Prevalence of various enterococcus species and their antibiotic resistance pattern among urinary isolates in tertiary care center in South Eastern Rajasthan

Harshad Singh Naruka[1,*], Anita E Chand[2], Harinandan Meena[3]

[1]Senior Demonstrator, [2]Senior Professor and Head, [3]Assistant Professor, [1]Dept. of Medical Microbiology, Government Medical College, Kota, Rajasthan, India

***Corresponding Author: Harshad Singh Naruka**
Email: narukaharshadsingh@gmail.com

Abstract

Introduction: In 1899 Thiercelin first described 'Enterococcus'. They are primarily member of gastrointestinal flora of human and animals. Enterococcus was grouped under streptococcus as group D streptococci but now it has been separated as individual genus. Clinically the most important species associated with human Infections are *E. faecalis* and *E. faecium*. They are frequently resistant to various group of antimicrobials. UTI is the most common infection among all Enterococcal infections and implicated in approximately 10 percent of all UTIs.

Materials and Methods: The samples were collected from all hospitalized and OPD patients of MBS Hospital, JK Lone Hospital & NMC Hospital. Government Medical College, Kota, Rajasthan. A total of 360 isolates were collected during the period of 2 years from April 2016 to April 2018 and processed at Microbiology Laboratory, Department of Microbiology, Government Medical College, Kota, Rajasthan.

Results: The present study shows a high incidence of enterococcal UTI among females (225) compare to males (135) with maximum Isolation among population between age group of 31- 40 (111) followed by 41-50 (59) and 21-30 (48). In present study the Ampicillin, Piperacillin, Ciprofloxacin, Norfloxacin, Nitrofurantoin, Gentamicin, Vancomycin and Linezolid antibiotic discs was tested and Linezolid was found to be most effective among all followed by Vancomycin, Nitrofurantoin, Ampicillin, Piperacillin, Ciprofloxacin, Norfloxacin. High level Gentamicin resistance (HLAR) was 60.27% in present study and Vancomycin resistance was 14.16 %.

Conclusion: Among all species of enterococci *E. faecium* were most resistant species among all. Ampicillin & Piperacillin resistance was considerably higher in present study in comparison to other studies. Resistance to Nitrofurantoin was also found highest among *E. faecium* 77.77% in present study and other compared studies.[19] By observing the various parameters of present study it can be concluded that enterococci which was thought to be a commensal organism is now emerging as a potential pathogen, particularly among hospitalised patients. In our institution Enterococcus isolates was more resistant to fluoroquinolones, aminoglycosides and β-lactams agents. Early detection of Enterococcal species and resistance to Aminoglycoside and Vancomycin can be helpful in limiting the morbidity in our hospital set up.

Keywords: Enterococcus, UTI, Antibiotic resistance.

Introduction

In 1899 Thiercelin described a saprophytic coccus from intestinal origin of almost all animals and term it "Enterococcus".[1] Enterococci are primarily member of gastrointestinal flora of human and animals.[1,2] Earlier the Enterococcus was grouped under streptococcus as group D streptococci but in past decades Enterococcus group has undergone various taxonomic changes and recognizes it as a separate genus, as it show no similar homology with streptococcus genus.[1,4] The current criteria of including bacteria in genus Enterococcus is based completely on molecular basis like DNA-DNA recognition experiments, 16 S RNA gene sequencing, analysis of whole cell protein profile etc.[1,5] Enterococcus genus has been phenotypically divided by Facklam & collaborators in 2002 into 5 groups based on acid formation in Mannitol & Sorbose broth as well as arginine hydrolysis.[1,3] Clinically the most important species associated with human Infections are *E. faecalis* and *E. faecium*.[6] Of these, *E. faecalis* is the most pathogenic species but *E. faecium* is of growing importance as it is more frequently resistant to antimicrobials.[7] Antimicrobial resistance can be classified as either intrinsic or acquired. Intrinsic resistance is related to inherent or natural chromosomally encoded characteristics present in all or most of the Enterococci. Enterococcal intrinsic resistance involves two major groups of antimicrobial therapeutic drugs: the aminoglycosides and β-lactams.[8] In addition to the intrinsic resistance traits, Enterococci have acquired different genetic determinants that confer resistance to several classes of antibiotics, including Chloramphenicol, Tetracycline, Macrolides, lincosamides, and Streptogramins, Aminoglycosides. Antibiotic resistance is a major factor involved in nosocomial infections. It allows the organisms of low pathogenicity like Enterococci, to endure in the environment in which antimicrobial agents are heavily used thereby providing a selective advantage. Enterococci have become the second most common agent recovered from nosocomial urinary tract infections (UTI). UTI is the most common infection among all Enterococcal infections; Enterococci have been implicated in approximately 10 percent of all UTIs.[9]

