Bangladesh J Med Microbiol 2014; 08 (01): 16-20
Bangladesh Society of Medical Microbiologists

Original Article

Antimicrobial Susceptibility Patterns of Enterococcus species Isolated from urinary tract infections

Jabin Akhter[1], Sharmeen Ahmed[2], Shaheda Anwar[3]

[1]Department of Microbiology, Bangladesh Institute of Tropical & Infectious Diseases, Chittagong;
[2, 3]Department of Microbiology & Immunology, Bangabandhu Sheikh Mujib Medical University, Dhaka

Abstract

Enterococci were frequently considered to be commensal organism and were ignored when isolated in clinical laboratory. But recently due to its capability of causing variety of infections, especially in hospitalized patients and difference in antimicrobial sensitivity of each species to varying antibiotics the present study was undertaken with a view at characterizing the clinical isolates of enterococcus, recovered from the cases of urinary tract infections (UTI). A total of 59 *Enterococci* were isolated from UTI patient for a period of 1 year. In the present study it was the 3rd leading organism causing UTI. They were speciated by an identification system based on the phenotypic characteristics of enterococcus species and their antimicrobial sensitivity pattern was determined. Most of the isolates were *E.faecalis* 42(71.18%) followed by *E.faecium* accounted for 10(16.94%), *E.avium* 1(2.43%), *E.raff* 1(2.43%) and 5(8.47%) remained unidentified. *E.faecium* showed increased resistance to amoxicillin (90%), co-trimoxazole (80%), ciprofloxacin (70%), gentamycin (80%), ceftriaxone (90%), and cefuroxime (80%). Most of the E.faecalis was resistant to amoxicillin (66.66%), co-trimoxazole (71.42%), ciprofloxacin (76.19%), gentamycin (71.42%), ceftriaxone (64.28%) and cefuroxime (80.95%). Multidrug-resistant enterococci are emerging as a leading nosocomial uropathogen. Identification of species along with knowledge of the antimicrobial resistance profile may ultimately contribute to development of strategies for prevention and to formulate treatment guidelines for infections caused by enterococci.

Key words: Antimicrobial Susceptibility Patterns, Enterococcus species, UTI.

Introduction:

Enterococci, an indigenous flora of the intestinal tract, oral cavity and the genitourinary tract of the humans and animals, are known to be relatively a virulent in healthy individuals, but have become important opportunistic pathogens, especially in hospitalized patients[1].Recent years have witnessed increased interest in enterococci not only because of their ability to cause serious infections like urinary tract infection (UTI), endocarditis, bacteremia, intra-abdominal infections but also because of their increasing resistance to many antimicrobial agents[2]. Among the 28 or more species identified *Enterococcus faecalis* causes 80-90 percent of human enterococcal infections while *E. faecium* accounts for majority of the remainder.

Although enterococci were not previously thought of as nosocomially spread pathogens, recent studies have confirmed this route of transmission[1] accounting for approximately 10% of hospital acquired infections[3], 10-20% hospital acquired urinary tract infections. A center for disease control (CDC)[4] survey of nosocomial infection, *enterococci* ranked third most common cause of hospital-acquired infections (HAI) after *Escherichia coli*, *S.aureus* and *Pseudomonas aeruginosa* and accounted for 13.9% of urinary tract infection[5]. The increasing resistance to antibacterial agent such as penicillin, aminoglycoside, trimethoprim and also to glycopeptide such as vancomycin and teicoplanin created an increasingly worrisome problem in clinical practice. This emphasizes the need for their identification from the clinical specimens and also differentiates them from other group D streptococci which are generally more sensitive

✉ ***Corresponding author:***
Dr. Jabin Akhter
Microbiologist
Bangladesh Institute of Tropical & Infectious Diseases
Chittagong.
E-mail: jabinakhter@yahoo.com
Cell: +88 01712171427

to the antimicrobial agents. The study was designed to determine the frequency of isolation of different species of enterococci causing UTI and the antimicrobial resistance pattern of the isolated species,

Materials & Methods

In this study a total 1203 urine samples were tested for the isolation of Enterococci from urine of suspected urinary tract infection patients of outpatient and inpatient department of BSMMU, Dhaka from January 2010 to December 2010. The urine specimens having pus cells (more than 5 cells per high power field from un-centrifuged urine) on microscopy were included. All samples were initially cultured on chromogenic agar media (Difco laboratories, Detroit, USA). They were sub cultured on blood agar plates at 37°c for 24 hrs. All suspected colonies were identified by standered microbiological methods including gram staining, catalase test and esculin hydrolysis test, growth in 6.5% NaCI and at pH 9.6[6].

