



“STUDY OF BACTERIOLOGICAL PROFILE AND ANTIBIOTIC SENSITIVITY PATTERN AMONG URINARY ISOLATES,,

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ABSTRACT **Background:** The study was performed to determine the bacteriological profile and antibiotic sensitivity pattern of culture sample of patients with cancer, the study was undertaken to formulate an antibiotic policy for the treatment of infection.

The various enterococcus species and their antibiotic resistance pattern among urinary isolates.

Materials and Methods: This observational study was carried out in the Department of Microbiology Patna medical college and hospital patna, Bihar, A total of 220 Enterococcus species isolated from urine samples were included in the study. Only one isolate per patient was included in the study.

Results: A total of 220 cases were sent for urine culture and sensitivity test the high incidence of enterococcal UTI among females (135) compare to males (85) So Male to Female ratio was 0.63. Isolation of enterococci was maximum among population between age group of 30- 40 (90) followed by 40-50 (36) and 20- 30 (30). Most common species found in present study was Enterococcus faecalis 130 (59.09%) followed by *E. faecium* 60 (27.27%), *E. durans* 20 (9.09%) and *E. avium* 10(4.54%). 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 (88.18%), followed by vancomycin sensitive to (65%) on isolates, Nitrofurantoin sensitive to (51.36%) on isolates, Ampicillin sensitive to (36.36%) isolates, Piperacillin sensitive to (32.27%) isolates, and other was least effective.

Conclusion: uti is most commonly seen in female of reproductive age group and most common causative agent organism is *E. coli*, *E. faecalis* and *E. faecium* found to be the most prevalent species which confer resistance to various groups of antibiotics.

KEYWORDS : Culture, Escherichia coli, Multidrug Resistance; High-level Gentamicin Resistance; Staphylococcus aureus, sensitivity, urinary tract

INTRODUCTION

Urinary tract infection is one of the commonest infection occurring in all age groups worldwide it is more common in females as compared to males, especially females of reproductive age group, Enterococci contain a C-carbohydrate that reacts with Lancefield Group D antisera. This study was conducted to determine the bacteriological profile and antibiotic sensitivity patterns in uti and the disease associated with it, they were considered Group D Streptococci.¹

DNA analysis and other properties have placed them in their own genus. Enterococci are regular inhabitants of the bowel. They are found in the intestine of nearly all animals, from cockroaches to humans. Enterococci are readily recovered outdoors from vegetation and surface water probably because of contamination by animal excrement or untreated sewage. In humans, typical concentrations of Enterococci in stool are up to 10⁸ CFU per gram. Although the oral cavity and vaginal tract can become colonized, Enterococci are recovered from these sites in fewer than 20% of cases.² Enterococci, leading cause urinary tract infection (UTI), are becoming resistant to many and sometimes all standard therapies. Enterococci are not very virulent, but they have become prominent as a cause of nosocomial infections as a result of their multiple antibiotic resistance.³ *Enterococcus species* are Gram positive cocci that have emerged over the last decades as very important opportunistic nosocomial pathogens causing Urinary tract infections (UTI).⁴ Currently they are an important cause of nosocomial infections with increasingly common isolates that are resistant to multiple antibiotics.¹ The Centre for Disease Control (CDC) and Prevention's National Nosocomial Surveillance Survey listed Enterococci as the second most common cause of nosocomial UTI.⁵ The genus Enterococci includes many species, but commonly implicated species in human infections are *E. faecalis* and *E. faecium*.⁶ Recently there is an increase in the rate of isolation of *E. faecium* and other species from clinical specimens.⁷

The natural ability of enterococci to acquire, accumulate, and share extra chromosomal elements encoding virulence traits or antibiotic resistance genes, in part, explains their increasing importance as nosocomial pathogens.^{8,9} Acquired resistance to various antimicrobial agents and available antibiotics currently limits the therapeutic options.⁸ The increasing resistance to antibacterial agents such as penicillin, aminoglycosides, trimethoprim, and also to glycopeptides such as vancomycin and teicoplanin, created an increasingly worrisome problem in clinical practice. Furthermore *Enterococci* have

different mechanisms for the transfer of resistance genes, to other more pathogenic Gram positive bacteria such as *Staphylococcus aureus* which is very important clinically. Since some species such as *E. casseliflavus* and *E. gallinarum* are less commonly associated with clinical infections and are inherently resistant to glycopeptides, screening for resistant strains by clinical laboratories are recommended in order to identify them to the species level.¹⁰