Materials and Methods

The samples were collected from all hospitalized and OPD patients of MBS hospital, JK Lone Hospital & NMC hospital. Government Medical College, Kota, Rajasthan. A total of 360 isolates were collected during the period of 2 years from April 2016 to April 2018 and processed at

Microbiology Laboratory, Department of Microbiology, Government Medical College, Kota, Rajasthan. All the isolates were identified as enterococci by gram staining, Motility, Bile esculin hydrolysis, salt tolerance test and further up to species level by following standard identification scheme by G.I. Barrow.[10]

All the isolates identified as enterococci were tested for their antibiotic susceptibility using Kirby-Bauer disc diffusion method and interpreted according to CLSI guidelines.[11,12] Antibiotic discs (Himedia) used for testing susceptibility of enterococcal isolates were Ampicillin 10 µg, Piperacillin 75 µg, Ciprofloxacin 5 µg, Norfloxacin 10 µg, Nitrofurantoin 300 µg, Vancomycin 30 µg, Linezolid 30 µg, Gentamicin high content 120 µg.

Results

In the present study samples were processed in department of Microbiology Govt. Medical College Kota. 360 isolates of enterococci were recovered in present study and distribution of isolates from samples collected from patients of both OPD and IPD was 256 and 104. A total 43 isolates was from catheterized patients among both OPD & IPD.

The present study shows a high incidence of enterococcal UTI among females (225) compare to males (135) so Male to Female ratio was 0.6. Isolation of enterococci was maximum among population between age group of 31- 40 (111) followed by 41-50 (59) and 21-30 (48) (Table-1) (Fig. 1).

Table 1: Distribution of enterococcus UTI in various age and sex

Age	Male	Female	Total
0-10	04	16	20
11-20	10	15	25
21-30	16	32	48
31-40	37	74	111
41-50	28	31	59
51-60	19	24	43
61-70	10	20	30
71and above	11	13	24
Total	135	225	360

Most common species found in present study was Enterococcus faecalis 222 (61.66%) followed by *E. faecium*108 (30%), *E. durans* 22 (6.11%) and *E. avium* 8 (2.22%)(Fig. 1).

