Int J Pharm Bio Sci 2015 Jan; 6(1): (B) 579 – 584

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

CHANGING PARADIGM OF ENTEROCOCCAL INFECTIONS

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ABSTRACT

Enterococci are increasingly recognized as an important nosocomial pathogen. The present study was done to establish the changing paradigm of blood culture enterococcal isolates and emergence of *Enterococcus faecium* as a nosocomial pathogen. A total of 150 Enterococcal isolates were obtained during the study period from January 2013 to March 2014. 68.3% and 31.7% of adult and 53% and 43% of paediatric blood culture enterococcal isolates were *Enterococcus faecalis* and *Enterococcus faecium* respectively. Antimicrobial susceptibility testing by Kirby Bauer disc diffusion revealed vancomycin resistance of 7.2% and 1.8% for adult 15.3% and 26 % among the paediatric *E. faecalis* and *E. faecium* isolates respectively. The present study demonstrates the changing spectrum of enterococcal species causing bacteremia and increasing prevalence of vancomycin resistance among *E. faecium* isolates. This emphasises the need for speciation of various enterococcal isolates and knowledge of antimicrobial resistance profile is necessary for management of serious enterococcal infections.

KEY WORDS: **VRE – Vancomycin resistant enterococci, HLG- high level gentamicin, MDR- multidrug resistant, CLSI- clinical and laboratory standard institute, MIC- minimum inhibitory concentration,**

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INTRODUCTION

Enterococci are Gram positive cocci which occur mainly in pairs and short chains and they are part of the normal human microbiome mainly the gastrointestinal tract of humans and animals. The pathogenicity of these organisms remain controversial as they can be part of the normal human microbial flora[1–4]. The major infections caused by various *Enterococcus* species are infective endocarditis, deep seated intra abdominal infections, post surgery wound infections, urinary tract infections, septic arthritis, bacteraemia, septicaemia and less frequently respiratory tract infections, skin and soft tissue infections[5]. Now over the past few decades, these organisms have emerged as predominant Gram positive nosocomial pathogens and have gained importance worldwide because of their multi-drug resistant (MDR) phenotype. Among the various species under the genus Enterococcus, the predominant species causing infections are *Enterococcus faecalis* and *Enterococcus faecium* although their relative contributions in causing various infections vary and have changed over the past few years[6]. The other enterococcal species causing human infections include *E. avium, E. gallinarum, E. mundtii, E. durans* and *E. Casseliflavus* [7]. Historically, majority of the invasive infections were caused by *Enterococcus faecalis* followed by *Enterococcus faecium.* However, over the past few years, increasing number of *E.faecium* strains has been found in the hospitals globally[8,9]. This recent upsurge in *E.faecium* infections has dragged the attention because of the antimicrobial resistance pattern (resistant to ampicillin, vancomycin, aminoglycosides) and the various virulence traits exhibited by this species which would have contributed in the emergence of this pathogen as a successful nosocomial pathogen[1–4,10–13]. Infections associated with *E.faecium* are associated with significant morbidity and mortality and hence the management of the infections caused by this species remains a therapeutic challenge[14]. The present study was undertaken to demonstrate the changing spectrum of enterococcal species isolated from blood culture and to evaluate the antimicrobial resistance profile of the various enterococcal species.

STUDY DESIGN

Present study is a retrospective study conducted in the Department of Microbiology in a 1650 bedded tertiary care centre in South India. Records of the various Enterococcal species isolated from blood culture from January 2013 to March 2014 were retrospectively retrieved and analyzed.

