

## Bacterial Uropathogens with Special Reference to Vancomycin-Resistant Enterococci and their Gastrointestinal Colonization: A Cross-Sectional Study from a Tertiary Care Center

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### Abstract

**Background:** Urinary tract infections are among the most common infectious diseases encountered in clinical practice. Although *Escherichia coli* remains the predominant uropathogen, *Enterococcus* species have emerged as important pathogens, particularly in healthcare-associated infections. The increasing prevalence of Vancomycin-resistant *Enterococcus* is a serious therapeutic and infection control challenge worldwide. This study sought to determine the distribution of bacterial uropathogens, characterize *Enterococcus* species with emphasis on vancomycin resistance and assess gastrointestinal colonization as a potential reservoir for infection.

**Materials and Methods:** In a prospective manner, a total of 250 urine samples were collected from patients presenting with suspected UTIs. Isolation and identification of uropathogens were done using standard microbiological techniques. The *Enterococcus* isolates were screened phenotypically for vancomycin resistance and differentiated into species level, *Enterococcus faecalis* and *Enterococcus faecium*. Stool samples from the patients with enterococcal UTIs were cultured to assess the gastrointestinal colonization.

**Results:** Of the total 250 urine specimens analyzed, 147 (58.8%) had evidence of bacteriuria. *E. coli* was most predominantly isolated (56.5%), followed by *Enterococcus* spp. (25.2%), *Klebsiella pneumoniae* (12.2%), and then by *Staphylococcus* spp. (6.1%). Of the total 37 *Enterococcus* species isolated, 12 (32.4%) were Vancomycin-resistant *Enterococcus* (VRE), and 25 (67.6%) were susceptible to Vancomycin. Of 12 Vancomycin-resistant *Enterococcus* species, 7 (58%) were *E. faecium*, while 5 (42%) were *E. faecalis*. Among the non-VRE species, 15 (60%) were *E. faecium*, and 10 (40%) were *E. faecalis* gastrointestinal colonization were seen.

**Conclusion:** Enterococci species, such as VRE, constitute a large percentage of uropathogens, and their ability to colonize the gastrointestinal tract adds to the importance of the gastrointestinal tract as a reservoir. Strict surveillance is required to contain the spread of resistance among enterococci.

**Keywords:** Urinary Tract Infection, Enterococcus, Vancomycin-Resistant Enterococci, Gastrointestinal Colonization, Antimicrobial Resistance.

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### Introduction

Urinary tract infection (UTIs) ranks among the most frequent bacterial infections worldwide and a

leading source of morbidity in all age groups [1]. UTIs are responsible for huge healthcare costs

associated with antibiotic consumption, which in turn fuels antimicrobial resistance. Even though *Escherichia coli* stands out as the predominant causative agent of community-acquired UTIs, the causative agents of Nosocomial UTIs include multi-drug-resistant organisms [2].

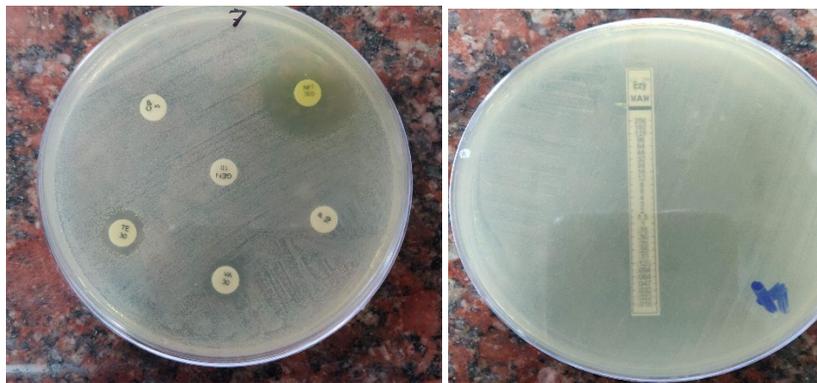
*Enterococcus* spp. are gram-positive cocci and are described as a part of the normal gut flora, but they have emerged as potential opportunistic pathogens<sup>3</sup>. They are now considered one of the top causes for health-care-related infections, including UTIs, bacteraemia, and endocarditis [4]. Among the *Enterococcus* spp., *Enterococcus faecalis* and *Enterococcus faecium* are the most common species [5].

One of the major concerns linked to enterococcal infections is their ability to develop antimicrobial resistance. Another concern associated with enterococcal infections is the development of Vancomycin-resistant *Enterococcus* (VRE). This has become one of the major concerns in hospitals across the world [6,7]. The gastrointestinal tract serves as the primary reservoir for *Enterococcus* colonization and plays a crucial role in the epidemiology and transmission of VRE [8]. Knowing the patterns and means of colonization by uropathogens and resistance patterns plays a critical role in the control and management of infections and antibiotic resistance. This paper was therefore conducted with the aim of analyzing the bacterial spectrum of UTIs with special reference to

*Enterococcus* contamination, vancomycin resistance, and gastrointestinal colonization.

### Materials & Methods

The study was conducted after getting institutional ethical committee approval. The study was carried out in the Department of Microbiology in a tertiary care teaching hospital over a period of six months. All age group patients presenting with clinical suspicion of UTI formed the population for this study. A total of 250 mid-stream urine samples were collected using aseptic techniques. The samples were then submitted to the microbiology laboratory within two hours of being collected. Urine cultures were inoculated with 0.001mL of urine using a loop into blood agar and Mac-Conkey agar. Plates were incubated aerobically at 37°C for 18 to 24 hours. Bacteriuria was said to be present when cultures grew  $10^5$  CFU/mL or more. Bacterial isolates were obtained by standard microbiology and biochemical tests. The biochemical identification and differentiation of *Enterococcus* species was done by Gram staining, catalase negativity, bile esculin hydrolysis, and growth in 6.5% sodium chloride. Detection of vancomycin resistance was carried out using vancomycin E strip and disk diffusion method according to CLSI guidelines<sup>10</sup>. Stool specimens were obtained from patients whose urine cultures were positive for *Enterococcus* species. Specimens were plated on selective agar Bile esculin azide agar to identify enterococcal colonization through standard techniques.