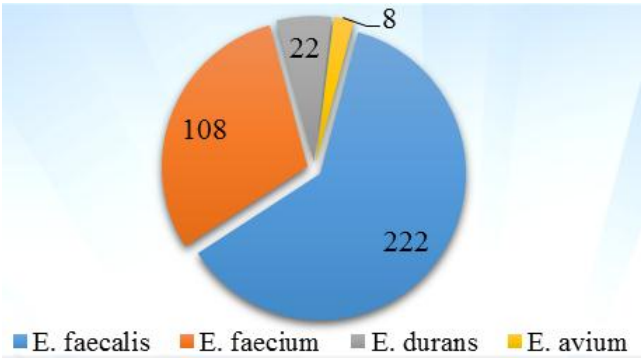

Fig. 1: Distribution of various enterococcal species among total isolates

In present study the Ampicillin, Piperacillin, Ciprofloxacin, Norfloxacin, Nitrofurantoin, Gentamicin, Vancomycin and Linezolid antibiotic discs was tested by Kirby-Bauer disc diffusion method. Linezolid was most effective among all showed sensitivity for 321(89.16%), intermediate to 28(7.77%) and resistance for 11(3.05%) isolates, followed by vancomycin sensitive to 235(65.27%) intermediate to 74(20.55%) and resistance for 51(14.16 %) isolates, Nitrofurantoin sensitive to 178(49.44%) intermediate to 11(3.05%) and resistance for 171(47.5%) isolates, Ampicillin sensitive to 120(33.33%) and resistance for 240(66.66%) isolates, Piperacillin sensitive to 99(27.5%) intermediate to 57(15.83%) and resistance for 204(56.66%) isolates, Ciprofloxacin sensitive to 74(20.55%) intermediate to 6(1.66%) and resistance for 280(77.77%) isolates, Norfloxacin sensitive to 47(13.05%) intermediate to 11(3.05%) and resistance for 302(83.88%) isolates. High level Gentamicin was tested for HLAR detection, this was sensitive to 143(39.72%) and resistance for 217(60.27%) isolates (Fig. 2).

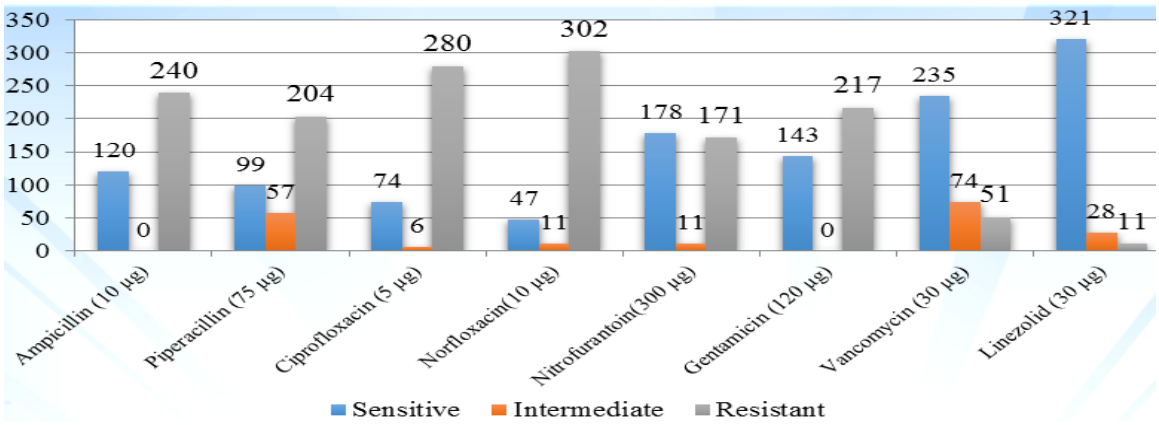

Fig. 2: Antibiotic sensitivity pattern among all enterococcus by Kirby -Bauer method

Antibiotics sensitivity pattern among different Enterococcus Species

Antibiotic susceptibility pattern among various enterococcus species has been shown in following Table-2. *E. faecalis* show highest sensitivity for Linezolid 195(87.83%) followed by Vancomycin 159(71.62%), Nitrofurantoin 136(61.26%), Ampicillin 80(36.03%), Piperacillin 73(32.88%), Ciprofloxacin 54(24.32%) and least for Norfloxacin (37(16.66%).

E. faecium show highest sensitivity for Linezolid 98 (90.74%) followed by Vancomycin 51(47.22%), Ampicillin 25(23.14%), Piperacillin 24(22.22%), Nitrofurantoin 22(20.37%), Ciprofloxacin and Norfloxacin show equal sensitivity 7(6.48%).

E. durans show highest sensitivity for Linezolid 22(100%) followed by Vancomycin 20(90.9%), Nitrofurantoin 15(68.18%), Ampicillin and Piperacillin show equal sensitivity 11(50%), Norfloxacin 7(31.81%), and least for Ciprofloxacin 5(22.72%).

E. avium showed highest sensitivity for Linezolid 6(75%) followed by vancomycin 5(62.5%), ampicillin, Piperacillin and Nitrofurantoin show same result 4(50%) and least for ciprofloxacin and Norfloxacin 2(25%).

