Original article:

Study on vancomycin-resistant enterococci in faecal samples from non-hospitalized individuals at MMIMSR, Haryana, India

Shyamsundar Bera[1], Dr. Sonia Mehta[2], Dr. Manisha Bhatt Dwivedi[3], Dr.Varsha A singh[4], Rajdeep Paul [5], SumiNongrum[6]

Abstract:

Background: Enterococci, formerly classified with fecal streptococci, have been recognized to be of fecal origin since the beginning of this century. ***Method:*** This study was undertaken to determine the prevalence of stool colonization with vancomycin resistant Enterococcus (VRE) and also to evaluate the risk factors for colonization with vancomycin resistant Enterococcus among non- Hospitalized individuals at MMIMSR, Mullana.Test was performed for VRE isolates collected over a period of 6 months (Oct2015- March 2016). Faecal samples were collected by using sterile container from non- hospitalized individuals then to Cultures using Mac Conkey and Blood agar. After presumptive diagnosis as an enterococcus spp, 50 Enterococcal isolates were then again cultured on special VRE screen agar media to identify vancomycin resistant Enterococcus. ***Result:*** The results were further supported by modified Kirby-bauer disk diffusion method with vancomycin (30µg) as per CLSI guideline. A total of 29 (58%) *Enterococcus faecalis* and 21 (42%) *Enterococcus faecium* were detected among the faecal isolates and 2 (4%) were VRE. According to CLSI guideline isolates showing diameter of zone of inhibition ≤16mm were considered among the VRE. Chronic diseases, previous hospital stay (more than 15 days) and repeatedly antibiotic consumption was found to be significant risk factor for non-hospitalized individuals. ***Conclusion:*** There is need for programs to promote greater attention about antibiotics usage in the general population. Education of Health care workers with implementation and observation of hand-washing practices constitutes a very effective step in preventing the spread Prolonged use of vancomycin drug should not be recommended by the physician.

Keywords: VRE; CLSI; Vancomycin; Nosocomial infection; VanA gene; VanB gene.

Bangladesh Journal of Medical Science Vol. 18 No. 02 April'19. Page : 334-339
DOI: https://doi.org/10.3329/bjms.v18i2.40705

Introduction

Enterococci, formerly classified with fecal streptococci, have been recognized to be of fecal origin since the beginning of this century. The usual ecological niche for *Enterococcus* species is the intestines of humans and other animals[1, 2]. In addition, *Enterococcus* and *Streptococcus* have been proposed as indicators of fecal contamination in water because of their high abundance in feces and their long survival in the environment. Although the ratio of fecal coliforms to fecal streptococci has been ruled out as an indicator [3], the identification of species associated with a given environment or host might provide additional information about the origin of isolates and the source of fecal contamination[4, 5]. Previously vancomycin was the reserved drug for the recent enterococcal infection but the appearance of vancomycin resistance strains has been increasing

1. Shyamsunder Bera-MSc., Medical Microbiology, Molecular Biologist, Kolkota Medical College, Kolkota, West Bengal, India.
2. Dr. Sonia Mehta-MBBS, MD, Professor, Department of Microbiology, MMIMSR, Mullana, Ambala, Haryana, India.
3. Dr. Manisha Bhatt Dwivedi – MBBS, MD, Professor and Head, Department of Anaesthesia, MMIMSR, Mullana, Ambala, Haryana, India.
4. Dr. Varsha A. Singh-MBBS, MD, Professor and Head, Department of Microbiology, MMIMSR, Mullana, Ambala, Haryana, India.
5. Rajdeep Paul-MSc., Medical Microbiology.
6. Sumi Nongrum-MSc., Medical Microbiology, Tutor, Department of Microbiology, MMIMSR, Mullana, Ambala, Haryana, India.

