

# ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF ENTEROCOCCUS SPECIES ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTION

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## 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. Vancomycin resistant enterococci along with other multidrug resistant bacteria are the main concerns for physicians. Enterococci may also act as vehicle for spread of resistant genes. The objective of this study was to determine the antimicrobial susceptibility pattern of different enterococcal species isolated from urine samples.

**Material & Methods:** This cross-sectional study was conducted at Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi. Hundred enterococcal isolates cultured from urinary specimens were processed from October 2011 to March 2012. In-vitro drug-susceptibility tests of enterococci isolated were performed on Mueller Hinton agar.

**Results:** A total of 100 urine specimens yielding growth of enterococci were studied. Among these only 7 samples were from OPD, the rest were from different wards. The mean age was  $55 \pm 24.58$  (range 8-89) years. Sixty-six (66%) of the isolates were from males while 34(34%) from females. Enterococcus faecalis was isolated in 81(81%) cases while Enterococcus faecium in 19(19%). Regarding sensitivity, 100% isolates were susceptible to linezolid, 99% to teicoplanin, 97% to vancomycin, 88% to imipenem, 87% to co-amoxiclav, 81% to nitrofurantoin, 75% to ampicillin, whereas the susceptibility to ciprofloxacin and tetracycline was 42% and 38% respectively.

**Conclusion:** Enterococci have emerged from being harmless commensals to versatile lethal pathogens. The rising antibiotics resistance is worrisome as the commonly used antibiotics for the treatment of nosocomial UTI are less effective.

**KEY WORDS:** Enterococcus; Enterococcus faecalis; Enterococcus faecium; Urinary tract infections; Urine culture; Antibiotics.

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## INTRODUCTION

Urinary tract infection (UTI), a very common disease among general practice patients is caused by various Gram positive and Gram negative bacteria. Enterococcus species are Gram positive cocci (GPCs) that have emerged, over the last decades, as very important opportunistic nosocomial pathogens causing UTIs.<sup>1,2</sup> The two most common Enterococcus species are Enterococcus faecalis and Enterococcus faecium. Enterococci are widespread in nature. In humans, they are the most abundant Gram

positive cocci colonizing the intestine. From GIT, they may migrate to cause infections including those of urinary tract. The risk factors for enterococcal UTI include old age, diminished host immunity, the use of broad-spectrum antibiotics and indwelling catheters. Enterococci have been implicated in approximately 10% of all UTIs and in up to approximately 16% of nosocomial UTIs.<sup>2,3</sup> Vancomycin Resistant Enterococci (VRE) along with multidrug resistant Mycobacterium tuberculosis (MDR-MTb), extended spectrum beta lactamase (ESBL) and metallo-beta lactamase (MBL) producing isolates and methicillin resistant *S. aureus* (MRSA) are the main concerns for today's physician. VREs are not only resistant to treat but they may act as vehicle for spread of resistant genes to other species especially *S. aureus*.<sup>2</sup>

There are several important characteristics of the enterococci due to which they grow and survive

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in harsh conditions. Their ability to form biofilms is of particular importance in the development of urinary tract infections especially in catheterized patients.<sup>4-6</sup> 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 UTIs and other enterococcal infections. Acquired antimicrobial resistance is also important. It allows virulent enterococci to survive for extended periods of time in hospital settings.<sup>7-9</sup> Because of the indiscriminate use of antibiotics enterococci have acquired resistance against several classes of antimicrobial agents, including chloramphenicol, tetracyclines, glycopeptides, quinolones and nitrofurantoin.<sup>10,11</sup>

The irrational use of antibiotics in our set up has immensely contributed to the antimicrobial resistance and emergence of multidrug resistant urinary isolates.<sup>12</sup> The objective of this study was to determine the antimicrobial susceptibility pattern of different enterococcal species isolated from urine samples as it would help in making optimal empirical choices. Moreover a policy for the judicious use of high-risk antibiotics can be devised which would definitely be helpful in delaying the emergence of more resistant and virulent strains of enterococci.

## MATERIAL AND METHODS

This laboratory based cross-sectional study was carried out at the Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan from October 2011 to March 2012. The specimens were collected from Combined Military Hospital, Military Hospital and Armed Forces Bone Marrow Transplant Centre, Rawalpindi.

Non-probability consecutive sampling technique was used and 100 *Enterococcus* species isolated from urine samples were included in the study. Isolates from the same patients were excluded from the study.

