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. 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 (98.57%) and imipenem (94.76%). 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 enterococcal 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. Enterococci have been recognized as being potentially pathogenic for humans since the turn of the century. 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. In the last decade, enterococci have become the surgical wound infections and nosocomial urinary tract infections and the third most frequently reported cause of bacteremia in humans. They also cause endocarditis, neonatal sepsis, intraabdominal pelvic infection and rarely endophthalmitis, meningitis.

Enterococcus faecalis and Enterococcus faecium are the most prevalent species cultured from humans, accounting for more than 90% of clinical isolates. 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. 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.

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

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 Hospital 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
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Antimicrobial susceptibility pattern of enterococci isolated from clinical specimens at Mymensingh Medical College Hospital, Mymensingh, Bangladesh. J Bacteriol Mycol Open Access. 2016;3(3):241–244. DOI: 10.15406/jbmoa.2016.03.00061

Citation: Boby F, Ahmed S, Paul SK, et al. Antimicrobial susceptibility pattern of enterococci isolated from clinical specimens at Mymensingh Medical College Hospital, Mymensingh, Bangladesh. J Bacteriol Mycol Open Access. 2016;3(3):241–244. DOI: 10.15406/jbmoa.2016.03.00061

with esculin hydrolysis. Species identification was done by PCR to detect E. faecalis and E. faecium using species specific primers. Standard disc diffusion techniques as recommended by the Clinical and Laboratory Standards 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 methol 100°C for 10 minutes. 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.

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 (Figures 1). (Table 1) (Figure 2).

Observations of polymerase chain reaction

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

320 bp 433 bp 822 bp 433 bp 822 bp

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

Table 2 Detection of species from total enterococcal isolates by PCR (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

Species No. of isolates Percentage

E. faecalis 80 77.14

E. faecium 0 0

E. gallinarum 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 harboring the vanB gene in our hospital.
Table 3 Antibiotic susceptibility pattern of Enterococci (N=105)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Sensitive</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>39(37.14)</td>
<td>66(62.86)</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>33(31.43)</td>
<td>72(68.57)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>28(26.67)</td>
<td>77(73.33)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>93(88.57)</td>
<td>12(11.43)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>26(24.76)</td>
<td>79(75.24)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>89(84.76)</td>
<td>16(15.24)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>105(100)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Linezolid</td>
<td>105(100)</td>
<td>0(0)</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>19(18.10)</td>
<td>86(81.90)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>39(37.14)</td>
<td>66(62.86)</td>
</tr>
</tbody>
</table>

Clinical specimens

Urine (n=705) | Enterococcus | Percentage |
<table>
<thead>
<tr>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>87</td>
<td>12.34</td>
<td></td>
</tr>
</tbody>
</table>

Pus (n=99) | Enterococcus | Percentage |
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>17</td>
<td>17.17</td>
<td></td>
</tr>
</tbody>
</table>

Wound swab (n=63) | Enterococcus | Percentage |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.58</td>
<td></td>
</tr>
</tbody>
</table>

Total (n=867) | Enterococcus | Percentage |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>105</td>
<td>12.11</td>
<td></td>
</tr>
</tbody>
</table>

Figure in parenthesis indicates percentage

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>vanA</th>
<th>vanB</th>
<th>vanC1, vanB</th>
<th>vanC2/C3</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=80</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>N=0</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(100)</td>
<td>0(0)</td>
</tr>
<tr>
<td>N=01</td>
<td>0(1)</td>
<td>2.5</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>N=9</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(100)</td>
<td>0(0)</td>
</tr>
</tbody>
</table>

Values in the parenthesis indicate percentage

Discussion

Nosocomial infections with enterococci are a major concern at many hospitals throughout the world, including in Bangladesh. 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.6

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.,6 was 11%. In Bangladesh, Saleh et al.,6 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.6 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.15 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 Akhtar16 that were almost similar to our present study.14,17 The isolation rate of Enterococcus Faecalis from India by Mendiratta et al.,18 (85.3%) and Jada & Jayakumar et al.,19 (100%) from Kancheepuram were higher than our present study.5,13 Reasons could be the predominance of E. faecalis in the endogenous flora of the body.6 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 strains17 (Table 2).

Resistance to a number of antimicrobial drugs is a characteristic of the genus Enterococcus. The most recent and important resistance in Enterococcus is vancomycin resistance has been increasingly reported from all parts of the world.15 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.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 Akhtar14 in Bangladesh.14 In the present study, Enterococcus isolates showed higher resistance to gentamicin (75.24%), ciprofloxacin (73.33%), ceftiraxone (62.86%), ampicillin (62.86%), and cotrimoxazole (60.95%) which coincides with the reports of other studies.14 Another study in Kolkata, showed that 92% of isolates were resistant to ciprofloxacin, 91.4% to gentamicin,11 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 0/1 (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.14,15 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 genes 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.21,22

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.

Limitations

The limitations of this study were 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.

Acknowledgements

Doctors, staffs and patients of different wards of Mymensingh Medical College Hospital. Professor Nobumichi Kobayashi, Sapporo Medical University of Japan for providing the PCR primers and technical assistance.

Conflict of interest

The author declares no conflict of interest.

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