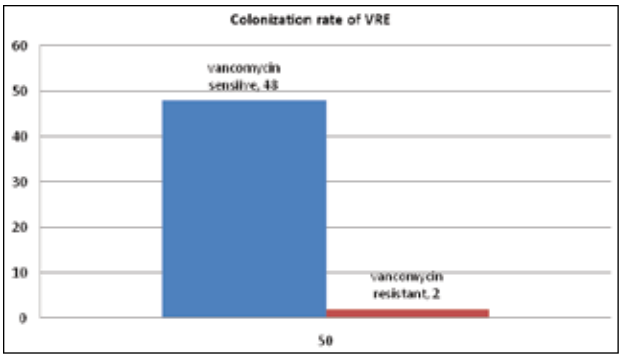
Correspondence to: Dr. Sonia Mehta, Dept. of Microbiology, MMIMSR, Mullana, India. Email: drsoni-aagar@gmail.com

reported. Although,Enterococci are normally present in gastro intestinal tract. So it readily passes through faecal matter. Those patients are treating with Glycopeptide like antibiotics are more at risk of picking up VRE. The most likely modes of transmission proximity from patient to patient are colonized with VRE especially those with diarrhoea. Either by direct contact through transient carriage of VRE on the hands of personnel or indirectly by infected environmental surfaces and other equipment for patient care. Most infections with these micro-organisms are aspectable to the patient's own flora. VRE are capable of prolonged survival on hands, gloves and environmental surfaces such as over-bed tables and call bells, door handles and stethoscopes[6]. Other Risk factors for bacteremia with VRE include hemodialysis, receipt of corticosteroids, anti-neoplastic agents or total parenteral nutrition, surgery, severity of illness, indwelling bladder catheters, antimicrobial administration, neutropenia and mucositis[7]. The susceptibility of Enterococcus species easily accomplish to genes resistance and the presence of some distinctive mechanisms conferring resistance to antibiotics like glycopeptides and aminoglycosides have cruelly deficient the choices available for treating severe infections due to these organisms. The emergence of multi-drug resistant enterococci has lead to a development which is almost as bad as the pre antibiotic era. Since many of these multi-drug resistant strains have developed resistance to almost all accessible antibiotics. Linezolid, Daptomycin are commonly choice able drug against VRE. Until recent times, the VRE strains were found sensitive to Linezolid. Resistance to Linezolid is gradually developing, pretense several questions on the virulence factors and their survival mechanisms[8]. Quinupristin or dalfopristin both are selective drug on VRE in such circumstance.

Material & Methods

This study was carried out in the department of Microbiology, Maharishi Markandeshwar institute of medical science and research (MMIMSR), Mullana, India, from October 2015 to March 2016.The stool samples were selected randomly from the samples of non-hospitalized individuals reaching lab for routine investigationswhich were stool microscopy and culture.50 isolates of *Enterococcus* species obtained from faecal samples were then further processed.

Faecal sample were collected by using culture swab then subjected to culture on blood agar or MacConkey agar. On MacConkey agar dark magenta color bacterial colony were seen. Identification of enterococcus spp. was done by different biochemical



Fegure 1:

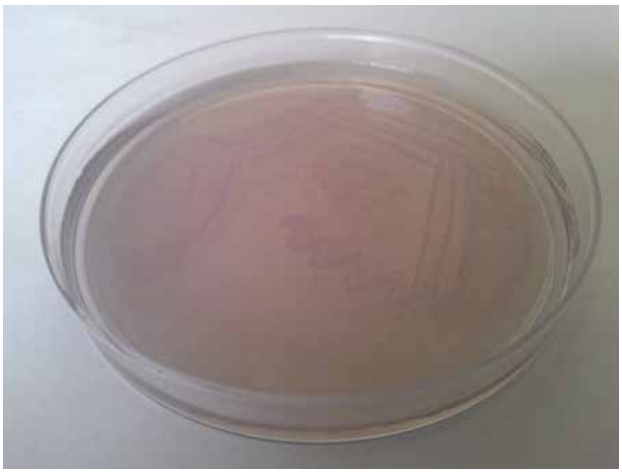


Fig-2: Enterococcus Growth On Vre Screen Agar Media

Fig-3:kirby-bauer Disk Diffusion Method Showingvre

reaction like catalase, bile-esculine, PYR test & salt tolerance test. After presumptive diagnosis of enterococcus spp. further strain was cultured on VRE screen agar Media[9] containing 6µg/lit Vancomycin drug.After incubation of 24 hours at 37°c growth seen on special media. The results were further supported by modified Kirby-bauer disk diffusion method with vancomycin (30µg) as per CLSI guideline. The isolates showing diameter of zone of inhibition ≤16 were considered as vancomycin resistant.

