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URINARY TRACT ENTEROCOCCAL INFECTIONS AND THEIR ANTIMICROBIAL RESISTANCE

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ABSTRACT

Context : Enterococci have emerged as second most common cause of nosocomial infections since the last decade. The most frequent infections caused by enterococci are urinary tract infections (UTIs). Enterococci also have become resistant to wide range of antibiotics.

Aim : The present study was done to determine the prevalence of different enterococcal species in urinary tract infections and their antimicrobial resistance with special reference to vancomycin and high level aminoglycoside resistance.

Material and methods : Study was conducted on 80 enterococcal urine isolates. The isolates were identified by standard microbiological techniques and antibiotic sensitivity testing was done by Kirby – Bauer disc diffusion method according to CLSI guidelines.

Results : Maximum enterococci were isolated from male patients in the age group of 0-20 years. Most of the enterococci (71.25%) were non hemolytic and multidrug resistant. *E.faecalis* (65%) was the predominant species isolated. Linezolid and nitrofurantoin showed good antienterococcal activity. Vancomycin resistance was seen in 11 (13.75%) isolates and high level aminoglycoside resistance was seen in 44 (55%) of isolates. *E.faecium* isolates were more drug resistant than *E.faecalis* isolates.

Conclusion : Steps should be taken to regularly screen enterococcal isolates for vancomycin and high level aminoglycoside resistance and treat enterococcal infections effectively to limit spread of multidrug resistant enterococcal infections.

KEYWORDS : Enterococcus, VRE, *E.faecium*, High level aminoglycoside resistance

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INTRODUCTION

Enterococci have emerged as second most common cause of nosocomial infections since the last decade[1]. The infections are recognized by 3 t's – tough, tenacious and often times troublesome[2]. Prior to 1990's, enterococci were recognized as an important cause of bacterial endocarditis for almost a century. However during the past decade there has been a worldwide trend in increasing occurrence of enterococcal infections, a shift in the spectrum of enterococcal infections and emergence of antimicrobial resistance in enterococci. The most frequent infections caused by enterococci are urinary tract infections (UTIs)[3]. In a CDC survey of nosocomial infections, enterococci accounted for 13.9% of UTIs, second only to *E.coli* as a sole agent of nosocomial UTIs[1].

Enterococci are intrinsically resistant to a wide range of antibiotics that most notably include beta lactams and aminoglycosides, frequently used to treat infections with gram positive cocci[4]. In addition enterococci have ability to acquire resistance to antimicrobial agents through transfer of plasmids, transposons, chromosomal exchange or mutations[5].

The incidence of vancomycin resistant enterococci (VRE) has increased significantly over the last decade. Vancomycin resistance in enterococci not only leaves fewer options for disease management, but also is important due to potential risk of vancomycin resistance gene transfer from enterococci to *Staphylococcus aureus*[6]. Enterococci are also showing acquired high level resistance to aminoglycosides (HLAR). VRE along with HLAR is making the treatment of these infections extremely difficult.

Traditionally 19 species of enterococcus have been recognized so far. Though *E.faecalis* and *E.faecium* account for most of infections, other species are also isolated.

Considering all these facts, the present study was done to know the species prevalence of enterococci in urinary tract infections and their antimicrobial resistance with special reference to vancomycin and high level aminoglycoside resistance in our tertiary health care centre.

MATERIAL AND METHODS

A total of 80 enterococci isolated in significant numbers from urine were included in the study. Ethical clearance was obtained from the institutional ethical committee.

Mid stream clean catch urine was collected from the patients in a wide mouthed sterile container. Samples were processed without any delay on Blood agar and MacConkey agar following standard calibrated loop technique[7]. Enterococci isolated in significant number (10^5 CFU/ml) were included in the study. The isolates were identified by colony morphology, Gram's stain, catalase reaction, growth on bile esculin agar and tolerance to 6.5% NaCl. Species identification was done using standard microbiological techniqes[8]. Antimicrobial sensitivity testing was done according to CLSI guidelines[9] by disc diffusion method of Kirby-Beaur using Mueller-Hinton agar.

Various antibiotics tested were Ampicillin (10µg), Tetracycline (30µg), Erythromycin (15 µg), Ciprofloxacin (5µg), Gentamicin (10µg), Vancomycin (30µg), Linezolid (30µg), Nitrofurantoin (300µg). For high level aminoglycoside resistance detection Gentamycin (120µg) and Streptomycin (300µg) discs were used. The source of antimicrobials was Hi-Media Ltd (Mumbai) India. Standard strain *E.faecalis* ATCC 29212 and *E.faecalis* ATCC 51299 were used as susceptible and resistant quality control strains.

