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 E. faecalis 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. gallinarum 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 (88.57%) and imipenem (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 multiple drug resistant enterococci should be considered as danger alarm for serious enterococci infections and further study in large scale is needed.

Keywords: Bangladesh; Enterococci; VanA; VanB; VanC1; VanC2/C3; Vancomycin resistant enterococci; Antibiotic sensitivity

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 encephalitis, 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 24 h 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, Ciprofloxacin, Ceftriaxone, Cefuroxime, Cotrimoxazole, Linezolid. For molecular study, DNA was extracted by heat method 100°C for 10 minutes [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 & 2).

**Observations of polymerase chain reaction**

| Lane | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 |
|------|----|----|----|----|----|----|----|----|----|----|----|----|

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

**Observations of polymerase chain reaction**

| Lane | 1  | 2  | 3  | 4  | 5  | 6  | 7  | 8  | 9  | 10 | 11 | 12 |
|------|----|----|----|----|----|----|----|----|----|----|----|----|

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

vanA 5′-CATGAATAGAATAAAAGTTGCAATA-3′ 1,030
5′-CCCTTTTAAGCTATACGATCAA-3′
vanB 5′-GTGACAAACCGGAGGCGAGGA-3′ 433
5′-CCGCCATCCTCTGCAAATAAA-3′
vanC1 5′-GGTATCAAGGAAACCTC-3′ 822
5′-CTTCCGCCATCATAGCT-3′
vanC2/C3 5′-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′

初段denaturation at 94ºC for 5 min
Followed by 30 cycles of in an automated DNA thermal cycler and each cycle consist of:
- Denaturation at 94ºC for 1 min
- Annealing at 54ºC for 1 min
- Extension at 72ºC for 60 sec
The final stage was an extension cycle at 72ºC 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. gallinarum (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      |

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 harboring the vanB gene in our hospital.

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

| Species      | No. of Isolates | Percentage |
|--------------|----------------|------------|
| E.faecalis   | 80             | 77.14      |
| E.aecium     | 0              | 0          |
| E.gallinaram | 1              | 0.95       |
| Other species| 24             | 22.86      |
| Total        | 105            | 100        |

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)     |
| Linezolid         | 105 (100) | 0 (0)     |
| Cefuroxime        | 19 (18.10)| 86 (81.90)|
| Ceftriazone       | 39 (37.14)| 66 (62.86)|

Table 4: Detection of vancomycin resistant genes from the genotypically confirmed enterococcal species.

| Name of Antibiotics | Drug Resistant Genes | E. faecalis | E. aecium | E.gallinaram |
|---------------------|----------------------|-------------|-----------|--------------|
| 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 Brazil 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 5 years (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. faecalis 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 which 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 meca 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 harboring the 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].

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 vanA and vanB 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.

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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References

1. Islam TAB, Shamsuzzaman SM (2015) 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 3(10): 787-790.

2. Murray BE (1990) The Life and Times of the Enterococcus. Clinical Microbiology Reviews 3(1): 46-65.

3. Trivedi K, Cupakova S, Karpisková R (2011) Virulence factors and antibiotic resistance in enterococci isolated from food-stuffs. Veterinarini Medicina 56(7): 352-357.

4. Sood S, Mahotra M, Das BK, Kapil A (2008) Enterococcal infections & antimicrobial resistance. Indian J Med Res 128(2): 111-121.

5. Jada S, Jayakumar K (2012) Prevalence of Enterococcus species from various clinical specimens in Tiruchyai 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 3(4): 154-160.

6. Srivastava P, Mehta R, Nirwan PS, Sharma M, Dahiya SS (2013) Prevalence and antimicrobial susceptibility of Enterococcus species isolated from different clinical samples in a tertiary care hospital of North India. Nat J Med Res 3(4): 389-391.
7. Giraffa G (2002) Enterococci from foods. FEMS Microbiology Reviews 26(2): 163-171.

8. Kariyama R, Mitsuhata R, Chow JW, Clewell DB, Kumon H (2000) Simple and reliable multiplex PCR assay for surveillance isolates of vancomycin-resistant enterococci. J Clin Microbiol 38(8): 3092-3095.

9. Neto JAD, Silva LDMD, Martins ACP, Tiraboschi RB, Domingos ALA, et al. (2003) Prevalence and bacterial susceptibility of hospital acquired urinary tract infection. Acta Cirúrgica Brasileira 18(5): 36-38.

10. Saleh AA, Ahmed SS, Ahmed M, Sattar ANI, Miah MRA (2009) Changing trends in Uropathogens and their Antimicrobial sensitivity pattern. Bangladesh J Med Microbiol 3(2): 9-12.

11. Higuita NIA, Huycke MM (2014) Enterococcal Disease, Epidemiology, and Implications for Treatment. Infectious Diseases Section pp. 45-70.

12. AlJarousha A, Saed AM, Afifi H (2008) Prevalence of multidrug resistant enterococci in nosocomial infection in Gaza Strip. J Al-Aqsa Uiv 12: 15-24.

13. Mukherjee K, Bhattacharjee D, Chakraborti G, Chatterjee SS (2016) 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 3(6): 1565-1567.

14. Akhter J (2011) Virulence factors and antimicrobial susceptibility pattern of different species of Enterococci causing urinary tract infection.

15. Mendiratta DK, Kaur H, Deotale V, Thamke DC, Narang R, et al. (2008) Status of high level aminoglycoside resistant Enterococcus faecium and Enterococcus faecalis in a rural hospital of central India. Indian J Med Microbiol 26(4): 369-371.

16. Reena G, Sumanta C, Kumkum B, Sujata B (2012) 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 13(2): 17-22.

17. Mohanty S, Jose S, Singhal R, Sood S, Dhawan B, et al. (2005) Species prevalence and antimicrobial susceptibility of Enterococci isolated in a Tertiary Care Hospital Of North India. Southeast Asian J Trop Med Public Health 36(4): 963-965.

18. Mathur P, Kapil A, Chandra R, Sharma P, Das B (2003) Antimicrobial resistance in Enterococcus faecalis at a tertiary care centre of northern India. Indian J Med Res 118: 25-28.

19. Zahan NA, Hossain MA, Musa AK, Shamsuzzaman AK, Mahamud MC, et al. (2009) PCR for mecA gene of methicillin resistant Staphylococcus aureus: Mymensingh Med J 18(1): 21-26.

20. Al Akydy AG, Daoud H, Mulhem MM (2014) 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 4(4): 488-491.

21. Domingo MC, Hulettsky A, Giroux R, Boissinot K, Picard FJ, et al. (2005) High prevalence of glycopeptide resistance genes vanB, vanD, and vanG not associated with enterococci in human fecal flora. Antimicrob Agents Chemother 49(11): 4784-4786.

22. Schooneveldt JM, Marriott RK, Nimmo GR (2000) Detection of a vanB Determinant in Enterococcus gallinarum in Australia. J Clin Microbiol 38(10): 3902.