animals, are known to be relatively avirulent in healthy individuals, but have become important opportunistic pathogens, especially in hospitalized patients [1].

Recent years have witnessed increased interest in *Enterococci* not only because of their ability to cause serious infections like endocarditis, bacteremia, intra-abdominal and urinary tract infection, but also because of their increasing resistance to many anti-microbial agents [2]. Infections by *Enterococci* have traditionally been treated with cell wall active agents in combination with an aminoglycoside, however emergence of high level resistance to aminoglycosides, β lactam antibiotics and to vancomycin by some strains has led to the failure of synergistic effects of combination therapy [3, 4].

Although 23 species in genus *Enterococcus* have been recognized, most common species are *E. faecalis* followed by *E. faecium*. *E. faecium* is more resistant species than *E. faecalis* and emergence of vancomycin resistance in it has caused an increase in frequency of its isolations [5].

Nevertheless, the incidence of other species of *Enterococci* is underestimated because of frequent misidentification [6]. Hence proper identification to species level is essential for proper management and prevention of this bacterial infection in any health care institution.

Hence the present study was conducted to know the species prevalence and the high level aminoglycoside resistance of Enterococcal isolates.

MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology from June 2011 – June 2012. Various clinical specimens such as urine, pus, abdominal drain fluid and blood were processed for the isolation of *Enterococci*. The specimens were also plated on blood agar and Mac Conkey's agar for the isolation of concomitant organisms along with *Enterococci*.

Enterococci were identified on the basis of characteristic colony morphology, gram staining and catalase test, and confirmed by Bile – esculin hydrolysis, salt – tolerance, heat tolerance tests. Enterococcal strains were further identified to the species level by using the conventional physiological tests devised by Facklam and Collins [7].

Anti-microbial susceptibility testing was done by Kirby – Bauer disc diffusion method as per the recommendations of CLSI [8]. The MIC of vancomycin was determined by E-test. The source of media and antibiotic discs were Hi-media ltd. Standard strains *E. faecalis* ATCC 29212 was used as control.

RESULTS

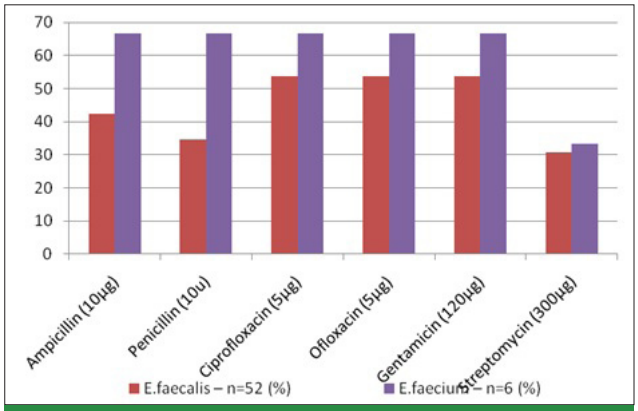
Result of the 250 samples tested, comprising urine – 120, pus – 75, body fluids – 30 and blood – 25, a total of 58 enterococcal isolates 52 –*E. faecalis* (89.6%) and 6 – *E. faecium* (10.4%) were obtained [Table/Fig-1]. 50 isolates were pure culture of *Enterococci*, remaining 8 isolates were associated with E.coli, Proteus, Pseudomonas and Staphylococcus aureus.

Entercoccus Species Isolated	No of Isolates	Percentage (%)
E.faecalis	52	89.6
E.faecium	6	10.4
Total	58	100

[Table/Fig-1]: Total number of Enterococcus species isolated

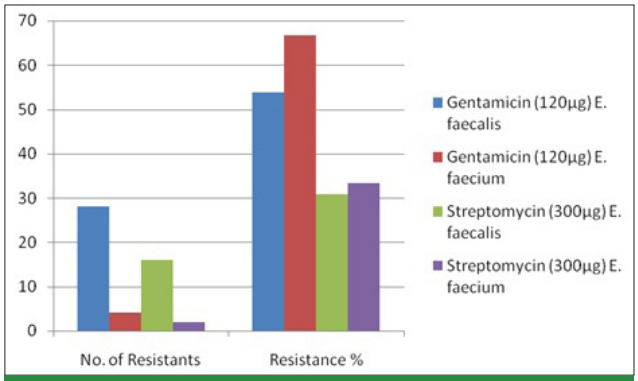
The majority of isolates were from urine 36 (62%), *E. faecalis* 34, *E. faecium* 2, 6 from non surgical wounds (10.3%), 8 from surgical wound (13.8%), 6 from blood (10.3%) and 2 from body fluids (3.4%).

Both *E. faecalis* and *E. faecium* exhibited 100% sensitivity for Vancomycin, Linezolid, Teicoplanin. Nitrofurantoin was used only for urinary isolates and was 100% sensitive. The resistence pattern of *E. faecalis*: Ampicillin (54%), Penicillin (35%), Ciprofloxacin (54%), Ofloxacilin (54%), Gentamicin (54%), Streptomycin (31%) [Table/Fig-2].



