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Antimicrobial susceptibility pattern of *enterococci* isolated from clinical specimens at mymensingh medical college hospital, Mymensingh, Bangladesh

Abstract

Enterococci are part of the normal intestinal flora of humans and animals but have also emerged as important pathogens responsible for serious infections in hospital and community acquired infections. The aim of this study was to investigate the prevalence of *Enterococcus* with their antimicrobial susceptibility pattern from patients of Mymensingh Medical College Hospital, Bangladesh during the period from July 2015 to June 2016. Samples were collected and identification of *enterococcus* was done by standard microbiological procedure and biochemical tests. Multiplex PCR was performed by using species specific primers for *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*),vanA, vanB, vanC1, vanC2/C3 genes for vancomycin resistance. A total of 105 *enterococci* were isolated from 1201 different clinical specimens (from urine (931), pus (175) and wound swab (95) respectively) from July, 2015 to June, 2016. The prevalence of *enterococci* was 12.34% in urine, 17.17% in pus and 1.58% in wound samples. Out of 105 *enterococci*, 80 (77.14%) were *Enterococcus faecalis*,1 (0.95%) was *E. gallinaram* and 24 (22.86%) were other species. All the species were highly resistant to aminoglycosides (Gentamicin) (75.24%) and ciprofloxacin (73.33%). Regarding antibiotic sensitivity, all the *enterococci* were (100%) sensitive to linezolid and vancomycin followed by nitrofurantoin 93 (88.57%) and imipenem 89 (84.76%). No vancomycin resistant *enterococci* were identified by disk-diffusion method. But by PCR, vanB was found in 1/80 (1.25%) of the *E. faecalis* isolates and 01 (100%) of the *E. gallinarum*.vanC1 was detected in 1 (100%) of *E. gallinarum* isolates. The vanA and vanC2/C3 gene was not found in any isolates. For the first time, one isolate of *E. gallinarum* has been found harboring the vanB gene in our hospital. The presence of multidrug resistant *enterococci* should be considered as danger alarm for serious *enterococcals* infections and further study in large scale is needed.

Keywords: Bangladesh; *enterococci*, VanA, VanB, VanC1, VanC2/C3, vancomycin resistant *enterococci*; antibiotic sensitivity

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Introduction

Enterococci are the member of normal flora in the gut of humans and animals.[1] *Enterococci* have been recognized as being potentially pathogenic for humans since the turn of the century.[2] Though they are not considered to be highly virulent, their intrinsic resistance and ability to acquire resistance to several broad spectrum antibiotics allow them to cause super infections in patients already receiving antimicrobial therapy.[1] In the last decade, *enterococci* have become the surgical wound infections and nosocomial urinary tract infections and the third most frequently reported cause of bacteraemia in humans.[3] They also cause endocarditis, neonatal sepsis, intraabdominal pelvic infection and rarely enopthalmitis, meningitis.[4]

Enterococcus faecalis and *Enterococcus faecium* are the most prevalent species cultured from humans, accounting for more than 90% of clinical isolates.[5] Species identification is useful for epidemiological investigation of an outbreak and also for clinical decisions, particularly with regard to therapy, as antimicrobial susceptibility differs by species.[6] Biochemical tests for species identification are not performed routinely as they are laborious and time consuming; so to overcome the problem, the use of molecular methods has been suggested.[1]

Enterococci have a tremendous capacity to acquire high level of resistance to penicillins, aminoglycosides and vancomycin making the treatment options limited for clinicians.[6] Vancomycin resistant *enterococci* (VRE) are possibly the most serious concern that has recently emerged in human clinical infections.[7]

The present study was undertaken with the objective of identification of *enterococci* from clinical specimens, to determine the antimicrobial susceptibility pattern of the isolates and to detect vancomycin resistant *enterococci* phenotypically and genotypically.

Methods

A total of 105 *enterococci* were isolated from 1201 different clinical specimens (from urine (931), pus (175) and wound swab (95) respectively). This study was carried out in the Department of Microbiology, Mymensingh Medical College during the period from July, 2015 to June, 2016. Ethical permission was taken from the institutional ethical review committee. The isolates were collected from in and out patients departments of Mymensingh Medical College Hospital (MMCH).