Mid stream urine samples were collected in a sterile screw capped containers. Urine microscopy was done for pus cells and was cultured with semi-quantitative dipstick method using bacteruritest strips (Mast Laboratories, Bootle Merseyside, England) on Cysteine lactose electrolyte deficient agar (Oxoid-Unipath Ltd., Basingstocke, UK). The plates were incubated aerobically at 37°C for 24-48 hours. Any significant growth obtained was identified using general appearance of the colonies and characters like pigment production, hemolysis and negative catalase. Gram stain was done to see for the characteristic morphology of Gram positive cocci in pairs or short chains. Entero set consisting of growth on

esculin agar in the presence of 40% bile, 6.5% NaCl and arabinose test was used to identify enterococci. Motility was observed in a wet mount by hanging drop method to detect *Enterococcus casseliflavus* and *Enterococcus gallinarum* which like *E. faecium* ferment arabinose but are motile.<sup>2</sup>

Antimicrobial susceptibility testing of the isolates was carried out using modified Kirby-Bauer disc diffusion method on Mueller-Hinton agar as recommended by Clinical and Laboratory Standards Institute (CLSI).<sup>13</sup> Isolates were interpreted as susceptible or resistant according to the sensitivity zones of the particular antimicrobial as recommended by CLSI.

Age in years and gender were demographic while isolation of enterococci and their sensitivity to different antibiotics were research variables. Mean and standard deviation (SD) were calculated for quantitative variables like age while the other three variables were analyzed for frequency (number) and relative frequency (%).

## RESULTS

A total of 100 urine specimens yielding growth of enterococci were studied. Among these only 7 samples were from OPD, the rest were from different wards. The mean age of these patients was  $55 \pm 24.58$  (range 8-89) years. Sixty-six (66%) of enterococcal isolates were from male patients while 34 (34%) were from female patients. *Enterococcus faecalis* was isolated in 81 (81%) cases while *Enterococcus faecium* in 19 (19%). None of the isolate was motile.

Three of 100 isolates were vancomycin resistant enterococci (VRE) whereas only one isolate was resistant to teicoplanin. The susceptibility pattern of enterococcal species against other antibiotics is given in Table 1.

## DISCUSSION

In an international survey by Kahlmeter G<sup>12</sup>, enterococci were among the leading causative agents of UTI. A study carried out at AFIP, Rawalpindi<sup>14</sup> in 2004 from 144 enterococcal isolates it was found that 99% isolates were susceptible to vancomycin, 91% to imipenem, 88% to nitrofurantoin and 72% to ampicillin. Our results have revealed almost comparable results with slightly lesser susceptibility percentages to the antimicrobials mentioned.

In a Brazilian study<sup>15</sup> carried out in 2009, only 38% were susceptible to ciprofloxacin. Our study also revealed results with quinolones as only 42% of total enterococcal isolates were susceptible to ciprofloxacin. This fact is significant on account of the fact that quinolones are considered as very potent urinary antimicrobial and are being used extensively

Table 1: In vitro susceptibility of the enterococcal isolates to different antimicrobials. (n=100)

S. No.	Antimicrobial disc (Oxoid)	Susceptible	Intermediate	Resistant
1.	Linezolid	100 (100%)	-	-
2.	Teicoplanin	99 (99%)	-	1 (1%)
3.	Vancomycin	97 (97%)	-	3 (3%)
4.	Imipenem	88 (88%)	-	12 (12%)
5.	Co-amoxiclave	87 (87%)	5 (5%)	8 (8%)
7.	Nitrofurantoin	81 (81%)	-	19 (19%)
6.	Ampicillin	75 (75%)	-	25 (25%)
8.	Ciprofloxacin	42 (42%)	4 (4%)	54 (54%)
9.	Tetracycline	38 (38%)	3 (3%)	59 (59%)

in both outdoor and indoor setups.

A similar rising trend of enterococcal resistance to various antibiotics was noted in another Indian study by Shinde et al<sup>16</sup> in a tertiary care hospital in Mumbai.

Our study has revealed very encouraging results for nitrofurantoin as 81% of our isolates were susceptible. In this era of ever increasing antimicrobial resistance it is mandatory that such antimicrobials are given due importance. Clinicians and pharmaceutical industry be encouraged to use and market nitrofurantoin because of its efficacy and low cost. Another antimicrobial which has given increasingly encouraging results is linezolid as all our isolates (100%) remained susceptible to this antimicrobial. It is imperative that clinical trials of linezolid are undertaken for the patients suffering from enterococcal UTIs so that effective empirical treatment strategy could be chalked out for this antimicrobial.

Since this was a cross-sectional study, further standard monitoring is vital to establish consistent information about resistance pattern of enterococci.

## CONCLUSION

Enterococci have emerged from being harmless commensals to versatile lethal pathogens. The rising antibiotics resistance is worrisome as the commonly used antibiotics for the treatment of nosocomial UTI are less effective. Injudicious usage of antibiotics must be curtailed and local antibiotic policies must be formulated.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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None declared.