High level Gentamicin was used to detect high level resistance for aminoglycosides among all enterococcus species *E. faecium* isolates show maximum resistance for aminoglycoside 92(85.18%), followed by *E. faecalis* 120(54.05%), *E. avium* 2(25%) and *E. durans* 3(13.63%) where the N value was 108, 222, 8, and 22 respectively.

Table 2: Sensitivity and resistance pattern to various antibiotics in different Enterococcus Species

Enterococcus spp.		Amp* (%)	Pip* (%)	Cip* (%)	Nor* (%)	Nit* (%)	Van* (%)	Lz* (%)	HLG* (%)
E. faecalis N=222	S	80 (36.03)	73 (32.88)	54 (24.32)	37 (16.66)	136 (61.26)	159 (71.62)	195 (87.83)	102 (45.94)
	I	0 (0)	33 (14.86)	7 (3.15)	1 (0.45)	8 (3.6)	44 (19.81)	23 (10.36)	0 (0)
	R	142 (63.96)	116 (52.25)	161 (72.52)	184 (82.88)	78 (35.13)	19 (8.55)	4 (1.8)	120 (54.05)
*E. faecium*N =108	S	25 (23.14)	24 (22.22)	7 (6.48)	7 (6.48)	22 (20.37)	51 (47.22)	98 (90.74)	16 (14.81)
	I	0 (0)	7 (6.48)	3 (2.77)	0 (0)	2 (1.85)	27 (25)	4 (3.7)	0 (0)
	R	83 (76.85)	77 (71.29)	98 (90.74)	101 (93.51)	84 (77.77)	30 (27.77)	6 (5.55)	92 (85.18)
E. durans N=22	S	11 (50)	11 (50)	5 (22.72)	7 (31.81)	15 (68.18)	20 (90.9)	22 (100)	19 (86.36)
	I	0 (0)	2 (9.09)	2 (9.09)	1 (4.54)	1 (4.54)	2 (9.09)	0 (0)	0 (0)
	R	11 (50)	9 (40.9)	15 (68.18)	14 (63.63)	6 (27.27)	0 (0)	0 (0)	3 (13.63)
E. avium N=8	S	4 (50)	4 (50)	2 (25)	2 (25)	4 (50)	5 (62.5)	6 (75)	6 (75)
	I	0 (0)	2 (25)	0 (0)	1 (12.5)	1 (12.5)	1 (12.5)	1 (12.5)	0 (0)
	R	4 (50)	2 (25)	6 (75)	5 (62.5)	3 (37.5)	2 (25)	1 (12.5)	2 (25)

*Amp=Ampicillin, Pip=Piperacillin, Cip=Ciprofloxacin, Nor=Norfloxacin, Nit=Nitrofurantoin, Van=Vancomycin, Lz=Linezolid, HLG=High level Gentamicin.

Discussion

Enterococcus species are continuously emerging as important pathogen specially in hospital environment and can cause different type of infection which are usually difficult to treat due to limited antibiotic options and higher incidence of drug resistance to various antibiotics. Enterococcus is a most important cause of urinary tract infection (UTI) caused by gram positive bacteria.

In the present study 360 urinary isolates of enterococci were studied during the period of 2 years from April 2016 to April 2018, among them more cases were from females (225) then males (135) which could be due to close proximity of anal orifice and urethra in females or due to poor hygiene.

E. faecalis: Among all groups of antibiotics *E. faecalis* show highest resistance for Fluoroquinolones in comprision to other study Norfloxacin resistance was higher in present study. Ampicillin resistance in present study is higher

compared to other studies. Resistance to Nitrofurantoin is also higher among *E. faecalis* in present study compared to study done by varun goel et al.

Aminoglycoside resistance was detected by Gentamicin 120 µg disc, in present study *E. faecalis* show considerably high resistance to Gentamicin the result is similar to study of Sanal C. Fernandes et al.