The samples were cultured on blood agar and chromogenic agar media and incubated at 37°C for 24h aerobically. *Enterococci* were identified by colony morphology, Gram staining, absence of catalase production, tolerance to 6.5% NaCl, growth on bile esculin agar

with esculin hydrolysis.[1] Species identification was done by PCR was done to detect *E.faecalis*and *E. faecium*by using species specific primers.[8] Standard disc diffusion techniques as recommended by the Clinical and Laboratory Standard Institute were performed for susceptibility testing of Ampicillin, Gentamicin, Nitrofurantoin, Imipenem, Vancomycin, Ciprofloxacillin, Ceftriaxone, Cefuroxime, Cotrimoxazole, Linezolid. For molecular study, DNA was extracted by heat mehod 100°C for 10mintues.[1] Multiplex PCR was performed by using species specific primers for *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*),vanA, vanB, vanC1, vanC2/C3 genes for vancomycin resistance.[8]

Primer sets for multiplex PCR

Multiplex PCR oligonucleotide primers were used. The sequences from 5′ to 3′ ends of these oligonucleotide primers were as follows[8] (Figures 1) (Figure 2).

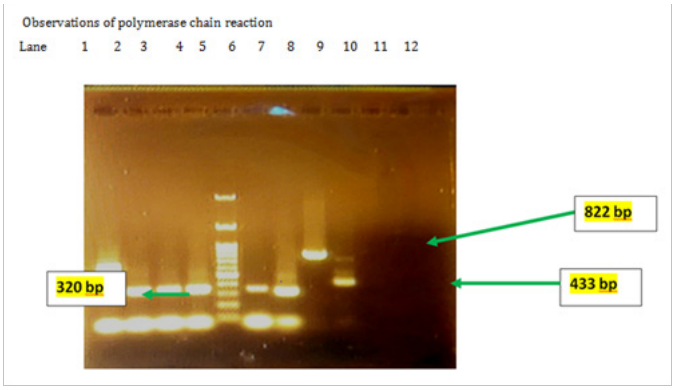

Figure 1 Multiplex PCR was done to detect the 16s *r*RNA (320bp), vanB gene (433bp) and vanC1gene (822bp) for VRE. Lane 7, 8 and 9 showing bands of the amplified product of 16s *r*RNA (320bp), vanC1 (822bp) gene and vanB gene (433bp) respectively.

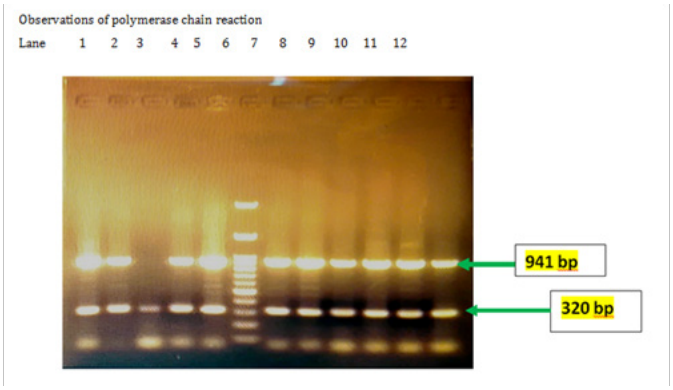

Figure 2 Multiplex PCR was done to detect the 16s *r*RNA (320bp), *E. faecalis* (941bp). Lane 1 to 12 showing bands of the amplified product of 16s *r*RNA (320bp) and 1, 2, 4, 5, 7-12 showing bands of the amplified product of *E. faecalis* (941bp).

Primers nucleotide sequence product size (bp)

vanA5′-CATGAATAGAATAAAAGTTGCAATA-3′ 1,030

5′-CCCCTTTAACGCTAATACGATCAA-3′ vanB 5′-GTGACAAACCGGAGGCGAGGA-3′ 433

5′-CCGCCATCCTCCTGCAAAAAAA-3′ vanC1 5′-GGTATCAAGGAAACCTC-3′ 822

5′-CTTCCGCCATCATAGCT-3′ vanC2/C35′-CGGGGAAGATGGCAGTAT-3 484

5′-CGCAGGGACGGTGATTTT-3′ *E. faecalis* 5′-ATCAAGTACAGTTAGTCTTTATTAG-3′ 3 (modified) 941

5′-ACGATTCAAAGCTAACTGAATCAGT-3′ *E. faecium*5′-TTGAGGCAGACCAGATTGACG-3′ 658

5′-TATGACAGCGACTCCGATTCC-3′

rrs (16S rRNA) 5′-GGATTAGATACCCTGGTAGTCC-3′ 320

5′-TCGTTGCGGGACTTAACCCAAC-3′

Initial denaturation at 94°C for 5min Followed by 30 cycles of in an automated DNA thermal cycler and each cycle consist of:

Denaturation at 94°C for 1 min Annealing at 54°C for 1 min Extension at 72°C for 60 sec	30 cycles

The final stage was an extension cycle at 72°C for 10 min.