RESULTS

A total of 80 enterococci were isolated in significant numbers from urine samples received in microbiology laboratory.

TABLE – 1
AGE AND SEX DISTRIBUTION OF ISOLATES

Age group (yrs)	Sex		Total (%)
	Male	Female	
0-20	14 (17.5%)	22 (27.5%)	36 (45%)
21-40	6 (7.50%)	10 (12.50%)	16 (20%)
41-60	7 (8.75%)	8 (10%)	15 (18.75%)
≥61	2 (2.50%)	11 (13.75%)	13(16.25%)
Total	29 (36.25%)	51 (63.75%)	80 (100%)

Table-1 shows age and sex distribution of isolates. Maximum isolates (45%) were seen in 0-20 years age group followed by 21-40 years age group (20%). 51 (63.75%) enterococci were isolated from females as compared to 29 (36.25%) from males.

TABLE – 2
HEMOLYTIC ACTIVITY OF ISOLATES

Species (no)	Non hemolytic	α-hemolytic	β-heomolytic
E.faecalis (52)	34 (65.38%)	17 (32.69%)	1 (1.92%)
E.faecium (26)	21 (80.76%)	3 (11.53%)	2 (7.69%)
E.gallinarum(1)	1 (100%)	0(0%)	0(0%)
E.durans (1)	1 (100%)	0(0%)	0(0%)
Total (80)	57 (71.25%)	20 (25%)	3 (3.75%)

Table-2 shows hemolytic activity of enterococcal isolates. Four species of enterococci, E.faecalis (52), E.faecium (26), E.gallinarum (1), E.durans (1) were isolated from urine samples. Most of the isolates (71.25%) were non-hemolytic. 25% were α-hemolyticand only 3.75% wereβ-hemolytic.

TABLE – 3
ANTIMICROBIAL RESISTANCE PATTERN OF ENTEROCOCCAL ISOLATES (n=80)

Antimicrobial agent	*E.faecalis* (n=52)	*E.faecium* (n=26)	*E.gallinarum* (n=1)	*E.durans* (n=1)
Ampicillin	35 (67.30%)	15 (57.69%)	1 (100%)	0(0%)
Tetracycline	18 (34.61%)	14 (53.84%)	1 (100%)	1 (100%)
Erythromycin	27 (51.92%)	8 (30.76%)	0(0%)	1 (100%)
Ciprofloxacin	38 (73.07%)	21 (80.76%)	1 (100%)	0(0%)
Gentamycin	34 (65.38%)	19 (73.07%)	1 (100%)	1 (100%)
Linezolid	4 (7.69%)	3 (11.53%)	0(0%)	0(0%)
Nitrofurantoin	2 (3.84%)	2 (7.69%)	0(0%)	0(0%)

Table-3 shows resistance pattern of enterococcal isolates. All the isolated four species were multidrug resistant and more than 50% drug resistance was seen to most of the drugs tested except for linezolid and nitrofurantoin. Linezolid and nitrofurantoin showed better activity against enterococcal isolates.

TABLE – 4
VANCOMYCIN AND HIGH LEVEL AMINOGLYCOSIDE RESISTANCE AMONG ENTEROCOCCAL ISOLATES

Enterococcal species	No (%) of isolates resistant to			
	Vancomycin	HLGR	HLSR	HLGR+ HLSR
E.faecalis (52)	7 (13.46%)	32 (61.53%)	38 (73.07%)	28 (53.84%)
E.faecium (26)	4 (15.38%)	17 (65.38%)	19 (73.07%)	15 (57.69%)
E.gallinarum (1)	0(0%)	0(0%)	1 (100%)	0(0%)
E.durans (1)	0(0%)	1 (100%)	1 (100%)	1 (100%)
Total (80)	11 (13.75%)	50 (62.50%)	59 (73.75%)	44 (55%)

HLGR : High level gentamycin resistance
HLSR : High level streptomycin resistance

Table-4 shows vancomycin and high level aminoglycoside resistance in enterococcal isolates. Vancomycin resistance was seen in 11 (13.75%) of isolates. High level gentamycin resistance (HLGR) was seen in 50 (62.50%) isolates and High level streptomycin resistance (HLSR) was seen in 59 (73.75%) isolates. Both HLGR + HLSR was seen in 44 (55%) isolates. E.faecium isolates were more resistant to all drugs when compared to E.faecalis.