Identification of species

Enterococcal strains were identified to the species level by using conventional physiological tests devised by Facklam and Collins[7] which are based on carbohydrate fermentation using 1% solution of following sugars: mannitol, sorbitol, arabinose raffinose, sorbose, lactose, and; by pyruvate utilization in 1% pyruvate broth; arginine decarboxylation in Moellers decarboxylase broth; motility was determined in modified Difco motility medium, and pigmentation was observed after overnight growth on tryptic soy agar. If the culture produced pigment, yellow was observed on a cotton swab that was used to pick up growth from the tryptic soy agar plate. All of the medium formulations and interpretations of tests used are described in the Manual of Clinical Microbiology, 4th ed[6].

Antibiotic sensitivity testing:

Antibacterial resistance pattern of 9 antimicrobial agents were performed with Amoxicillin, Cotrimoxazole, Ciprofloxacin, Nitrofurantoin, Ceftriaxone, Gentamycin, Cefuroxime, Imipenem, Vancomycin using standard disk diffution method (Kirby- Bauer sensitivity test)[8] susceptibility test. All test were performed on Muller Hinton agar (Oxoid Co, Hampshire, UK), and results read after 24 hrs of incubation at 37°c.

Results

A total of 59 enterococci were isolated from urine specimens (N=1,203) during the study period corresponding to 8.44% of all positive urine cultures. In this study significant growth of different organism occurred in 55.36% of cases. Based on the biochemical reactions, only 55 enterococci could be speciated. *E. faecalis* (71.18%) was the most common species isolated followed by *E.faecium* accounted for 10(16.94%), *E.avium* 1(2.43%), *E.raff* 1(2.43%) and 5(8.47%) remained unidentified.

On the basis of the results of four physiologic tests (acidification of mannitol, sorbitol, and sorbose broths, as well as failure to deaminate arginine), Enterococci were placed in three groups identified as I, II, and III (Table I). Twelve species and 1 variant species of Enterococci were placed in three groups. Table II showed one *E.avium* and one *E.raff* from group I are speciated by using acidification of arabinose and raffinose broths. Fifty two enterococci from group II are speciated using acidification of pyruvate and arabinose broth. Fourty two *E. faecalis* utilized pyruvate but did't produce acid from arabinose and 10 *E.faecium* produced acid from arabinose but did'nt utilized pyruvate (Table III). Five cultures remained unidentified; they did not fit into any of the three groupings listed in

TABLE 1. Key tests for identification of Enterococcus groups

Species	No. of strains	group	Reaction (% positive) Mannitol	Sorbitol	Sorbose	Arginine
E. avium□	1□	1□	+□	+□	+□	-
E. raffinosus□	1					
E. malodoratus						
E. pseudoavium						
E. faecalis□	42□	Il□	+□	V□	-□	+
E. solitarius						
E. gallinarum						
E. faecium□	10					
E. casseliflavus						
E. mundtii						
E. durans□	0□	III□	-□	-□	-□	+
E. hirae						
E. faecalis *						

+, Positive reaction; -, negative reaction; *Asaccharolytic variant.

TABLE II. Identification of group I Enterococcus species

Species	No. of strains	Reaction Arabinose	Raffinose
E. avium□	1□	+□	-
E. raffinosus□	1□	+□	+

Key reactions, Mannitol, sorbitol, and sorbose (+) and arginine (-).
+, Positive reaction; -, negative reaction.

