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Original Research Article

Antibiotic resistance pattern of *Enterococcal* isolates from patients with urinary tract infectionKalpana Devi Venkatesan^{*1}, Senthil Chander², Ananthi B¹, Sophia Abigail¹ and Kalavathy Victor¹¹Department of Microbiology, A.C.S Medical College and Hospital, Chennai, Tamil Nadu, 600077 India²Department of General Medicine, A.C.S Medical College and Hospital Chennai, Tamil Nadu, 600077 India

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Chennai, Tamil Nadu, 600077 India***Article History:****Received:** 24/05/2017**Revised:** 08/06/2017**Accepted:** 10/06/2017**DOI:** <https://doi.org/10.7439/ijbr.v8i6.4182>**Abstract****Background:** *Enterococci* are the most common cause of healthcare associated urinary tract infections. The frequency of isolation of *Enterococci* from urinary tract of hospitalized patients has risen. *Enterococcus* is considered as an important nosocomial pathogen because of its intrinsic as well as acquired resistance to antibiotics. The purpose of this study is to determine the emergence of *Enterococci* as uropathogens and their antimicrobial susceptibility pattern.**Materials and methods:** This cross sectional study was conducted at the Department of Microbiology, A.C.S Medical College and Hospital. A total of 175 *Enterococcus* spp were isolated from patients with urinary tract infection. Identification and speciation of the *Enterococcal* isolates were done using conventional method. The antibiotic susceptibility testing of the *Enterococcal* isolates was performed by modified Kirby-Bauer disk diffusion method as per Clinical Laboratory Standard Institute (CLSI) guidelines.**Results:** A total of 175 urine specimens yielding the growth of *Enterococci* were studied. *Enterococcus faecalis* (77.7%) was the predominant species isolated followed by *E. faecium* (20%) and *E. durans* (2.3%). The sensitivity pattern of the isolates showed an increased resistance to antibiotics like erythromycin (66.3%), ciprofloxacin (56%) and penicillin (49.7%). The HLAR (High Level Aminoglycoside Resistance) to gentamycin was observed in 37.2%, however the susceptibility rate of nitrofurantoin was 86.9%, amoxicillin - clavulanic acid was 81.1% and ampicillin (21.7%). Among the isolated *Enterococci*, 5.7% were vancomycin resistant. All the *Enterococci* were found to be sensitive to linezolid.**Conclusion:** *Enterococci* have emerged from being harmless commensals to versatile lethal pathogens. In our study, *E. faecalis* was the most frequently isolated species followed *E. faecium*. Linezolid, vancomycin, nitrofurantoin and amoxicillin-clavulanic acid were found to be the most effective antimicrobials against *Enterococci* isolated from patients with UTI. However vancomycin or linezolid therapy should be restricted for use in patients infected with MDR strains only. Although the frequency of isolation of VRE is not very high in our setting, this may be the beginning. Tracking of antimicrobial susceptibility due to increasing resistance rate is very important in treatment of *Enterococcal* infections.**Keywords:** *Enterococci*, Urinary Tract Infection, Vancomycin Resistance.**1. 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 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.

2. Materials and methods

The present study was a prospective cross sectional study conducted in the Department of Microbiology at A.C.S Medical College and Hospital, Chennai over a 1 year period from January, 2016 to December, 2016. A total of 175 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. The disks used were Penicillin (10U/disc), Ampicillin (10µg), Erythromycin (15µg), Amoxycillin- clavulanic acid (30µg), Nitrofurantoin (300µg), Vancomycin (30µg) and Linezolid (15µg). For High Level Gentamycin Resistance (HLGR) detection, gentamycin 120µg disk was used. Isolates were interpreted as susceptible, intermediate or resistant according to the sensitivity zones of the particular antimicrobial as recommended by CLSI. HLGR was indicated by no zone, and susceptibility, by a zone of diameter of ≥ 10 mm. [12]

3. Results

Among the 175 *Enterococcal* isolates, 136 (77.7%) were from female patients while 39(22.3%) were from male patients. All the isolates were further speciated as *E. faecalis*(77.7%), *E. faecium* (20%) and *E. durans*(2.3%). (Fig-1) Antibiotic susceptibility testing of the isolates were done by Kirby Bauer disk diffusion method. The antibiotic susceptibility pattern of the *Enterococcal* isolates is shown in Table-1. Among the total isolates, resistance to erythromycin was 66.3%, ciprofloxacin-56%, penicillin-49.7%. The HLAR to gentamycin was observed in 37.2% isolates with 29.4% in *E. faecalis* and 65.7% in *E. faecium*. However the sensitivity to nitrofurantoin was 86.9%, amoxicillin -clavulanic acid 81.1% and ampicillin-78.3%. Linezolid has the 100% susceptibility followed by vancomycin with 94.3%.