[Table/Fig-2]: Antibiotic resistance percent in Enterococci by Kirby – Bauer disk diffusion method

High level gentamicin disc (120µg) and high level streptomycin disc (300µg) were used for detection of high level aminoglycoside resistance in *Enterococci*. Among the 58 isolates, 28 strains of *E. faecalis* exhibited HLGR (53.8%) and 8 strains showed both HLGR and HLSR (15.3%) 20 strains showed only HLGR and was sensitive to streptomycin (38.4%). The remaining 24 isolates of *E. faecalis* were sensitive to gentamicin (46%). Among 6 isolates of *E. faecium* 4 strains exhibited HLGR (66.6%) [Table/Fig-3].



[Table/Fig-3]: High level aminoglycoside resistance pattern (HLAR)

High level streptomycin disc (300µg) detected totally 16 HLSR strains (30.76%), 8 strains were both HLGR and HLSR, 8 showed only HLSR (15.3%) among *E. faecalis* isolates. [Table/Fig-3] 2 isolates of *E. faecium* (33.3%) exhibited HLSR. MIC of Gentamicin was performed by E-test. The strip with MIC range (0.064-1024µg) was used. 28 isolates of *E. faecalis* and 4 isolates of *E. faecium* had MIC > 512µg (resistant). MIC of vancomycin determined by E-test, strips with MIC range (0.016-256µg) was used. All 58 isolates showed MIC < 4µg (sensitive).

DISCUSSION

Enterococci are commensals of the gastrointestinal tract of human beings. Over the past 2 decades they have become

important nosocomial pathogens probably due to inherent resistance to antibiotics (cephalosporins), ability to adhere to indwelling medical devices, and ability to survive adverse environmental conditions [9]. Correct speciation is very important since there is variation in resistance to antibiotics by particular enterococcal species [10].

In this study *E. faecalis* is the predominant species isolated (89.61%) followed by *E.faecium* (10.4%) in accordance with other studies of Gary Cotter et al., [11]; Simonson et al., [12], Mohammad Rahbar [13]. In contrary Baragundi Mahesh et al., [14] reported *E. faecium* as the predominant species (47.50%). Majority of the isolates were from urine, similar to other studies [15, 16].

This study showed *E. faecium* to be more resistant to ampicillin than *E. faecalis*. this correlates with the study by Jyotsna Agarwal et al., [17] and Steven Gordon et al., [18]. Penicillin resistance was more in *E.faecium* than *E.faecalis*. In contrast L.A Sechi et al., reported 60% of *E. faecalis* isolates resistant to penicillin [19].

This study showed 100% sensitivity to vancomycin, teicoplanin and Linezolid, similar to results observed in other studies [20,16]. High level gentamicin resistance was seen in both *E. faecalis* and *E.faecium*, consistent with other studies [21, 22]. In our study among *E. faecalis* and *E. faecium* isolates resistant pattern was more for gentamicin than streptomycin, which is similar to previous studies [23, 24]. *E. faecium* strains were observed to be more resistant to the tested antimicrobials in accordance to studies from India and outside [20, 21].

Even though there are studies suggestive of arising resistance pattern to vancomycin in *Enterococci* [25, 3] no such pattern was observed in our isolates and isolates have MIC range between 1-4µg/ml for vancomycin.

CONCLUSION

This study showed increasing high level aminoglycoside resistence in hospital setting.Therfore emphasising the rational and restricted use of antibiotics to minimise the selection and spread of such strains.

REFERENCES

[1] Murray BE. The life and times of *Enterococci*. *Clin Microbiol Rev.* 1990; 3: 46-65.

[2] Moellering RC. Jr Emergence of *Enterococci* as a significant pathogen. *Clin Infect Dis.* 1992; 14: 1173-78.

[3] Jesudason MV, Pratima VL, Pandian R,I Abigail S. Characterization of penicillin resistant *enterococci*. *Indian J Med Microbiol.* 1998; 16(1): 16-8.

[4] Patterson JE, Zervos M. High level gentamicin resistance in *Enterococci*us, microbiology, genetic basis and epidemiology. *Rev Infect Dis.* 1990; 12: 644-51.

[5] Rice LB. Emergence of Vancomycin resistant *Enterococci* Emerg Infect Dis 2001; 7: 183-87.

[6] Vittal P Prakash, Sambasiva R Rao, Subhash C Parija. Emergence of unusual species of *enterococci* causing infections, South India. *BMC Infect disease.* 2005 Mar; 5:14.

[7] Facklam RR, Collins MD. Identification of *enterococcus* species isolated from human infection by a conventional test scheme. *J Clin Microbiol.* 1989; 27: 731-34.

[8] Clinical and Laboratory standards Institute. Performance Standards for Antimicrobial susceptibility testing; 20th informational supplement. CLSI/NCCLS M100-S20. Wayne(PA). The Institute; 2010.

[9] Shabnam Qamer, Jonathan Sandoe, Kevin G Kerr. Use of colony morphology to distinguish different Enterococcal strains and species in mixed culture from clinical specimens. *Journ. of Clin Micro.* June 2003: p 2644-46.