**Figure 1: (A). Showing antibiotic susceptibility testing of Vancomycin resistant *Enterococcus*. (B). Showing E test for Vancomycin resistant *Enterococcus***

Data analysis was performed using SPSS version 25.0 software. Categorical variables were presented as frequencies and percentages. Chi-square or Fisher's exact tests were used for comparisons between proportions.  $p < 0.05$  was taken to be statistically significant.

### Results

Out of 250 urine samples processed, 147 (58.8%) demonstrated significant bacterial growth, while 103 (41.2%) were culture negative.

Among the 147 culture-positive samples, *Escherichia coli* was the most frequently isolated organism (83 isolates; 56.5%). *Enterococcus* species accounted for 37 isolates (25.2%), followed by *Klebsiella pneumoniae* (18 isolates; 12.2%) and *Staphylococcus* species (9 isolates; 6.1%). Of the 37 *Enterococcus* isolates, 12 (32.4%) were

identified as vancomycin-resistant Enterococcus (VRE), while 25 (67.6%) were vancomycin susceptible. Of the 12 isolates of Vancomycin resistant Enterococcus 7 (58 %) were *E. faecium* and 5 (42%) were *E. faecalis*. Out of non-VRE isolates 15 (60%) were *E. faecium* and 10 (40%) were *E. faecalis*.

Among the 25 non-VRE Enterococcus isolates, 12 (48%) were also isolated from stool samples. In contrast, 4 (33.3%) of the 12 VRE isolates showed concurrent gastrointestinal colonization. The difference between the two groups was not statistically significant ( $p > 0.05$ ). The results were summarized in table 1.

**Table 1: Distribution of Uropathogens and Characterization of Enterococcus Isolates**

Parameter	Number (n)	Percentage (%)
Total urine samples processed	250	100
Culture positive samples	147	58.8
Culture negative samples	103	41.2
<b>Distribution of uropathogens (n = 147)</b>		
<i>Escherichia coli</i>	83	56.5
<i>Enterococcus</i> spp.	37	25.2
<i>Klebsiella pneumoniae</i>	18	12.2
<i>Staphylococcus</i> spp.	9	6.1
<b>Characterization of Enterococcus isolates (n = 37)</b>		
Vancomycin-resistant Enterococcus (VRE)	12	32.4
Vancomycin-susceptible Enterococcus (non-VRE)	25	67.6
<b>Species distribution among VRE (n = 12)</b>		
<i>Enterococcus faecium</i>	7	58.0
<i>Enterococcus faecalis</i>	5	42.0
<b>Species distribution among non-VRE (n = 25)</b>		
<i>Enterococcus faecium</i>	15	60.0
<i>Enterococcus faecalis</i>	10	40.0
<b>Gastrointestinal colonization</b>		
Non-VRE isolates with stool positivity	12 / 25	48.0
VRE isolates with stool positivity	4 / 12	33.3

## Discussion

This study shows the changing face of epidemiology in UTIs, particularly focusing on the rising incidence of Enterococcus and the increasing trend of vancomycin resistance among them. The positivity rate among the urine cultures conducted in this study, which was 58.8%, was found to be comparable with similar settings of care [13].

As seen worldwide, *E. coli* dominated the uropathogens, secures its position based on established virulence factors for easy colonizing and infection of the urinary tract [1,14]. Nonetheless, the significant rate of Enterococcus isolates (25.2%) signifies the rising trend of these bacteria, especially in healthcare-associated UTIs [16].

VRE isolation in almost a third of Enterococcus isolates is a worrying finding and also supported by reports indicating an increasing incidence of VRE in the hospital environment [6,14]. Vancomycin resistance is also more likely linked with *E. faecium*, which is usually causally associated with nosocomial transmission and drug resistance [1]. This is reflected in the likely distribution of species in this study.

The gastrointestinal tract is a key component in enterococcal ecology. The fact that a large number of VRE and non-VRE isolates recovered were also isolated from stool specimens supports the suggestion that UTIs due to Enterococcus may occur as a result of endogenous colonization of the gastrointestinal tract [8].

The declined rate of colonization in VRE isolates may be affected by antibiotic selective pressure and changes in the underlying community of gut microbiota [18]. Concerning the worldwide perspective of infectious diseases, it can be said that VRE has many challenges, especially in resource-limited countries, which have limitations for diagnosis or treatment. The results highlighted the importance of a comprehensive approach to the management of VRE, with a focus on infection control and prudent use of antimicrobial agents [20].

The limitations of this study were that phenotypic approaches were used for species identification as well as resistance gene confirmation in a smaller number of isolates. Hence genotypic study in a large number of isolates will give more detail about the prevalence & colonization of VRE.

## Conclusion

Species of *Enterococcus* are major uropathogens, and there is a considerable prevalence of vancomycin resistance among them. The role of colonizing the bowel in the pathogenesis of enterococcal urinary tract infections seems to be very significant. Improvement in the infrastructure of antimicrobial and resistance surveillance is the key to combatting the increasing problem of VRE.

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