BACTERIAL ISOLATES

All the blood samples sent to Microbiology laboratory for culture and sensitivity during the time period of January 2013 to March 2014, were cultured aerobically using conventional monophasic blood culture broth containing Brain Heart infusion broth (BHI) broth (Hi Media Laboratories Ltd, Mumbai, India). Sodium polyanethol sulphonate (SPS) was used in the concentration of 0.125mg/L as an anticoagulant to enhance the bacterial recovery. Inoculated blood culture bottles were incubated at 37^0 C for 7 consecutive days. Blind subculture of the broth was routinely done on day one, day two and finally on seventh completed day on 5% sheep blood agar and MacConkey agar. Identification of *E.faecalis* and *E.faecium* were done using the standard biochemical tests, including the ability of the organism to hydrolyze esculin in the presence of bile, fermentation of arabinose (fermented/not fermented-*E.faecium/E.faecalis)*, growth in the presence of 6.5% sodium chloride, lack of pigmentation and motility. All consecutive, non-duplicate isolates of Enterococcus species were included in the study. Antimicrobial susceptibility testing of the isolates to a panel of antibiotics including β-lactam group, high level aminoglycosides, tetracycine , vancomycin, teicoplanin and Linezolid was performed by Kirby- Bauer disc diffusion method as per the standard procedure given in CLSI January 2013 M02-A10 document. MIC for vancomycin was done for all the isolates by agar dilution method using Brain Heart Infusion containing 4 µg/ml of vancomycin. One or more colony or film of growth indicated resistance to vancomycin[15].

RESULTS

A total of 150 Enterococcal isolates were obtained out of which 101(67.3%) were from adult and 49 (32.68%) were from paediatric blood culture samples sent to microbiology lab for culture and sensitivity. Table 1 depicts ICU and Non-ICU distribution of adult and paediatric *E.faecalis* and *E. faecium* isolates from blood culture. The antimicrobial resistance profile of *E.faecalis* and *E.faecium* isolates by Kirby-Bauer disc diffusion method are summarised in the table 2. The disc diffusion results of vancomycin were simultaneously confirmed with vancomycin screen agar test containing Brain Heart Infusion (BHI) agar and 4µg/ml of vancomycin.

Table I
ICU and Non-ICU distribution of adult and paediatric Enterococcus faecalis and E. faecium isolates

ADULT (N=101)				PAEDIATRIC (N=49)			
Enterococcus faecalis (N= 69) 68.3%		*Enterococcus faecium* (N=32) 31.7%		*Enterococcus faecalis* (N=26) 53%		*Enterococcus faecium* (N=23) 46%	
I CU	NON-ICU	ICU	NON-ICU	ICU	NON-ICU	ICU	NON-ICU
19	50	14	18	17	9	15	8

Table II
Antimicrobial resistance profile of Enterococcus faecalis and Enterococcus faecium isolates by Kirby-Bauer disc diffusion method.

ADULT (n=101)	Va	A	T	HLG	Te	Lz
Enterococcus faecalis (69)	7.2%	50%	62%	68%	5.7%	0%
Enterococcus faecium (32)	1.8%	75%	75%	78%	15%	0%
PAEDIATRICS (n=49)						
Enterococcus faecalis (26)	15.3%	57%	53%	53.8%	7.6%	0%
Enterococcus faecium(23)	26%	95%	69.5%	82.6%	26%	0%

DISCUSSION

Traditionally Enterococcus species are considered as low grade pathogens since they lack few of the major virulence factors present in other bacteria. However, the incidence of infections caused by these enterococcal species is increasing and now they have been recognised as the one of the most common nosocomial pathogen worldwide. Among the various enterococcal species, the most commonly isolated human pathogen is *E.faecalis* followed by *E.faecium*. Earlier, it was a common practice to clump all the

