

A STUDY TO DETERMINE THE PREVALENCE OF VARIOUS ENTEROCOCCUS SPECIES AND THEIR ANTIBIOTIC RESISTANCE PATTERN AMONG URINARY ISOLATES: AN OBSERVATIONAL INVESTIGATION.

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Abstract

Aim: To determine the prevalence of various enterococcus species and their antibiotic resistance pattern among urinary isolates.

Materials and Methods: The samples were collected from all hospitalized patients department of microbiology, Netaji Subhas Medical College and Hospital, Bihta, Patna, Bihar India from Oct 2019 to March 2020. A total of 200 Enterococcus species isolated from urine samples were included in the study. Only one isolate per patient was included in the study.

Results: The present study shows a high incidence of enterococcal UTI among females (120) compare to males(80) so Male to Female ratio was 0.66. Most common species found in present study was Enterococcus faecalis 124 (62%) followed by *E. faecium* 56 (28%), *E. durans* 14 (7%) and *E. avium* 6 (3%). 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 (87.50%), followed by vancomycin sensitive to (64.50%) on isolates, Nitrofurantoin sensitive to (50.50%) on isolates, Ampicillin sensitive to (36%) isolates, Piperacillin sensitive to (31%) isolates, and other was least effective. High level Gentamicin was tested for HLAR detection, this was sensitive to (42%) on isolates.

Conclusion: *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*.

Introduction:

Urinary tract Infection is one of the most common infectious conditions in clinical practice and an important cause of nosocomial infection. *E. coli*, other Gram negative rods and *Staphylococcus saprophyticus* are the most frequent infecting organisms of the urinary tract^{1,2}. *Enterococcus* species are Gram positive cocci that have emerged over the last decades as very important opportunistic nosocomial pathogens causing Urinary tract infections (UTI)^{3,4}. Currently they are an important cause of nosocomial infections with increasingly common isolates that are resistant to multiple antibiotics¹. The Centre for Disease Control (CDC) and Prevention's National Nosocomial Surveillance Survey listed Enterococci as the second most common cause of nosocomial UTI⁵. 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 specimens⁷.

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 options⁸. 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 level¹⁰.

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.

Methods and Materials

A competitive evaluation study was carried out in the department of microbiology, Netaji Subhas Medical

College and Hospital, Bihta Patna, Bihar India from Oct 2019 to March 2020, after taking the approval of the protocol review committee and institutional ethics committee. A total of 200 isolates were collected during the period of 6 months processed at a Microbiology Laboratory, Department of Microbiology.

A total of 200 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 were 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

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

Age	Male	Female	Total
0-10	3	8	11
11-20	5	8	12
21-30	13	15	28
31-40	30	49	79
41-50	13	18	31
51-60	10	18	28
Above 60	6	5	11
Total	80	120	200

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

Enterococcus spp.		Amp* (%)	Pip* (%)	Cip* (%)	Nor* (%)	Nit* (%)	Van* (%)	Lz* (%)	HLG* (%)
<i>E. faecalis</i> N=124	S	48 (38.70)	42 (33.87)	34 (27.41)	18 (14.51)	78 (62.90)	85 (68.54)	108 (87.09)	56 (45.16)
	I	0 (0)	20 (16.13)	5 (4.03)	2 (1.61)	4 (3.23)	22 (17.74)	14 (11.29)	0 (0)
	R	76 (61.30)	62 (50)	85 (68.54)	104 (83.87)	42 (33.87)	17 (13.70)	2 (1.61)	68 (54.84)
<i>E. faecium</i> N=56	S	13 (23.21)	12 (21.43)	4 (7.14)	4 (7.14)	11 (19.64)	28 (50)	50 (89.28)	10 (17.86)
	I	0 (0)	4 (7.14)	0 (0)	0 (0)	1 (1.78)	14 (25)	3 (5.35)	0 (0)
	R	43 (76.79)	40 (71.43)	52 (92.86)	52 (92.86)	44 (78.57)	14 (25)	3 (5.35)	46 (82.14)
<i>E. durans</i> N=14	S	7 (50)	7 (50)	4 (28.57)	5 (35.71)	9 (68.28)	13 (92.85)	14 (100)	13 (92.85)
	I	0 (0)	2 (14.29)	1 (7.14)	1 (7.14)	1 (7.14)	1 (7.14)	0 (0)	0 (0)
	R	7 (50)	5 (35.71)	9 (64.28)	8 (57.14)	4 (28.57)	0 (0)	0 (0)	1 (7.14)
<i>E. avium</i> N=6	S	4 (66.67)	3 (50)	3 (50)	1 (16.67)	3 (50)	3 (50)	3 (50)	5 (83.33)
	I	0 (0)	1 (16.67)	0 (0)	1 (16.67)	1 (16.67)	1 (16.67)	1 (16.67)	0 (0)
	R	2 (33.33)	2 (33.33)	3 (50)	4 (66.67)	2 (33.33)	2 (33.33)	2 (33.33)	1 (16.67)

*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 especially in hospital environment and can cause different type of infection which is 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 200 urinary isolates of enterococci were studied during the period of 6 month from Oct 2019 to March 2020, among them more cases were from females (120) then males (80) 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

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.^{16,17} Vancomycin resistance among *E. faecalis* in present study is comparatively higher than a study from south India done by Sanal C. Fernandes et al.¹⁵ and lower from study of north India AIIMS done by varun goel et al.¹⁴

Linezolid resistance among *E. faecalis* in present study was 1.67% 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.¹⁸, 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.^{20,21}

Ampicillin resistance was considerably higher in present study as compare to most of studies except study done by Yaeghob Sharifi et al.¹⁸ which showed higher resistance than present study, similarly Piperacillin resistance was also high 53.50% in present study. Resistance to Nitrofurantoin was found highest among *E. faecium* 78.57% in present study and other compared studies.¹⁹

Gentamicin resistance for *E. faecium* was 82.14% also highest compared to other species in present and other studies.^{22,23} Vancomycin resistance among *E. faecium* 25% which is higher than other studies.^{14,15} Linezolid resistance was highest (5.35) among *E. faecium* than 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

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.

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