Natural intrinsic tolerance to Aminoglycosides has been shown by enterococci. This property is due to two main factors, poor entry of antibiotic and inactivation of antibiotic by covalent modification of the hydroxyl or amino groups by naturally occurring enterococcal enzymes. In addition to this enterococci can modify the ribosomal target by the action of ribosomal RNA (rRNA) methyltransferase known as EfmM.[13,14] Vancomycin resistance among *E. faecalis* in present study is comparatively higher than a study from south India done by Sanal C. Fernandes et al and lower from study of north India AIIMS done by varun goel et al

Linezolid resistance among *E. faecalis* in present study was 1.8% which is higher than other studies.

***E. faecium*:** Among all species of enterococci *E. faecium* were most resistant species among all. They show highest resistance to Fluoroquinolone, followed by Aminoglycosides, Nitrofurantoin, Ampicillin, Piperacillin, Vancomycin and Linezolid.

Fluoroquinolone resistance among *E. faecalis* was highest then other species and comparatively it was lower than other studies of Yaeghob Sharifi et al, Saraswathy MP et al. and was higher than study of Varun goel et al.

E. faecium shares all resistance mechanism with E. faecalis in addition efflux pump mediated by *NorA* gene.[13,15,16]

Ampicillin resistance was considerably higher in present study as compare to most of studies except study done by Yaeghob Sharifi et al which showed higher resistance than present study, similarly Piperacillin resistance was also high 71.29% in present study. Resistance to Nitrofurantoin was found highest among *E. faecium* 77.77% in present study and other compared studies.[19]

Gentamicin resistance for *E. faecium* was 85.18% also highest compared to other species in present and other studies.[17,18] Vancomycin resistance among *E. faecium* 27.77% which is higher than other studies.[19,20] Linezolid resistance was highest among *E. faecium* then other species of enterococci in this study.

E. durans and *E. avium* was isolated in lesser amount and *E. avium* was more resistant to almost all groups of antibiotics studied. As the number of isolates of *E. durans* and *E. avium* was less so significant comparison can not be done.

Conclusion

Enterococci is a notorious pathogen as it has intrinsic resistance/tolerance to different group of antibiotics and is a main causative agent of gram positive UTI. By observing the various parameters of present study it can be concluded that enterococci which was thought to be a commensal organism is now emerging as a potential pathogen, particularly among hospitalised patients. *E. faecalis* and *E. faecium* found to be the most prevalent species which confer resistance to various groups of antibiotics. *E. faecium* found to be more resistant species then *E. faecalis*. In our institution Enterococcus isolates was more resistant to fluoroquinolones, aminoglycosides and β-lactams agents like Ampicillin and Piperacillin. This may be due to selection pressure of these antibiotics in our set up. Early detection of Enterococcal species and resistance to Aminoglycoside and Vancomycin can be helpful in limiting the morbidity in our hospital set up.

Conflicts of Interest: None.