It is crucial to provide accurate and complete description of antimicrobial susceptibility pattern and current possibility for treating *Enterococcal* urinary tract infection. Therefore the purpose of our current study is to determine the antimicrobial susceptibility pattern of *Enterococcus spp* isolated from patients with urinary tract infection.

MATERIALS AND METHODS

This was a observational study conducted in the Department of Microbiology at patna medical college and hospital patna, bihar, A total of 220 isolates were collected during the study period and processed a Microbiology Laboratory, Department of Microbiology.

A total of 220 Enterococcus species isolated from urine samples were included in the study. Only one isolate per patient was included in the study.

Early morning Clean Catch Midstream Urine Samples were collected into a wide mouthed sterile screw capped container from clinically suspected patients. Urine samples were cultured over routine culture media; MacConkey agar and Cysteine Lactose Electrolyte Deficient agar with a sterile standard loop. These plates were incubated aerobically at 37°C for 24 to 48 hours. Urinary tract infection was defined as the presence of $\geq 10^5$ colony forming units per mL in the culture of an appropriately collected urine specimen. Enterococci was identified by standard microbiological methods including Gram staining, colonial morphology, growth in 6.5% sodium chloride broth and esculin hydrolysis.¹¹ The isolates were subjected to antimicrobial susceptibility testing by Kirby-Bauer disk diffusion method, as per Clinical and Laboratory Standards Institute (CLSI) recommendations using commercially available 6mm disks (HIMEDIA, Mumbai, India) on Mueller Hinton agar^{12,13}. Antibiotic discs (Himedia) used for testing susceptibility of enterococcal isolates were Ampicillin 10µg, Piperacillin 75 µg, Ciprofloxacin 5 µg, Norfloxacin 10µg, Nitrofurantoin 300 µg, Vancomycin 30 µg, Linezolid 30 µg, Gentamicin high content 120 µg.

RESULTS

A total 220 patients clinically suspected of having UTI were included in this study and sent for culture sensitivity tes, a high incidence of enterococcal UTI among females (135) compare to males (85) So Male to Female ratio was 0.63. Isolation of enterococci was maximum among population between age group of 30- 40 (90) followed by 40-50 (36) and 20-30 (30). (Table- 1) Most common species found in present study was *Enterococcus faecalis* 130 (59.09%) followed by *E. faecium* 60 (27.27%), *E. durans* 20 (9.09%) and *E. avium* 10(4.54%). 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 (88.18%), followed by vancomycin sensitive to (65%) on isolates, Nitrofurantoin sensitive to (51.36%) on isolates, Ampicillin sensitive to (36.36%) isolates, Piperacillin sensitive to (32.27%) isolates, and other was least effective . High level Gentamicin was tested for HLAR detection, this

was sensitive to (43.18%) on isolates.

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

Age in years	Male	Female	Total
Below 10	4	8	12
10-20	5	9	14
20-30	10	20	30
30-40	35	55	90
40-50	15	21	36
50-60	11	17	28
Above 60	5	5	10
Total	85	135	220

Antibiotics sensitivity pattern among different *Enterococcus* Species .Antibiotic susceptibility pattern among various enterococcus species has been shown in following Table-2