The PCR products were analysed by 1% agarose gel (Alpha Imager, Germany) electrophoresis and photographed using a gel documentation system (Alpha Imager, Germany).

Results

A total of 105 *enterococci* were isolated from urine, pus and wound swab with the prevalence of 12.34%, 17.17% and 1.58% respectively (Table 1). *E. faecalis* were the most frequently identified *Enterococcus* species (77.14%), followed by was *E. gallinaram* (0.95%) and (22.86%) were other species (Table 2).

Table 1 Distribution of *Enterococci* obtained from different clinical specimens by phenotypic method (N=105)

Clinical Specimens	*Enterococci*	Percentage
Urine (n=705)	87	12.34
Pus (n=99)	17	17.17
Wound swab (n=63)	1	1.58
Total (n=867)	105	12.11

Table 2 Detection of species from total enterococcal isolates by PCR(N=105)

Species	No. of isolates	Percentage
E.faecalis	80	77.14
E.faecium	0	0
E.gallinaram	1	0.95
Other species	24	22.86
Total	105	100

In the present study, the *Enterococcus* isolates showed the highest rate of resistance in case of gentamycin, Cefuroxime and ciprofloxacin were 75.24%, 81.90% and 73.33% respectively. Regarding antibiotic sensitivity, all the *enterococci* were (100%) sensitive to linezolid and vancomycin followed by nitrofurantoin 93 (88.57%) and imipenem 89 (84.76%) (Table 3). None of the isolates were resistant to vancomycin and linezolid by disk-diffusion method. But by PCR,vanB was found in 1/80 (1.25%) of the *E. faecalis* isolates and 01 (100%) of the *E. gallinarum.*VanC1 was detected in 1 (100%) of *E. gallinarum* isolates. The vanA and vanC2/C3 gene was not found in any isolates (Table 4). For the first time, one isolate of *E. gallinarum* has been found harboringthe vanB gene in our hospital.

Table 3 Antibiotic susceptibility pattern of *Enterococci*(N=105)

Name of antibiotic	Sensitive	Resistant
Ampicillin	39(37.14)	66(62.86)
Cotrimoxazole	33(31.43)	72(68.57)
Ciprofloxacin	28(26.67)	77(73.33)
Nitrofurantoin	93(88.57)	12(11.43)
Gentamicin	26(24.76)	79(75.24)
Imipenem	89(84.76)	16(15.24)
Vancomycin	105(100)	0(0.0)
Linezolid	105(100)	0(0.0)
Cefuroxime	19(18.10)	86(81.90)
Ceftriaxone	39(37.14)	66(62.86)
Clinical specimens	***Enterococci***	**Percentage**
Urine(n=705)	87	12.34
Pus(n=99)	17	17.17
Wound swab(n=63)	1	1.58
Total(n=867)	105	12.11

Figure in parenthesis indicates percentage

Table 4 Detection of vancomycin resistantce genes from the genotypically confirmed enterococcal species

Name of antibiotics	Drug resistant genes	*E. faecalis*	*E. faecium*	*E.gallinaram*
		N=80	N=0	N=01
	vanA	0(0)	0(0)	0(0)
	vanB	01(1.25)	0(0)	01(100)
	vanC1- vanB	0(0)	0(0)	01(100)
Vancomycin				
	vanC2/C3	0(0)	0(0)	0(0)

Values in the parenthesis indicate percentage

Discussion

Nosocomial infections with *enterococci* are a major concern at many hospitals throughout the world including in Bangladesh.[1] Intestinal colonization with resistant *enterococcal* strains is more common than clinical infection. Colonized patients are a potential source for the spread of organisms to the health care workers, the environment and other patients. According to recent surveys, *Enterococci* remain in the top 3 most common pathogens that cause nosocomial infections.[5]