TABLE III. Identification of group II Enterococcus species

Species	No. of strains	Arabi-nose	raffinose	Reaction pyruvate	Motility	Pigment
E. faecalis	42	-	-	+	-	-
E. faecium	10	+	-	-	-	-

Key reactions, Mannitol and arginine (+) and sorbose (-).
+, Positive reaction; -, negative reaction;

Resistance to several antimicrobial agents was prevalent among the Enterococci isolates recovered in the hospital. This study investigated the species occurrence and antibacterial resistance pattern of *enterococci* isolated from UTI individuals (Table IV). *E.faecium* was found to be more multidrug resistant than *E.faecalis*. Amoxicillin resistance was found in 66.66% *E. faecalis* whereas *E. faecium* was 90%. High rate of resistance to cotrimoxazole was found in *E.faecium* (71.42%). Gentamycin resistance was observed among 30 of 42(71.42%) *E.faecalis* and of 8/10(80%). Among the Enterococci isolates 76.19% *E.faecalis* and 80% *E.faecium* resistant to ciprofloxacin. But regarding the antimicrobial sensitivity pattern, Enterococcal isolates were best sensitive to vancomycin (98.30%), followed by imipenem (94.91%) and nitrofurantoin (86.44%).

Table IV: Antibiotic resistant pattern of Enterococcus species (N=52)

Name of antibioticsa	No. of resistant isolates	
	E.faecalis **N=42**	***E.faecium*** **N=10**
Amoxicillin	28(66.66)	9(90)
Cotrimoxazole	30(71.42)	8(80)
Ciprofloxacin	32(76.19)	8(80)
Nitrofurantoin	06(14.28)	2(20)
Ceftriaxone	27(64.28)	9(90)
Gentamycin	30(71.42)	8(80)
Cefuroxime	34(80.95)	8(80)
Imipenem	2(4.76)	1(10)
Vancomycin	0(0)	1(10)

*Parenthesis indicates percentage

Discussion

Enterococci are not generally regarded as highly virulent bacterial pathogens; however, resistance to many antimicrobial drugs complicates the treatment of enterococcal infections. Acquired resistance to high concentrations of ampicillin, aminoglycoside, and glycopeptide antibiotics, specifically vancomycin, has exacerbated this problem. As a measure of infection control, it is essential to differentiate *Enterococci* from other Gram positive bacteria inherently resistant to vancomycin. In this study *Enterococci* were differentiated from other Gram positive bacteria by standard biochemical tests.

In the present study, prevalence of enterococcal urinary tract infection was 8.44% which almost correlate with the findings of *Barros et al(2009)*[9]. They found 6.2% of the urine culture was positive for enteroccocal species in their study. Bagshaw *et al(2010)*[10] recorded *enterococci* as the third most frequent uropathogen in ICU acquired urinary tract infections after *E. coli* and *P. aeruginosa*. In the present study it was also the 3rd leading organism causing UTI, but was ranked behind *E.coli* & *Enterobacter*. In this study, 13.84% *Enterococci* was isolated from patient on indwelling urethral catheter. This finding is similar to a study conducted in U.S. acute-care hospitals where the incidence of *Enterococci* causing nosocomial catheter-associated urinary tract infections was 16% & 13% in hospital wide & ICU setup respectively[11]. Risk factors for enterococcal UTI include urinary tract instrumentation, indwelling catheterization, genitourinary tract disease and prior antimicrobial exposures. In this study, significant growth of different organisms occurred in 55.36% of cases. Increased number of significant growth in the study is due to inclusion of symptomatic patient having white cell count> 5/HPF of urine on microscopy. In a study by *Lakshmi et al(2004)* found 20% pure growth and 4% mixed growth in urine culture[12]. They cultured all urine samples irrespective of pus cell which may be the reason for lower percentage of growth than that of the present study.