4. Discussion

Enterococci have become important nosocomial pathogens worldwide and are associated with a high mortality. [13] *E. faecalis* is the predominant *Enterococcal* species, which accounts for 80-90% of all clinical isolates, which is followed by *E. faecium*(5-15%).[14,15] However a progressive increase in *E. faecium* infections has been reported.[3] In our study, *E. faecalis*(77.7%) was the major species isolated followed by *E. faecium* (20%) and *E. durans* (2.3%). Our observations on the incidence of Enterococcus spp. were comparable with previous reports. [8,16,17]

In hospital settings, *Enterococci* have emerged as one of the leading therapeutic challenges because of the intrinsic as well as the ever increasing acquired antibiotic resistance. The intrinsic resistance of *Enterococci* involves

cotrimoxazole, aminoglycosides and cephalosporins which are commonly used to treat UTI. Acquired antimicrobial resistance is also important. Because of the indiscriminate use of antibiotics, *Enterococci* have acquired resistance against several classes of antimicrobial agents, including chloramphenicol, tetracyclines, glycopeptides, quinolones and nitrofurantoin.[17]

Knowledge of the antimicrobial resistance profile is essential to formulate treatment guidelines for infections caused by *Enterococci*. In the present study, highest prevalence of resistance was observed against erythromycin (66.3%) followed by ciprofloxacin (56%). Almost half of the isolates were resistant to penicillin (49.7%). However amoxicillin- clavulanic acid showed a higher susceptibility rate of 81.1% followed by ampicillin showing 78.3%. The HLAR to gentamycin was observed in 37.2%. HLAR to gentamycin is universally reported in the range of 1-48%[18] although with an increasing trend recently.[19] HLAR to gentamicin nullifies the efficacy of combination therapy, which is used to treat serious *Enterococcal* infections. Nevertheless, empirically chosen combination therapy with ampicillin and gentamycin would be effective in 54.25% of nosocomial infections. Hence it is necessary to distinguish the HLAR strains from simply resistant strains. [18]

In the present study, susceptibility pattern of Enterococci against nitrofurantoin is very promising. Most of the *Enterococcal* isolates (86.9%) were susceptible to nitrofurantoin, the result being consistent with the several other studies which have shown susceptibility rate of 86.44%[20] and 81% [17]. However Yadav *et al*[21] has shown a lower susceptibility rate of 36.84% whereas Sanjay *et al*[16] has shown that 93% of the isolates were susceptible to nitrofurantoin which is slightly higher than the present study. Further in this study, most of the isolates which were found to be resistant to other available antibiotics were found susceptible to nitrofurantoin. In this era of ever increasing antimicrobial resistance it is mandatory that such antimicrobials are given due importance.

An antimicrobial which has given increasingly encouraging results is Linezolid as all the isolates (100%) remained susceptible to this antimicrobial. Vancomycin Resistant Enterococci have been increasingly reported from all parts of the world. In our study, 5.7% of the *Enterococcal* isolates were found to be resistant to Vancomycin which showed significant similarity to results reported from other studies ranging from 1.7%-20% in tertiary care hospitals in other parts of India.[16,17,21,22] The present method of VRE detection needs confirmation by MIC value estimation and detection of resistance encoding gene by molecular methods. The emergence of VRE has been attributed to the

inprudent use of vancomycin, colonization pressure and noncompliance with the infection control measures.[23] CDC Hospital Infection Control Practices Advisory Committee (HICPAC) had published recommendations to control the nosocomial transmission of VRE.[5] Aim of this recommendation is to minimize nosocomial transmission of VRE; hospitals must use a multidisciplinary approach. Tracking of antimicrobial susceptibility due to increasing resistance rate is very important in treatment of *Enterococcal* infections.

5. Conclusion

In our study, linezolid, vancomycin, nitrofurantoin and amoxicillin-clavulanic acid were found to be the most effective antimicrobials against Enterococci isolated from patients with UTI. However vancomycin or linezolid therapy should be restricted for use in patients infected with MDR strains only. Although the frequency of isolation of VRE is not very high in our setting, this may be the beginning. By increasing awareness regarding drug resistance and use of proper antimicrobials, further emergence of VRE and multidrug resistant Enterococci can be reduced.

Figure 1: Species distribution of the Enterococcal isolates

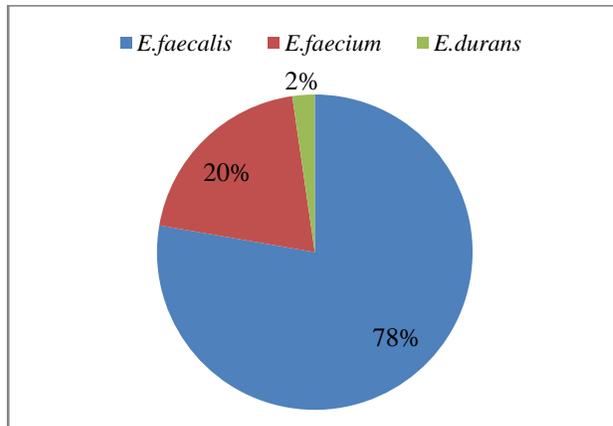


Table 1: In vitro susceptibility pattern of Enterococcus spp. isolated as uropathogens

Antibiotics	Sensitive N (%)	Intermediate N (%)	Resistant N (%)
Ampicillin	137(78.3)	-	38(21.7)
Amoxyclav	142(81.1)	03(1.7)	30(17.2)
Penicillin	88(50.3)	-	87(49.7)
Erythromycin	50(28.6)	09(5.1)	116(66.3)
Ciprofloxacin	63(36)	14(8)	98(56)
High level Gentamycin	108(61.7)	02(1.1)	65(37.2)
Nitrofurantoin	152(86.9)	-	23(13.1)
Vancomycin	165(94.3)	-	10(5.7)
Linezolid	175(100)	-	-