Ethical Clearance: The study was approved by ethics Committee of Maharishi Markandeshwar institute of medical science and research (MMIMSR), Mullana, India,

Result

50 isolates of *Enterococcus* species were obtained from stool samples of 67 non-hospitalized individuals screened over the 6 months study period .Out of this2 (4%) were VRE.Experiment was divided according to level of education, here we observed that school and college going students were highly infected. No significant result found in case of animal contact as well as abroad travel patients. Very highly significant result (P value= 0.004*) was found in patients who had history of previous hospital admission with surgical case. On the basic of antibiotic consumption, 4% patients were found as VRE but no statistically significant value was found in case of non-hospitalized individuals. The association is considered to be very statistically [p value= 0.021*] significant result showed inpatients those had history of chronic diseases.

Table 1: Risk factors for acquiring Vancomycin resistant Enterococcus Among non hospitalized individuals

Risk Factor	VRE		VSE		Total	P VALUE
	n	%	n	%		
Gender						
MALE	2	6.45	29	93.5	31	**0.258**
FEMALE	0	00	19	100	19	
Age						
< 10 years	0	0	7	10	7	
10-20 years	0	0	6	100	6	
20-41 years	2	8	23	92	25	
40-60 years	0	0	9	100	9	**0.720**
>60 years	0	0	3	100	3	
Level of education						
Pre school	0	00	2	100	2	
School	1	3.7	26	96.2	27	
College	1	6.25	15	93.7	16	**0.917**
University	0	00	5	100	5	
Animal Contact						
`YES	0	00	12	100	12	**0.417**
NO	2	5.2	36	94.7	38	
Travel abroad						
YES	0	00	2	100	2	**0.768**
NO	2	4.1	46	95.8	48	
Previous hospital admission						
YES with surgery	2	25	6	75	8	
YES without surgery	0	00	10	100	10	**0.004***
NO	0	00	32	100	32	
Antibiotic consumption						
YES	2	6.6	28	93.3	30	**0.239**
NO	0	00	20	100	20	
Chronic diseases						
YES	2	14.2	12	85.7	14	**0.021***
NO	0	00	36	100	36	

***Significance $P \leq 0.05$**

Table 2: Rate of VRE isolate in non-hospitalized individuals on the basis of previous hospital admission

PREVIOUS HOSPITAL ADMISSION	Yes With surgery	Yes without surgery	No	P VALUE
NO. OF PATIENT	08 (16%)	10(20%)	32 (64%)	
NO. OF VRE FOUND	2(4%)	0	0	0.004*

***Significance $P \leq 0.05$**

Table 3: Rate of VRE isolate in non-hospitalized individuals on the basis of antibiotic consumption

ANTIBIOTIC CONSUMPTION	YES	NO	P VALUE
NO. OF PATIENT	30 (60%)	20 (40%)	0.239
NO. OF VRE FOUND	2(4%)	0	

***Significance $P \leq 0.05$**

Discussions

Vancomycin resistance in enterococci has become a major therapeutic and infection control challenge, especially in centers[13]. In order to facilitate earlier efficient containment of the organism, the identification of colonized patientsis required. Furthermore certain measures have been recommended for use in hospitals where VRE have not yet been detected or remains rare. These measures include screening of all enterococci from clinical specimens for vancomycin resistance, the periodic surveillance of stools or rectal swabs from patients. Although the optimal method of screening stools for VRE has not yet been established, laboratories commonly utilize mediasupplemented with vancomycin[14]. As the vancomycin MICs for some strains of vanB VRE may be in the susceptible or intermediate range, screening is frequently done with media supplemented with vancomycin concentrations as low as 6 mg/ liter[15]. This is the concentration of vancomycin that is recommended by the National Committee for Clinical LaboratoryStandards for screening isolates of enterococci for vancomycin resistance[16]. As a result, enterococci intrinsically resistant to vancomycin may be detected upon screening, and a decision must be made about the implications for infection control[17, 18].