In this study, Table 1 shows that the prevalence of urinary tract infection (UTI) caused by *enterococci* was 12.34%. These findings agree with the report conducted in Brasil by Neto et al.,[9] was 11%. In Bangladesh, Saleh et al.,[10] showed that the frequency of *Enterococci* isolated from urine specimens of outpatient department BSMMU had increased significantly in 5years (2003-2008). The frequency of isolates was 11.38% and 13.29% in 2003 and 2008 respectively.[10] The explanation in favor of the higher rate of *enterococcal* infection should be its transmission from a healthcare worker's hands to a patient may involve direct inoculation to intravenous or urinary catheters.[11] In this study 1.58% *enterococci* was found in wound swab which is similar to a study conducted in Gaza. They found the prevalence of *enterococci* 1.9% in wound infection.[12] The prevalence rate of *enterococci* in pus was 17.17% in our study. Similar result was also reported by Mukherjee who found 16% isolation rate of *enterococci* from pus.[13]

In our study, the predominant species isolated was *E. faecalis* 80/105 (77.14%). Two other studies from Bangladesh reported isolation rates of *E. faecalis* as 62.5% by Islam and 71.18% by Akhter[14] that were almost similar to our present study.[1,14] The isolation rate of *Enterococcus Faecalis* from India by Mendiratta et al.,[15] (85.3%) and Jada & Jayakumar et al.,[5] (100%) from Kancheepuram were higher than our present study.[5,15] Reasons could be the predominance of *E. fecalis* in the endogenous flora of the body.[16] After *E. faecalis*, 1 isolate of *E. gallinarum* (0.95%)has been detected among 105 isolated *Enterococci* in our present study. This is similar to a study in North India by Mohanty et al who found 1(0.9%) isolate of *E. gallinarum* among 105 isolated *Enterococcus* strains[17] (Table 2).

Resistance to a number of antimicrobial drugs is a characteristic of the genus *Enterococcus*. The most recent and important resistance in *Enterococci* is vancomycin resistance has been increasingly reported from all parts of the world.[13] But in our study, regarding the antimicrobial sensitivity pattern, *enterococcal* isolates were best sensitive to vancomycin and linezolid. These findings agree with the report of other studies.[1,13] In our study, nitrofurantoin 93 (88.57%) and imipenem 89 (84.76%), were also most sensitive drug after vancomycin and linezolid which almost similar to a study by Akhter[14] in Bangladesh.[14] In the present study, *Enterococcus* isolates showed higher resistance to gentamycin (75.24%), ciprofloxacin (73.33%), ceftriaxone (62.86%), ampicillin (62.86%), and cotrimoxazole (60.95%) which coincides with the reports of other studies.[1,14] Another study in Kolkata, showed that 92% of isolates were resistant to ciprofloxacin, 91.4% to gentamicin,[13] which showed a drastic increase in resistance of the commonly used drugs comparable to our study (Table 3).

No vancomycin resistant *enterococci* were identified by disk-diffusion method in our study but by PCR, vanB was found in 1/80 (1.25%) of the *E. faecalis* isolates and 01 (100%) of the *E. gallinarum*. *VanC1* was detected in 1 (100%) of *E. gallinarum* isolates (Table 4). The reason behind this is either expression of the heterogeneous resistance of the isolates or carrying a non-functional or non-expressed *mec*A gene.[18,19] These results are almost similar to the study done by Domingo et al.,[20] they found VRE-associated vanB genes were 4.7% in only one hospital.[20] In this study, vanA and vanC2/C3 gene was not found in any isolates. One isolate of *E. gallinarum* has been found harboringthe vanB gene for the first time, in Bangladesh in our present study. A study conducted in Australia also reported vanB determinant in *E. gallinarum* and the second naturally acquired case.[21,22]

Conclusion

This study reveals the emergence of vancomycin resistant *E. faecalis* isolates carrying vanB gene and vancomycin resistant *E. gallinarum* with van B gene from this geographic region. This finding is alarming since it suggests the possibility of transfer of these plasmid borne van A and van B genes to other Gram-positive bacteria as well as to other plasmid free *Enterococci*both in the GIT and in hospital environment. It is, therefore, imperative to maintain a strict vigil on the spread of these organisms in the hospital and also from the hospital to the community.

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Limitations

The limitations of this study was non availability and high cost of the reagents and limited number of the case collection. As well as the study was done in a limited time period.

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Conflict of interest

The author declares no conflict of interest.