In the present study, regarding incidence of various species of *Enterococci* found almost similar to other studies, E.faecalis (71.18%) was the most commonly identified species isolated from urine specimens followed by *E.faecium* (16.94%). Other species of *Enterococcus* like *E.avium* & *E.raffinosus* accounted for 3.38% in the institution. Among 59 isolates ; 8.47% were unidentified. From different studies it was revealed that about 4% to 4.6% of enterococcus isolates were remained unidentified[7,13]. Increased percentage of unidentified isolates in the present study probably due to lack of application of complete range of tests to identify *enterococci*. *Desai et al(2001)*[14] applied full range of biochemical test and were able to speciate 95-100% of species. Correct speciation is very important since there is variation in resistance to antibiotics by particular *Enterococcal* species. However, species identification has been found to vary in different studies. *Ruoff et al(1990)* found six species of *Enterococci* in their study from a set of 206 cultures, with *E.faecalis* (91.8%) and *E. faecium* (6.3%) & one each of *E. casseliflavus, E.gallinarum, E.raffinosus, E. avium* in urine isolates[15]. *Chowdhury et al(2007)* isolated *E.faecalis* (82%), *E. feacium* (6 %), *E.raff* (4%),

E.casseliflavus (2%), & *E.disper* (2%) from urine[13]. The prevalence of other species of *Enterococci* found usually from 2-10% [7].

Resistance to several antimicrobial agents was prevalent among the *Enterococci* isolates recovered in the hospital. Knowledge of the antimicrobial resistance profile is essential to formulate treatment guidelines for infections caused by *Enterococci*.

Isolates of *E. faecium* were found in our study to be more multi-resistant than *E. faecalis*. *E. faecium* isolates were significantly more resistant to amoxicillin, cotrimoxazole, ciprofloxacin, ceftriaxone. *Almeida and colleagues* reported a higher percentage of resistance in *E. faecalis* to ampicillin, penicillin, aminoglycosides, chloramphenicol, ciprofloxacin, rifampicin, and erythromycin while *E. faecium* isolates exhibited more resistance to tetracyclines[16]. Regarding the unusual species of *Enterococcus*, the susceptibility patterns did not show any major differences.

Amoxicillin resistance in *E. faecium* was significantly higher and this finding is similar to the report of Jureen *et al(2003)*[18]. According to their study this kind of resistance in *Enterococci* may be due to the production of low-affinity penicillin-binding protein. Cotrimoxazole resistant *Enterococci* have been isolated worldwide[19]. In the present study, 70-80% of the *Enterococci* are gentamycin resistant. Gentamicin is one of the most commonly used aminoglycosides against *enterococci*. High level aminoglycoside resistance is a real problem. This resistance overcomes the synergy of killing combination therapy. Ampicilin and vancomycin are not bactericidal unless combined with an aminoglycoside. High level gentamycin resistance is most often associated with high-level resistance to all alternative aminoglysides.

The high resistance to ciprofloxacin seen in the present study may be due to the widespread usage of these antibiotics for UTI as a first-line treatment in our country. There are also reports of increasing resistance of *Enterococci* to ciprofloxacin [20]

Resistance of *Enterococci* to glycopeptides poses an increasing problem in clinical practice in many countries around the world21. The prevalence of vancomycin resistant *E. faecium* in health care institutions across United States is reported to be 15%[4]. In our isolates we did not found any vancomycin resistant *Enterococci*.

The susceptibility pattern of enterococci against nitrofurantoin is very promising[22]. In our study, *enterococci* not only showed significantly very high susceptibility against nitrofurantoin (86.44%) but most of the isolates, which were found resistant to other available antibiotics, were found susceptible to nitrofurantoin.

Conclusion

For long time, enterococci were considered to be commensal organism and were ignored when isolated in clinical laboratory. But recently due to its capability of causing variety of infections, especially in hospitalized patients and difference in antimicrobial sensitivity of each species to varying antibiotics has led to understanding the importance of identification of *Enterococcus* to species level. The increased resistance to major antibiotic classes emphasizes, once more, not only on the necessity for more discriminate use of new drugs but also for continuous efforts to find or design antimicrobial agents. Thus, we suggest intensified actions to promote more the rational use of antibiotics in health care settings, more surveillance studies in order to monitor changes in enterococcal resistance patterns and the adoption of measures to prevent the spreading of resistance isolates.