- Our results confirm that VRE are infrequently recoveredfrom stool samples. We were able to detect 4% VRE from non-hospitalized individuals. In contrast SNawal Hijazi et al reported a very high rate (43.8%) of VRE in non-hospitalized individuals.
- Karine Gambarotto reported11.8% of the subjects from the community were found to be VRE carriers. A total of 65 VRE strains were isolated: 12 (18
- Similarly Enditz HP reportedVRE were isolated from 12 of 624 (2%) and 4 of 200 (2%) hospitalized patients and patients living in the community, respectively.

imilarly, Ruoff reported that 2% of 302 consecutive isolates of enterococci from clinical specimens were identified as either *E. gallinarum*or *E.casseliflavus*[19] with a conventional test scheme. In another study, of 705 enterococcal isolates collected from eight tertiary- care hospitals, 1.1% was found to be either *E. gallinarum*or *E. casseliflavus*[20].It appears that approximately 4% of the outpatients were colonized with VRE in their gastrointestinal tracts. Thus, we found similarrates among outpatients in our community and patients who were hospitalized or receiving care at our hospital. Unfortunately, this study did not allow us to obtain more clinical information on the outpatients colonized with VRE. Nevertheless, these results suggest that some patients in the community carry these organisms and bring them into the hospital upon admission.

Blaimont[18] et al. reported Vancomycin resistance was more prevalent in E. faecium and high level gentamicin resistance in E. faecalis. It is noteworthy that no resistant strains to any of the antibiotics tested, were found in the faecal samples of healthy individuals except one E. faecium strain resistant to ampicillin.[21]

According to our study 2% of Nonhospitalized

individuals carrying VRE were school pass, while only 2% were with higher education. This result suggests that the level of education may be a significant factor. Education is usually allied with increased awareness of the dangers of antibiotic use. Un-educated people may not comply with antibiotic use instructions because they either can't read them or do not understand them properly. None of them from non –hospitalized individuals with VRE were in contact with animals. In contrast, the study done by Bates J[22]et al in Europe, the explained isolation of VRE from healthy volunteers, animals, and environmental sources indicates that these organisms are part of the normal human flora and suggests that the food chain may be the origin of VRE.

None of the patient gave history of travel abroad; hence the present study could not access the role of VRE positivity with this parameter. But study had done by Mutters NT[23] et al (2015) in Germany. They resulted 2.9% and it could have influence the immergence of VRE.Since VRE is a hospital acquired pathogen. In the present study, VRE positive patients had a history of previous hospital admission and under surgical treatments and got a significant (P value-0.004*) findings. In contrast, the study compared with Tacconelli E[24] et al (2004) in Boston. They showed VRE positivity in previous hospitalization.Non-hospitalized individuals with VRE positive gave history of multiple antibiotics and could not explain the class of antibiotic; this might be the reason.

The present study also observed association of chronic diseases with increase risk of VRE. 4% of non-hospitalized individuals with VRE suffered from chronic diseasesand also found Significant result (P value-0.021*) in this situation. High risk of colonization in this patient might due to prolonged antibiotic therapy and their lower immunity. In similarly, VRE are now being seenwith increasing frequency among patients with chronic renal failure.