enterococci together without any specific species identification. However, the antimicrobial resistance profile of *E.faecium* is clearly different from that of *E. faecalis* .In the present study we have found that the ratio of *E. faecalis* to *E. faecium* is 2:1 among the adult patients and 1:1 among the paediatric patients. This depicts a significant upsurge in the number of infections due to *E. faecium* where the traditional ratio is 1:9. The present study also demonstrates that the antimicrobial resistance profile of the *E.faecium* is clearly distinct from that of *E, faecalis.* In the present study, resistance of *E.faecium* was higher than *E.faecalis* isolates to all the antibiotics except for vancomycin resistance among the *E.faecalis* isolates from the adult blood culture samples. Karmarkar *et al* [16]also has shown higher resistance among the *E.faecium* isolates. In our study paediatric *E.faecium* isolates (26%) were showing higher resistance to vancomycin than the *E.faecalis* (15.3%) isolates. During the past few decades, *E.faecium* has gained much importance mainly because of the multi drug resistance profile shown by this organism. Vancomycin remains the drug of choice for those infections caused by resistant enterococci. With the first detection of Vancomycin resistant enterococci in 1986[1–3,17], the number of infections caused by vancomycin resistant enterococci is increasing worldwide. This calls for attention because Enterococci are notorious for their transfer of these antimicrobial resistance genes through plasmids to other Gram positive cocci[18,19]. This antimicrobial resistance and particularly to vancomycin is seen more among the *E.faecium* isolates. The percentage of vancomycin resistance is more among the paediatric *E.faecium* isolates (26%) compared to that of the *E.faecalis* isolates and the infections with *E.faecium* are often associated with increase in length of hospital stay, and overall morbidity and mortality. This is in accordance with other studies which show higher resistance to vancomycin among the *E.faecium* isolates[3,20]. The major reservoir of *Enterococcus faecium* is gastro intestinal tract of animals. Gastro intestinal colonisation of VRE is usually considered to precede infections with VRE[21,22]. Various predisposing factors were found to increase the proportion of VRE infections in our study population especially exposure to broad spectrum antibiotics which results in establishment of gastrointestinal colonisation with multidrug resistant organisms and the presence of indwelling devices and urinary catheters. VRE infections caused by *E.faecium* were more likely to be associated with the hospital acquired infections and all the laboratories should have effective detection methods for the detection of VRE for the effective implementation of control measures[23–25].

CONCLUSION

This study clearly demonstrates the increasing incidence of blood stream infections caused by *E.faecium* with an alarming increase in vancomycin resistance of 26 % among the paediatric *E.faecium* isolates and the need to develop active surveillance method to prevent the further spread of this multi drug resistant organism. To conclude, VRE bacteremia, especially by *E. faecium* is a major complication of prolonged hospitalization and treatment with inappropriate and broad spectrum/ extended spectrum antibiotics appears to be an important predisposing factor[11]. Hence judicial use of antibiotics has to be practiced and manipulation of the antimicrobial formulary like restriction of the use of antibiotics like clindamycin, cephalosporins and carbapenems and proper hand washing before handling the patients plays an important role preventing the spread of these multidrug resistant organisms[26].

LIMITATIONS

Molecular analysis of the isolates was not performed to assess the clonality of the strains which would have helped in finding out the dominant VRE clones in the hospital and to assess the inter hospital and intrahospital spread.