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

Enterococcus spp.		Amp* (%)	Pip* (%)	Cip* (%)	Nor* (%)	Nit* (%)	Van* (%)	Lz* (%)	HLG* (%)
	S	49 (37.69)	43 (33.07)	35 (26.92)	18 (13.07)	81 (62.31)	88 (67.69)	112 (86.15)	58 (44.62)
<i>E. faecalis</i> N=130	I	0 (0)	20 (15.38)	5 (3.85)	2 (1.54)	5 (3.85)	24 (18.46)	16 (12.31)	0 (0)
	R	81 (62.31)	67 (51.54)	91 (70)	111 (85.38)	44 (33.85)	18 (13.85)	2 (1.54)	72 (55.38)
	S	14 (23.33)	13 (21.67)	4 (6.67)	4 (6.67)	12 (20)	31 (51.67)	54 (90)	11 (18.33)
<i>E. faecium</i> N =60	I	0(0)	4(6.67)	0	0(0)	1(1.67)	14(23.33)	3(5)	0(0)
	R	46 (76.67)	43 (71.66)	56 (93.33)	56 (93.33)	47 (78.33)	15 (25)	3 (5)	49 (81.67)
	S	10 (50)	10 (50)	6 (30)	8 (40)	14 (70)	18 (90)	20 (100)	18 (90)
<i>E. durans</i> N=20	I	0 (0)	3 (15)	1 (5)	1 (5)	1 (5)	2 (10)	0 (0)	0 (0)
	R	10 (50)	7 (40)	13 (65)	11 (55)	5 (25)	0 (0)	0 (0)	2 (10)
	S	7 (70)	5 (50)	5 (50)	2 (20)	6 (60)	6 (60)	8 (80)	8 (80)
<i>E. avium</i> N=10	I	0 (0)	2 (20)	0 (0)	1 (10)	1 (10)	1 (10)	1 (10)	0 (0)
	R	3 (30)	3 (30)	5 (50)	7 (70)	3 (30)	3 (30)	1 (10)	2 (20)
	S	7 (70)	5 (50)	5 (50)	2 (20)	6 (60)	6 (60)	8 (80)	8 (80)

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

E. faecalis show highest sensitivity for Linezolid 112(86.15%) followed by Vancomycin 88(67.69%), Nitrofurantoin 81(62.31%), Ampicillin 49(37.69%), Piperacillin 43(33.07%), Ciprofloxacin 35(26.92%) and least for Norfloxacin 18(13.07%). *E. faecium* show highest sensitivity for Linezolid 54 (90%) followed by Vancomycin 31(51.67%), Ampicillin 14(23.33%), Piperacillin 13(21.67%), Nitrofurantoin 12(20%), Ciprofloxacin and Norfloxacin show equal sensitivity 4(6.67%). *E. durans* show highest sensitivity for Linezolid 20(100%) followed by Vancomycin 18(90%), Nitrofurantoin 14(70%), Ampicillin and Piperacillin show equal sensitivity 10(50%), Norfloxacin 8(40%), and least for Ciprofloxacin 6(30%). *E. avium* showed highest sensitivity for Linezolid 8(80%) followed by ampicillin, 7(70), vancomycin 6(60%), Piperacillin and Nitrofurantoin show same result 5(50%) and least for Norfloxacin 2(20%). High level Gentamicin was used to detect high level resistance for aminoglycosides among all enterococcus species *E. faecium* isolates show maximum resistance for aminoglycoside 49(81.67%), followed by *E. faecalis* 72(55.38%), *E. avium* 2(20%) and *E. durans* 2(10%).

DISCUSSION

UTI is a common problem faced by clinicians in every part of the world irrespective of the age and sex. 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 220 urinary isolates of enterococci were studied. The present study shows a high incidence of enterococcal UTI among

females (135) compare to males (85), 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 comparison to other study. Norfloxacin resistance was higher in present study. Ampicillin resistance in present study is higher also higher among *E. faecalis* in present study compared to study done by varun goel et al.¹⁴

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.¹⁵

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.^{16,17} Vancomycin resistance among *E. faecalis* in Linezolid resistance among *E. faecalis* in present study was 1.67% which is higher than other studies.

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 al¹⁸ , Saraswathy MP et al.¹⁹ and was higher than study of Varun goel et al.¹⁴

E. faecium shares all resistance mechanism with *E. faecalis* in addition

efflux pump mediated by *NorA* gene Amoxycilin sensitivity was 60.6% in our study where as other studies showed resistance to amoxycilin as high as 77.4% and 84%. Sensitivity to amikacin was 56.4% in our study which is low compared to study done, where sensitivity was as high as 82.6%. patients with clinical diagnosis of cystitis, pyelonephritis, Gentamicin resistance for *E. faecium* was 81.67% also highest compared to other species in present and other studies. Vancomycin resistance among *E. faecium* 25% which is higher than other studies.^{14,15} Linezolid resistance was highest 5% 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 cannot be done.