DISCUSSION

In the present study we have determined the species prevalence and antimicrobials resistance pattern of enterococcal isolates from urine samples. The study showed highest number of enterococcal isolates in 0-20 years age group. Similar results are reported in the study done by Orrette FA[10]. Enterococcal isolates from females (63.75%) were more than males (36.35%).

Of the 80 enterococcal isolates, 57 (71.25%) were non hemolytic, 20(25%) were α-hemolytic. Only 3 (3.75%) wereβ-hemolytic.Thus no hemolysis is more common in enterococcal urine isolates than α and β-hemolysis in present study. In contrast to present study, study done by Desai PJ et al[11], has shown more β hemolytic isolates than α hemolytic isolates.

From urine samples, *E.faecalis* was the most predominant isolate accounting for 52 (65%) isolates followed by 26(32.50%) *E.facium*, 1(1.25%) *E.gallinarum* and 1(1.25%) *E.durans*. Present study is in acceptance with the various studies[12,13,14,15] from India which have shown *E.faecalis* as predominant isolate from human infections. But few studies[15,16] have recently reported *E.faecium* as emerging predominant species isolated from UTI. This could be due to geographic variations in prevalence of different species of enterococci in different areas.

Present study revealed presence of multidrug resistance in enterococcal isolates. Similar results were obtained from various studies from different parts of the world.[5,12,17,18, ,19]

Drug resistance is rapidly acquired by enterococci by plasmids[20], conjugative transposition[21] or by mutations[5] in enterococci leading to rapid spread of multidrug resistant enterococcal infections.

The present study also reveals *E.faecium* to be more drug resistant than *E.faecalis*. Similar finding are reported by other workers also[14,22]. Linezolid and nitrofurantoin showed good

antienterococcal activity. Nitrofurantoin is a cheap drug and can be administered orally[23]. Thus nitrofurantoin and Linezolid can be kept as alternative to multidrug resistant enterococci. Daptomycin is also shown [24,25] to have good antienterococcal activity. But in the present study daptomycin was not tested for sensitivity as it was not included in CLSI document.

Vancomycin resistance in the present study was seen in 11 (13.75%) of isolates. *E.facium* showed more vancomycin resistance (15.38%) than *E.faecalis* (13.14%). Earlier Indian studies[12,13,14,21] have reported 0-5% of vancomycin resistance. This indicates an increasing trend of vancomycin resistance in enterococcal isolates. In the present study, one *E.gallinarum* was isolated and it was sensitive to vancomycin. But studies at CDC Atlanta[26]and Minnesota USA[27] have shown that disc diffusion test fails to detect vancomycin resistance in motile enterococci like *E.gallinarum*. So MIC test should be done to correctly decide vancomycin in such cases.

High level aminoglycoside resistance (HLGR+HLSR) was seen in 44 (55%) of isolates. The finding of the present study are well comparable with other studies.[4,12,17] Resistance to aminoglycosides is of great concern, since it eliminates the synergy of aminoglycosides with β-lactam antibiotics, which is the therapy of choice for enterococcal infections, thus limiting the therapeutic options.

In conclusion, *E.faecalis* is the predominant isolate from enterococcal UTI patients. Most of the enterococcal isolates are multidrug resistant. Vancomycin and high level aminoglycoside resistance is on the rise in enterococcal isolates. Linazolid and nitrofurantoin have still good antienterococcal activity. Steps should be taken to regularly screen enterococcal isolates for vancomycin and high level aminglycoside resistance and treat enterococcal infections effectively to limit the spread of multidrug resistant enterococcal infections.

REFERENCES

1. Schaberg DR, Culver DH, Ganes RP. Major trends in the microbial etiology of nosocomial infection. Am J Med 1991;91:725-755
2. Edwards DD. Enterococci attract attention of concerned microbiologists. ASM News 2000;66:540-5.
3. Low DE, Keller N, Barth A, Jones RN. Clinical prevalence, antimicrobial susceptibility and geographic resistance patterns of enterococci : results from the SENTRY Antimicrobial surveillance program, 1997-1999. Clin Infect Dis 2001;32:5133-45.
4. Latakapoor, V.S. Randara, Monorama Deb. Antimicrobial resistance of enterococcal blood isolates at a pediatric care hospital in India. Jpn Infect Dis 2005;58:101-103.
5. Mundy LM, Satim DF, Gilmore M. Relationships between enterococcal virulence and antimicrobial resistance. ClinMicrobiol Rev 2000;13:513-522.
6. Noble W, Virani Z, Cree R. Co transfer of vancomycin and other resistant genes from *E.faecalis* NCTC 12201 to *Staphylococcus aureus*. FEMS MicrobiolLett 1992;93:195-8.
7. Forbes BA, Sahm DF, WeisfeldAS.In Baily and Scott's diagnostic microbiology 11th edn.Mosby Publications. 2002: 927-38.
8. Facklam RR, Collins MD. Identification of enterococcus species isolated from human infections by a conventional test scheme. J ClinMicrobiol 1989;27:731-734.