REFERENCES

1. Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. Clin. Microbiol. Rev. 13(4):686–707(2000);.
2. Moellering RC. Vancomycin-resistant enterococci. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.;26(5):1196–9(1998).
3. Zirakzadeh A, Patel R. Vancomycin-Resistant Enterococci: Colonization, Infection, Detection, and Treatment. Mayo Clin. Proc.81(4):529–36(2006).
4. Fernandes SC, Dhanashree B. Drug resistance & virulence determinants in clinical isolatesof Enterococcus species. Indian J. Med. Res. 137(5):981–5 (2013).
5. Murray BE. The life and times of the Enterococcus. Clin. Microbiol. Rev. 3(1):46–65(1990).
6. Mundy LM, Sahm DF, Gilmore M. Relationships between enterococcal virulence andmicrobial resistance. Clin. Microbiol. Rev. 13(4):513–22(2000)
7. De Perio MA, Yarnold PR, Warren J, Noskin GA. Risk factors and outcomes associated with non-Enterococcus faecalis, non-Enterococcus faecium enterococcal bacteremia. Infect. Control Hosp. Epidemiol. Off. J. Soc. Hosp. Epidemiol. Am. 27(1):28–33(2006)
8. Noskin GA, Peterson LR, Warren JR. Enterococcus faecium and Enterococcus faecalis Bacteremia: Acquisition and Outcome. Clin. Infect. Dis.20(2):296–301(1995).
9. Stosor V, Peterson LR, Postelnick M, Noskin GA. Enterococcus faecium bacteremia: Does vancomycin resistance make a difference? Arch. Intern. Med. 158(5):522–7(1998).
10. Praharaj I, Sujatha S, Parija SC. Phenotypic & genotypic characterization of vancomycin resistant Enterococcus isolates from clinical specimens. Indian J. Med. Res 138(4):549–56(2013).
11. Gangurde N, Mane M, Phatale S. Prevalence of Multidrug Resistant Enterococci in a Tertiary Care Hospital in India: A Growing Threat. Open J. Med. Microbiol. 04(01):11–5(2014).
12. Mendiratta D, Kaur H, Deotale V, Thamke D, Narang R, Narang P. Status of high level aminoglycoside resistant *Enterococcus faecium* and *Enterococcus faecalis* in a rural hospital of central India. Indian J. Med. Microbiol. 26(4):369(2008).
13. De A, Bindlish A, Kumar S, Mathur M. Vancomycin resistant enterococci in a tertiary care hospital in Mumbai. Indian J. Med. Microbiol. 27(4):375 (2009)
14. R N Garrison DEF. Enterococcal bacteremia: clinical implications and determinants of death. Ann. Surg. 196(1):43–7(1982).
15. Clinical and Laboratory Standards Institute (January 2013)-Performance standard for antimicrobial susceptibility testing 23rd informational supplement CLSI document M100-S23.
16. Karmarkar MG, Gershom ES, Mehta PR. Enterococcal infections with special reference to phenotypic characterization & drug resistance. Indian J. Med. Res. 119 Suppl:22–5 (2004).
17. Sood S, Malhotra M, Das BK, Kapil A. Enterococcal infections & antimicrobial resistance. Indian J. Med. Res. 128(2):111–21(2008;).
18. Ray AJ, Pultz NJ, Bhalla A, Aron DC, Donskey CJ. Coexistence of Vancomycin-Resistant Enterococci and Staphylococcus aureus in the Intestinal Tracts of Hospitalized Patients. Clin. Infect. Dis.;37(7):875–81(2003)
19. Bush K. Vancomycin-Resistant Staphylococcus aureus in the Clinic: Not Quite Armageddon. Clin. Infect. Dis. 38(8):1056–7 (2004).
20. Corso AC, Gagetti PS, Rodríguez MM, Melano RG, Ceriana PG, Faccone DF, et al. Molecular epidemiology of vancomycin-resistant Enterococcus faecium in Argentina. Int. J. Infect. Dis. 11(1):69–75(2007).
21. Vignaroli C, Zandri G, Aquilanti L, Pasquaroli S, Biavasco F. Multidrug-resistant enterococci in animal meat and faeces and co-transfer of resistance from an Enterococcus durans to a human Enterococcus faecium. Curr. Microbiol.;62(5):1438–47(2011).
22. Tremblay C-L, Letellier A, Quessy S, Boulianne M, Daignault D, Archambault M. Multiple-antibiotic resistance of Enterococcus faecalis and Enterococcus faecium from cecal contents in broiler chicken and turkey flocks slaughtered in Canada and plasmid colocalization of tetO and ermB genes. J. Food Prot. 74(10):1639–48 (2011).

23. Asha Peter, Shini Zacharia, Radhakrishnan.E.K, Jyothis Mathew. Antimicrobial resistance trends with special reference to vancomycin resistance among different species of enterococci. Int. J. Pf Pharma Biosci. 4(1):356–63 (2013).
24. Salem-Bekhit M, Muharram M, Hefni H, Moussa I, Alanazy F. Prevalence and antimicrobial resistance pattern of multidrug-resistant enterococci isolated from clinical specimens. Indian J. Med. Microbiol. 30(1):44 (2012);
25. Rajkumar Kalyan, Mastan Singh,Jyotsna Agarwal. Vancomycin resistant enterococci at a tertiary care hospital in northern india. Int. J. Pf pharma biosci. 4(2):1090–4(2013).
26. Recommendations for preventing the spread of vancomycin resistance. Recommendations of the Hospital Infection Control Practices Advisory Committee (HICPAC). MMWR Recomm. Rep. Morb. Mortal. Wkly. Rep. Recomm. Rep. Cent. Dis. Control 44(RR-12):1–13 (1995).