References

1. Islam TAB, Shamsuzzaman SM. Isolation and species identification of *enterococci* from clinical specimen with their antimicrobial susceptibility pattern in a tertiary care hospital, Bangladesh. *Journal of Coastal Life Medicine*. 2015;3(10):787–790.
2. Murray BE. The Life and Times of the *Enterococcus*. *Clinical Microbiology Reviews*. 1990;3(1):46–65.
3. Trivedi K, Cupakova S, Karpiskova R. Virulence factors and antibiotic resistance in *enterococci* isolated from food-stuffs. *Veterinarni Medicina.* 2011;56(7):352–357.
4. Sood S, Malhotra M, Das BK, et al. Enterococcal infections & antimicrobial resistance. *Indian J Med Res*. 2008;128(2):111–121.
5. Jada S, Jayakumar K. Prevalence of *Enterococcus species* from various clinical specimens in Shrisathya Sai Medical College And Research Institute with special reference to speciation and their resistance to their resistance to vancomycin. *International Journal of Medical and Clinical Research*. 2012;3(4):154–160.
6. Srivastava P, Mehta R, Nirwan PS, et al. Prevalence and antimicrobial susceptibility of *Enterococcus species* isolated from different clinical samples in a tertiary care hospital of North India. *Natl J Med Res*. 2013;3(4):389–391.
7. Giraffa G. *Enterococci* from foods. *FEMS Microbiology Reviews.* 2002;26(2):163–171.
8. Kariyama R, Mitsuhata R, Chow JW, et al. Simple and reliable multiplex PCR assay for surveillance isolates of vancomycin-resistant *enterococci*. *J Clin Microbiol*. 2000;38(8):3092–3095.
9. Neto JAD, Silva LDMD, Martins ACP, et al. Prevalence and bacterial susceptibility of hospital acquired urinary tract infection. *Acta Cirúrgica Brasileira.* 2003;18(5):36–38.
10. Saleh AA, Ahmed SS, Ahmed M, et al. Changing trends in Uropathogens and their Antimicrobial sensitivity pattern. *Bangladesh J Med Microbiol.* 2009;3(2):9–12.
11. Higuita NIA, Huycke MM. Enterococcal Disease, Epidemiology, and Implications for Treatment. *Infectious Diseases Section*. 2014. p. 45–70.
12. AlJarousha A, Saed AM, Afifi H. Prevalence of multidrug resistant *enterococci* in nosocomial infection in Gaza Strip. *J Al-Aqsa Unv*. 2008;12:15–24.
13. Mukherjee K, Bhattacharjee D, Chakraborti G, et al. Prevalence and antibiotic susceptibility pattern of *enterococcus species* from various clinical samples in a tertiary care hospital in Kolkata. *International Journal of Contemporary Medical Research.* 2016;3(6):1565–1567.
14. Akhter J. *Virulence factors and antimicrobial susceptibility pattern of different species of Enterococci causing urinary tract infection.* 2011.
15. Mendiratta DK, Kaur H, Deotale V, et al. Status of high level aminoglycoside resistant *Enterococcus faecium* and Enterococcus faecalis in a rural hospital of central India. *Indian J Med Microbiol*. 2008;26(4):369–371.
16. Reena G, Sumanta C, Kumkum B, et al. Enterococcal infections and its antimicrobial resistance with special reference to VRE And HLAR in a tertiary care hospital in Eastern India. *Journal of Dental and Medical Science*. 2012;13(2):17–22.
17. Mohanty S, Jose S, Singhal R, 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(4):963–965.
18. Mathur P, Kapil A, Chandra R, et al. Antimicrobial resistance in *Enterococcus faecalis*at a tertiary care centre of northern India. *Indian J Med Res*. 2003;118:25–28.
19. Zahan NA, Hossain MA, Musa AK, et al. PCR for mecA gene of methicillin resistant *Staphylococcus aureus*. *Mymensingh Med J*. 2009;18(1):21–26.
20. Al Akydy AG, Daoud H, Mulhem MM. Disk diffusion method versus PCR for mecA gene in detection of oxacillin-resistant *Staphylococcus aureus* in university children's hospital in Damascus, Syria. *International Journal of Pharmacy and Pharmaceutical sciences.* 2014;6(4):488–491.
21. Domingo MC, Huletsky A, Giroux R, et al. High prevalence of glycopeptide resistance genes vanB, vanD, and vanGnot associated with *enterococci* in human fecal flora. *Antimicrob Agents Chemother*. 2005;49(11):4784–4786.
22. Schooneveldt JM, Marriott RK, Nimmo GR. Detection of a vanBDeterminant in *Enterococcus gallinarum* in Australia. *J Clin Microbiol*. 2000;38(10):3902.