9. Clinical and laboratory standards institute, performance standards for Antimicrobial susceptibility testing 15th – informational supplement. CLSI/NCCLS M 100-555 Wayne (PA).The Institute; 2005.
10. Orrett FA, Connors E. Enterococcal urinary tract infections – eight years experience at a regional hospital in Trinidad – West indies. Chinese Med J 2001;114(1):90-92.
11. Desai PJ, Pandit D, Mathur M, Gogate A. Prevalence, identification and distribution of various species of enterococci isolated from clinical specimens with special reference to urinary tract infection in catheterized patients. Indian J Med Microbiol 2001;19(3):132-37.
12. Mathur P. Kapil. A, Chandra R, Sharma P. Antimicrobial resistance in *E.faecalis* at a tertiary care centre of North India. Indian J Med Res 2003;188:25-28.
13. Taneja N, Roni P, Emmanueal R, Sharma M. Significance of vancomycin resistant enterococci form urinary specimens at a tertiary care centre in North India. Indian J Med Res 2004;119: 72-74.
14. Bhat KG, Paul C, Anantha Krishna NC. Drug resistant enterococci in SouthIndianHospital. Trop Doc 1998;28:106-107.
15. Kamakar MG, Gershom ES, Metha PR. Enterococcal infections with special reference to phenotypic characterization and drug resistance. Indian J Med Res 2004;119(5):22-25.
16. Iwen PC, Dominguez EA, Patil KD. Change in prevalence and antibiotic resistance of enterococcus species isolated from blood cultures over an 8 year period. Antimicrobo Agents Chemother 1997;41:494-95.
17. Baragundi MC, Sonth SB, Solabannawar SS, Patil CS, Yemul VL. Species prevalence and antimicrobial resistance pattern of enterococcal isolates in a tertiary health care centre. J Clin&Diag Res 2010;4:3405-3409.
18. Akhi MT, Farzazeh F, Oskouri M. Study of enterococcal susceptibility patterns isolated from clinical specimesn in Tabriz, Iran Pak J Med Sci 2009;25(2):21
19. Gupta V, Singla N. Antibiotic susceptibility pattern of enterococci. J Clin&Diag Res 2007;5:385-389.
20. Bunny GM, Leonard. BA, Hedberg PJ. Pheromone-inducible conjugation in enterococcus faecalis: inter bacterial and host parasite chemical communication. J Bacteriol 1995;177:871.
21. Clewell DB, Gawron-Burke C, Conjugative transposons and the dissemination of antibiotic resistance in streptococci. Annu Rev Microbiol 1986;40:635.
22. Arias CA, Reye SJ, Zuniga M. Multi centre surveillance of antimicrobial resistance in enterococci and staphylococci form Colombian hospitals. J Antimicrobial Chemother 2003;51:69-68.
23. Butt T, Leghar MJ, Mahmood A. In vitro activity of nitrofurantoin in enterococcus urinary tract infection. J Pakistan Med Ass 2004; 231-233.
24. Novais C, Souza JC, Coque TM, Piexel LV. In vitro activity of daptomycin against enterococci from nosocomial and community environments in Portugal. J AntimicrobChemother 2004;54(5): 964-66.
25. Mohr JF, Friedrich LV, Yenkelev S, Lamp KC. Daptomycin for the treatment of enterococcal bacteremia; results from the Cubicin outcomes registry and experience (CORE). International J of Antimicrobial agents 2009;33(6):543-548.
26. Jana M, Swenson, Bertha C Hill, ClydeThornsberry. Problems with disc diffusion test for detection of ancomycin resistance in Enterococci. J ClinMicrobiol 1989;27(9):2140-2142.

27. Kohner PC, Patel R, Whl JR, Garin KM, Hopkins MK, Wagener LT, et al. Comparison of agar dilution, E-test, disk diffusion and automated Vitek methods for testing susceptibilities of enterococcus spp. to vancomycin. J Clin Microbiol 1997;35(12): 3258-3263.