REFERENCES:

1. Murray BE. The life and Time of the *Enterococci*. Clinical Microbiology review.1990; 3:46-65
2. Moellering RC Jr. Emergence of enterococci as a significant pathogen. *Clin Infect Dis*. 1992; 14: 1173 - 1178
3. Chenoweth C and Schaberg D. The epidemiology of enterococci. *Eur J Clin Microbiol Infect Dis*.1990; 9:80-89
4. Centres for Disease Control. (1989-1993) Nosocomial *enterococci* resistant to vanomycin: United States, National Nosocomial Infection Surveilance. MMWR. 1993; 42: 597 - 599.
5. Schaerberg DR, Culver DH, Gayes RP. Major trends in the microbiology, etiology of nosocomial infections. *Am J Med*. 1991; 91:795-825
6. Fackland R, Sham DA, Teixeira LM: Enterococcus. In Manual of Clinical Microbiology 7th edition. Edited by: Murray PR, Baron EJ, Pfaller MA, Tenover FC, and Yolken RH. Washington: American Society of Microbiology.1999; 297-305.
7. Facklam RR and Collins MD. Identification of *Enterococcus* Species Isolated from Human Infections by a conventional test scheme. *J of Clin Microbiol*. 1989;27:731-734

8. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol.* 1966; 45(4):493-6

9. Barros M, Martinelli R, Rocha H. Enterococcal Urinary Tract Infections in a University Hospital. *The Brazilian Journal of Infectious Diseases*. 2009 ;13(4):294-296.

10. Bagshaw SM, Laupland KB. Epidemiology of intensive care unitacquired urinary tract infections. Curr Opin Infect Dis. 2006 ;19:67-71

11. Maki DG and Agger WA.. Enterococcal bacteremia: clinical features, the risk of endocarditis, and management. *Medicine (Baltimore)*.1988; 67:248-269.

12. Lakshmi V, Satheeshkumar T, Kulkarni G. Utility of Urichrom II- A Chromogenic Medium for Uropathogen. *Indian Journal of Medical Microbiology*. 2004; 22(3):153-158

13. Chaudhary U, Shamma M, Yadav A. Antimicrobial Susceptibility Patterns of Common and Unusual Enterococcus species Isolated from Clinical Specimens. *J Infect Dis Antimicrob Agents*. 2007; 24:55-62.

14. Desai PJ, Pandit D, Mathur M, Gogate A. Prevalence, Identification & Distribution of various species of *Enterococcus* isolates from clinical specimens with special reference to urinary tract infection in catheterized patients. *Indian J Med Microbiol*. 2001;19:132-137

15. Ruoff KL, Maza L, Murtagh MJ, et al. Species identities of enterococci isolated from clinical specimens. *J Clin Microbial*. 1990; 28: 435 - 437.

16. Coque T, Patterson J, Steckelberg J et al. Incidence of haemo-lysin, gelatinase and aggregation substance among *Enterococci* isolated from patients with endocarditis and other infections and from feces of hospitalized and community-based persons. *J Infect Dis*. 1995; 171: 1223-9

17. Almeida RT, Filho MR, Silveira CAN, Rodrigues IP, Filho JE, Nascimento RSD *et al*. Molecular Epidemiology and Antimicrobial Susceptibility of *Enterococci* Recovered from Brazilian Intensive Care Units. *The Brazilian Journal of Infectious Diseases*. 2004; 8(3):197-205

18. Jureen R, Top J, Mohn SC, et al. Molecular characterization of ampicillin resistance *Enterococcus* faecium isolated from hospitalized patient in Norway. *J Clin Microbial*. 2003; 41: 2330-2335 .

19. Coque TM, Singh KV, Weinstock GM, Murray BE. Characterization of dihydrofolate reductase genes from trimethoprim- susceptible and trimethoprim-resistant strains of Enterococcus faecalis. *Antimicro Agents Chemother*. 1999; 43: 141-7.

20. Schaerberg D, Dilon WI, Terpenning MS, et al. Increasing resistance of Enterococci to ciprofloxacin. *Antimicrob agent chemother*. 1992;36: 2533-2535.

21. Bambeke FV, Chauvel M, Reynolds P, et al. Vancomycin-dependent enterococcus faecalis clinical isolates and revertant mutants. *Antimicro Agents chemother* 1993; 43: 41-7.

22. Butt T, Leghari MJ, Mahmood A. In-Vitro Activity of Nitrofurantoin in Enterococcus Urinary Tract Infection. *JPMA*. 2004; 54:466.