References

1. Lucica M. Teixeria and Richard R. Faclam.. Enterococcus, Topley and Wilson's Microbiology and Microbial Infections, S. Peter Borriello, Patrick R. Murray, Guido Funke (ed.), (10th). Bacteriology Vol.2; P 2-10: Wiley,2006.
2. Paul B. Eckburg et.al. Diversity of the Human Intestinal Microbial Flora NIH Public Access Author Manuscript. *Sci* 2005;10;308(5728):1635–1638.
3. Francois lebreton et al. Enterococcus diversity, origins in nature and gut colonization.2014 feb 2.in:Gilmore MS, Clowell DB, Lke Y. et.al Ed.Enterococci:From commensal to leading cause of drug resistance infection. Boston:Massachusetts Eye and Ear infirmary; 1-59;2014.
4. Seema Sood et.al. Enterococcal infections & antimicrobial resistance. *Indian J Med Res* 128,August 2008;111-121.
5. Katie Fisher et.al.The ecology, epidemiology and virulence of Enterococcus. Microbiol 2009;155:1749–1757
6. Werner G, Coque TM, Hammerum AM, Hope R, Hryniewicz W, Johnson A, Klare I, Kristinsson KG, Leclercq R, Lester CH, Lillie M, Novais C, Olsson-Liljequist B, Peixe LV, Sadowy E, Simonsen GS, Top J, Vuopio-Varkila J, Willems RJ, Witte W, Woodford N 2008. Emergence and spread of vancomycin resistance among enterococci in Europe. *Euro Surveill* 13:1-11.
7. French GL 1998. Enterococci and vancomycin resistance. *Clin Infect Dis* 27: S75-S83.
8. Huycke MM, Sahm DF, Gilmore MS. Multiple-Drug Resistant Enterococci: The Nature of the Problem and an Agenda for the Future. *Emerg Infect Dis* 1998;4(2):239-249.
9. Felmingham D, Wilson PR, Quintana AI, Gruneberg RN, Enterococcus species in urinary tract infection. *Clin Infect Dis* 1992:15:295-305.
10. G.I. Barrow and R.K.A. Feltham. Cowan and Steel's Manual for the identification of medical bacteria. Cambridge univ. press 3rd ed. 2003;6: 59-67
11. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing-fourth informational supplement (Update). CLSI document. Wayne: CLSI; 2014: M100–S24.
12. Washing Winn, Stephen Allen, William Janda, Elmer Koneman, Gary Procop, Paul Schreckenberger. Antimicrobial susceptibility testing. Koneman's colour at las and textbook of diagnost ic microbiology. th 6 ed. Lippincott Williams and Wilkins. 2006; 996.
13. William R Miller, Jose M Munita, and Cesar A Arias. Mechanisms of antibiotic resistance in enterococci. *Expert Rev Anti Infect Ther* 2014;12(10):1221–1236.
14. Galimand M, Schmitt E, Panvert M, et al. Intrinsic resistance to aminoglycosides in Enterococcus faecium is conferred by the

16S rRNA m5C1404-specific methyltransferase EfmM. RNA. 2011; 17:251–62. [PubMed: 21159796]
15. Hawkey P. Mechanisms of quinolone action and microbial response. J *Antimicrob Chemother* 2003;51(1):29–35. [PubMed: 12702701]
16. Hooper D. Mechanisms of action and resistance of older and newer fluoroquinolones. *Clin Infect Dis* 2000;31(2):S24–28. [PubMed: 10984324]
17. K. Suresh et al., Isolation, Speciation and Determination of High Level Aminoglycoside Resistance of Enterococci. *National J Lab Med* 2013;2(1):12-15.
18. Vishal Shete, Naveen Grover, and Mahadevan Kumar. Analysis of Aminoglycoside Modifying Enzyme Genes Responsible for High-Level Aminoglycoside Resistance among Enterococcal Isolates. *J Pathog* 2017:P1-5.
19. Varun Goel, Dinesh Kumar, Rajendra Kumar, Purva Mathur, Sarman Singh. Community Acquired Enterococcal Urinary Tract Infections and Antibiotic Resistance Profile in North India. *J Lab Physicians* 2016;8:50-54.
20. Sanal C. Fernandes, H. and Dhanashree, B.2013. Drug resistance and virulence determinants in clinical isolatesof Enterococcus species. *Indian J Med Res* 137:981-985.
21. Yaeghob Sharifi, Alka Hasani, Reza Ghotaslou, Behrouz Naghili, Mohammad Aghazadeh, Mortaza Milani, Ahad Bazmani. Virulence and Antimicrobial Resistance in Enterococci Isolated from Urinary Tract Infections. *Adv Pharma Bull* 2013;3(1):197-201.
22. Saraswathy MP. Multidrug resistant Enterococci isolated from urine samples at a tertiary care hospital. *Indian J Microbiol Res* 2015;2(4):214-219.

How to cite this article: Naruka HS, Chand AE, Meena H, Prevalence of various enterococcus species and their antibiotic resistance pattern among urinary isolates in tertiary care center in South Eastern Rajasthan, *Int J Med Microbiol Trop Dis* 2019;5(1):18-22