CONCLUSION

UTI going into a complicated one, *E. coli* is the most common organism isolated in urine culture in most of the hospitals. *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 than *E. faecalis*.

REFERENCES

- Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC, editors. The gram positive cocci part II: Streptococci, Enterococci, and the Streptococci-like bacteria. Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Ch. 13. New York: JB Lipincott; 1997. p. 673-764.
- Available from: <http://www.microbewiki.kenyon.edu/index.php/enterococcus>. [Last accessed on 2014 May, 28].
- Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC, editors. Antimicrobial susceptibility testing. Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. New York: JB Lipincott; 1997. p. 945-1021.
- Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA. Manual of Clinical Microbiology. 10th edition. Washington D.C. ASM Press; 2011.
- Mendiratta DK, Kaur H, Devtale V, Thakare VC, Narang R, Narang P. Status high level aminoglycoside resistance in Enterococcus faecium and Enterococcus faecalis in rural hospital in central India. Journal of Clinical Microbiology 2008; 26(4): 369-71.
- Morrison D, Woodford N, Cookson B. Enterococci as emerging pathogens of humans. Journal of Applied Microbiology Symposium Supplement. 1997; 83:89-99.
- Desai PJ, Pandith D, Mathur M, Gogate A. Prevalence, Identification and distribution of various species of Enterococci isolated from clinical samples with special reference to urinary tract infections in catheterized patients. Indian Journal of Medical Microbiology 2001; 19:132-137.
- Sharifi Y, Hasani A, Ghotaslou R, Naghili B, Aghazadeh M, Milani M, Bazmani A. Virulence and antimicrobial resistance in enterococci isolated from UTI. 2013;3(1):197-201
- Klibi N, Gharbi S, Masmoudi A, Ben Slam K, Poeta P, Zaranga M, et al. Antibiotic resistance and mechanisms implicated in clinical Enterococci in a Tunisian hospital. Jour of Chemo 2006; 18(1):20-6.
- Mansouri SH, Shkibaie MR, Mahboob. Antibiotic Susceptibility in Enterococci isolated from patients in Kerman, Southeaster Iran. Iran Journal of Medical Science 2005; 30(2): 68-72.
- Fackland R, Sham DA, Teixeira LM: Enterococcus. In Manual of Clinical Microbiology. 7th edition. Edited by: Murray PR, Baron EJ, Pfaller MA, T enover FC, Tenover FC, Tenover FC. Washington: American Society of Microbiology; 1999:297-305.
- Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing-fourth informational supplement (Update). CLSI document. Wayne: CLSI; 2014: M100-S24.
- Washing Winn, Stephen Allen, William Janda, Elmer Koneman, Gary Procop, Paul Schreckenberger. Antimicrobial susceptibility testing. Koneman's colour at las and textbookof diagnost ic microbiology. th 6 ed. Lippincott Williams and Wilkins. 2006; 996.
- 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.
- Sanal C. Fernandes, H. and Dhanashree, B.2013. Drug resistance and virulence determinants in clinical isolates of Enterococcus species. Indian J Med Res 137:981-985
- 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.
- 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
- 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.
- Saraswathy MP. Multidrug resistant Enterococci isolated from urine samples at a tertiary care hospital. Indian J Microbiol Res 2015;2(4):214-219.
- Hawkey P. Mechanisms of quinolone action and microbial response. J Antimicrob Chemother 2003;51(1):29-35.
- Hooper D. Mechanisms of action and resistance of older and newer fluoroquinolones. Clin Infect Dis 2000;31(2):S24-28
- K. Suresh et al., Isolation, Speciation and Determination of High Level Aminoglycoside Resistance of Enterococci. National J Lab Med 2013;2(1):12-15.