Conclusion

The occurrence of VRE is a persisting clinical problem in all geographic areas and continues to be exacerbated by clonal propagation within the health care facility leading to limitedtherapeutic options. The steady pandemic spread of VRE along with acquisition of resistance to newerantimicrobials warrants continued surveillance of these versatile pathogens. The prevalence of VRE in faeces of non-hospitalized individuals at MMIMSR was 4%. However, asfor most bacteria described as causing human disease, enterococcialso possess properties that can be ascribed roles inpathogenesis. The natural ability of enterococci to readilyacquire, accumulate, and share extrachromosomal elementsencoding virulence traits or antibiotic resistance genes lendsadvantages to their survival under unusual environmentalstresses and in part explains their increasing importance asnosocomial pathogens. Aggregation substance, surface carbohydrates, or fibronectin-binding moieties may facilitate adherenceto host tissues. E. faecalis appears to have the capacity totranslocate across intact intestinal mucosa in models of antibiotic induced superinfection.[25]Faecal colonization, prolonged hospital stay, long time antibiotic treatment and chronic diseases had an important role in development of resistant to vancomycin. Hence it is concluded that future prospective group studies are needed to understand better the epidemiology of VRE transmission. In particular, the temporal acquisition of vancomycin resistance in enterococciin patients after starting antibiotic therapy is still to be defined.

Conflict of interest: None declared

Authors' Contribution:

Data gathering and idea owner of this study: Bera S, Mehta S, Dwivedi MB

Study design: Bera S, Mehta S, Dwivedi MB, Singh VA

Data gathering: Bera S, Mehta S, Paul R, Nongrum S

Writing and submitting manuscript: Bera S, Mehta S

Editing and approval of final draft: Bera S, Mehta S, Dwivedi MB, Singh VA, Paul R, Nongrum S

Reference:

1. Devriese L A, Collins MD, Wirth R. The genus *Enterococcus*.*In* A. Ballows, H. G. Tru¨per, M. Dworkin, W. Harder, and K. H. Schleifer (ed.), The prokaryotes. Springer-Verlag, New York, N.Y 1991:1465–1477.
2. Flahaud, S., P. Boutibonnes, and Y. Auffray. Les ente´rocoquesdans l'environnementproche de l'homme. Can. J. Microbiol 1997;43:699–708
3. Pourcher, A. M., L. A. Devriese, J. F. Herna´ndez, and J. M. Delattre. Enumeration by a miniaturized method of *Escherichia coli*, *Streptococcusbovis*and enterococci as indicators of the origin of faecal pollution of waters.J. Appl. Microbiol 199170:525–530.
4. Devriese, L. A., A. V. Kerckhove, R. Kilpper-Ba¨lz, and K. H. Schleifer. Characterization and identification of *Enterococcus* species isolated from the intestines of animals. Int. J. Syst. Bacteriol 1987;37:257–259
5. Leclerc, H., L. A.Devriese, and D. A. A. Mossel. Taxonomical changes in intestinal (faecal) enterococci and streptococci: consequences on their use as indicators of faecal contamination in drinking water. J. Appl. Bacteriol 199681:459–466.
6. Greenaway C, Miller MA: Mode of transmission of vancomycin-resistant enterococci (VRE) in long term care facilities, The Society for Healthcare Epidemiology of America, The Eighth Annual Scientific Meeting, Orlando, Florida, 1998.
7. Henning KJ, Delencastre H, Eagan J, Boone N, Brown A, Chung M et al: Vancomycin-resistant Enterococcus faecium on a pediatric oncology ward: duration of stool shedding and incidence of clinical infection. PediatrInf Dis J 1996 15, 848–54
8. Marion AK, Rose AD, Timothy FJ, Bryan PS, Kelly M, Susan C et al: Response to emerging infection leading to outbreak of Linezolid-resistant Enterococci. Emerg Infect Dis 2007;13:1024-30
9. Washington DC, 2nd Ed., 1974. Facklam and Carly, 1985, Mannual of ClinMiocrobiol, Lennette and others (Eds). 4th Ed. ASM,Washington DC.
10. Morris, J. G., Jr., D. K. Shay, J. N. Hebden, R. J. McCarter, Jr., B. E. Perdue, W. Jarvis, J. A. Johnson, T. C. Dowling, L. B. Polish, and R. S. Schwalbe. Enterococci resistant to multiple antimicrobial agents, including vancomycin. Ann. Intern. Med 1995;123:250–259
11. Boyce, J. M. Vancomycin-resistant enterococci: pervasive and persistent pathogens. Infect. Control Hosp. Epidemiol 1995;16:676–679.
12. Lior, L., M. Litt, J. Hockin, C. Kennedy, B. A. Jolley, M. Garcia, G. Gillis, A. Humar, I. Campbell, J. Brunton, H. Dedier, and J. Conly. 1996. Vancomycin-resistant enterococci on a renal ward in an Ontario hospital. Can. Commun. Dis. Rep. 22:125–128.
13. National Committee for Clinical Laboratory Standards. 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
14. Green, M., K. Barbadora, and M. Michaels. Recovery of vancomycin resistant gram-positive cocci from pediatric liver transplant recipients. J. Clin. Microbiol 199129:2503–2506.
15. Swenson, J. M., N. C. Clark, M. J. Ferraro, D. F. Sham, G. Doern, M. A. Pfaller, L. B. Reller, M. P. Weinstein, R. J. Zabransky, and F. C. Tenover. Development of a standardized screening method for detection of vancomycin-resistant enterococci. J. Clin. Microbiol1994;32:1700–1704.
16. Kaplan, A. H., P. H. Gilligan, and R. R. Facklam. Recovery of resistant enterococci during vancomycinprophylaxis. J. Clin. Microbiol 1988;26:1216–1218.
17. Ruoff, K., Murtagh M., Spargo J., Ferraro J., 1990 - Species identities of enterococci isolated from clinical specimens. J. Clin. Microbiol. 28:434-437
18. Gordon, S., J. M. Swenson, B. C. Hill, N. E. Pigott, R. R. Facklam, R. C. Cooksey, C. Thornsberry, Enterococcal Study Group, W. R. Jarvis, and F. C. Tenover. Antimicrobial susceptibility patterns of common and unusual species of enterococci causing infections in the United States. J. Clin. Microbiol 1992;30:2373–2378
19. Blaimont B, J. Charlier, and G. Wauters. Comparative distribution of Enterococcus species in faeces and clinical samples. Microb. Ecol. Health Dis 1995;8:87–92
20. Gordts, B., H. Van Landuyt, M. Ieven, P. Vandamme, and H. Goossens. Vancomycin-resistant enterococci colonizing the intestinal tracts ofhospitalized patients. J. Clin. Microbiol 1995;33:2842–2846.
21. Hospital Infection Control Practices Advisory Committee. Centers for Disease Control and Prevention. Recommendations for preventing thespread of vancomycin resistance. Infect. Control Hosp. Epidemiol 1995;16:105–113.
22. Morris, J. G., Jr., D. K. Shay, J. N. Hebden, R. J. McCarter, Jr., B. E. Perdue, W. Jarvis, J. A. Johnson, T. C. Dowling, L. B. Polish, and R. S. Schwalbe. Enterococci resistant to multiple antimicrobial agents, including vancomycin. Ann. Intern. Med 1995; 123:250–259.
23. Bates J, Jordens J, Griffiths D. Farm animals as a putative reservoir for vancomycin-resistant enterococcal infection in man. J. Antimicrob. Chemother 1994; 34:507–516.
24. Mutters NT, Gunther F, Sander A, Mischnik A, Frank U. Influx of multidrug-resistant organisms by country-to-country transfer of patients, BMC Infectious Dis 2015;15(466):2-6.
25. Tacconelli E, Karchmer AW, Yokoe D, Erika, D'Agata EMC: Preventing the Influx of Vancomycin Resistant Enterococci into Health Care Institutions, by Use of a Simple Validated Prediction Rule. Clin Infect Dis 2004; 39:964–70.
26. Wells, C. L., R P. Jechorek, and S. L. Erlandsen. Evidence for the translocation of Enterococcus faecalis across the mouse intestinal tract. J. Infect. Dis 1990; 162:82-90.