[10] PJ Desai, D Pandit, M Mathur, A Gogate. Prevalence, identification and distribution of various species of *enterococci* isolated from clinical specimens with special reference to UTI in catheterized patients. *Indian journal of Medical Microbiology.* 2001; 19(3): 132-37.

[11] Gary Cotter and Catherine C Adley. Comparison and evaluation of antimicrobial susceptibility testing of *Enterococci* performed in accordance with six National Committee standardized disk diffusion procedures. *Journal of Clinic Micro.* Oct 2001; p.3753-56.

[12] G.S Simonson, L Smabrekkel, DL Monnet, TL Sorensen, JK Moller, et al. KG Kristensson et al. Prevalence of resistance to ampicillin, gentamicin and vancomycin in *Enterococcus* faecalis and E.facium isolates from clinical specimens and use of antimicrobials in five Nardic hospitals. *Journal of Antimicrobial chemotherapy.* (2003) 51, 323-31.

[13] Mohammed Rahbar, Massoud Hajia, Mohammad Farzaneh Khan. Activity of Nitrofurantoin against isolates of vancomycin – resistant *enterococci* (VRE): A three layer survey in an Iranian hospital. *Iran journal of patho.* Nov. 2007; vol.2(4).

[14] Baragundi Mahesh, Suresh B Sonth, Shivakumar S. Solabannavar, Chidanand S. Patil, Vishwanath, Yemul, Species prevalence and antimicrobial resistance pattern of enterocoocal isolates in a tertiary health care centre. *Journal of clinical and diagnostic research*. 2010 Dec; (4): 3405-09.

[15] Maria Bitsori, Sofia Maraki, Maria Raissaki, Anna Bakantaki, Emmannouil Galanakis. Community acquired enterococcal urinary tract infections. *Paed Nephro.* 2005; 20: 1583-86.

[16] Neelam Taneja, Phulan Rani, Rekha Emmanuel, Meera Sharma. Significance of vancomycin resistant *enterococci* from urinary specimens at a tertiary care centre in northern India. *Indian J Med Res.* 2004 Feb: pp. 72-74.

[17] Jyotsna Agarwal, Rajkumar Kalyan and Mastan Singh. High level aminoglycoside resistance and β lactamase production in *Enterococci* at a tertiary care hospital in India. *Jpn. J. Infect. Dis.* 2009; 62: 158-59.

[18] Steven Gordon, Jana M Swenson, Bertha C Hill, Nan E Pigott, RR Facklam. Anti-microbial susceptibility patterns of common and unusual species of *Enterococci* causing infections in the United States. Sep. 1992; vol. 30, no. 9: p. 2373-78.

[19] I Dupre, S Zanetti, AM Schito, G Fadda, LA Sechi. Incidence of cirulence determinants in clinical *enterococcus* faecium and *enterococcus* faecalis isolates collected in Sardina, *Journal Med Microbiol.* 2003; 52: 491-98.

[20] Srujana Mohanty, Swapna Jose, Ritu Singhal, Seema Sood, Benu Dhawan et al. Bimal K Das et al. species prevalence and antimicrobial susceptibility testing of clinical isolates of *Enterococcus* faecium and *Enterococcus* faecalis. *Journ of clin. Microbiology.* 1992; vol. 30: (1) p. 41-45.

[21] M Louie, AE Simor, S Szeto, M Patel, B Kreiswirth, D E Low.

Susceptibility testing of clinical isolates of *enterococcus* facium and *enterococcus* faecalis. *Journ of clin. Micorbiology.* 1992; vol.30; (1) p.41-45.

[22] K H Keddy, K P Klugman, L D Vebowitz. Incidence of high– level gentamicin resistance in *enterococci* at Johannesburg Hospital. *S Afr Med J.* 1996: 86: 1273-76.

[23] Agarwal VA, John VI, Pathak AA. Concomitant high level resistance to penicillin and aminoglycosides in *Enterococci* at Nagpur, Central India. *Indian J Med Microbiol.* 1999; 17(2): 85-87.

[24] Udo EE, Noura A, Phillips OA, Chugh TD. Species prevalence and antibacterial resistance of *Enterococci* isolates in Kuwait hospitals. *J Med Microbiol.* 2003; 52: 163-68.

[25] Bhat KG, Paul C, Bhat MG. High level aminoglycoside resistance in *Enterococci* isolated from hospitalized patients. *Indian J Med Res.* 1997 May; 105: 198-99.

AUTHOR(S):

1. Dr. K. Suresh
2. Dr. B. Saipriya
3. Dr. G. Viswanath

PARTICULARS OF CONTRIBUTORS:

1. MD Professor
2. MBBS Post Graduate
3. MD, Professor & HOD, Department of Microbiology, J.J.M. Medical College, Davangere, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. K. Suresh,
Professor, Department of Microbiology,
JJM Medical College, Davangere,
Karnataka-577004, India.
Ph: 09986048719
Email: dr.sureshk@rediffmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Oct 31, 2012**
Date of Peer Review: **Jan 26, 2013**
Date of Acceptance: **Feb 10, 2013**
Date of Publishing: **Apr 15, 2013**