

## Epidemiological Investigation of The Antimicrobial Resistance Profile Associated with A Suspected Urinary Tract Infection in A Tertiary Care, Super-Specialty Hospital in India

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### Abstract

The purpose of this study was to evaluate the antibiotic resistance profiles of urinary tract infections (UTI) obtained from male and female participants of different ages. Methods: 266 urine samples from a variety of people were examined in this investigation. Within two hours, midstream urine samples were gathered, moved, and processed on a CLED medium. Gram staining, biochemical traits, and colony shape were used to identify the bacteria. ESBL detection, carbapenemase detection, and antimicrobial susceptibility testing were all conducted using the VITEK 2 Compact system (Biomérieux, Marcy-l'Étoile, France). Results: Gram-negative bacteria accounted for 78.69% of the 61 significant UTIs in both male and female patients, Gram-positive bacteria for 11.48%, and *Candida* species for 9.84%. The most prevalent uropathogens were *Pseudomonas aeruginosa* (11.47%), *Klebsiella pneumoniae* (26.22%), and *Escherichia coli* (37.70%). While amikacin (89.36%) and colistin (97.30%) were the most effective against both Gram-negative and Gram-positive uropathogens, tetracycline (88.89%) and ceftriaxone (77.14%) encountered substantial resistance. Only cases of pan-drug resistance (PDR) and multidrug resistance (MDR) were shown to be susceptible to colistin. Conclusions: According to this study, compared to Gram-positive uropathogens, a greater number of UTIs are caused by Gram-negative bacteria, especially *E. coli*. The best antibiotics for both Gram-positive and Gram-negative uropathogens were found to be amikacin and colistin.

**Keywords:** urinary tract infection; uropathogens; antibiotic susceptibility; antimicrobial resistance; Gram-positive bacteria; Gram-negative bacteria

### Background

Urinary tract infections (UTIs) are highly common, impacting individuals of all ages and genders globally, with millions of cases documented each year [1]. *Escherichia coli* is the predominant etiological agent in urinary tract infections, accounting for over 90% of community-acquired cases and 50% of hospital-acquired cases [2,3]. Besides *E. coli*, numerous additional infections, including *Klebsiella*, *Proteus*, *Acinetobacter*, *Pseudomonas*, *Staphylococcus*, *Enterococcus*, and *Streptococcus* species, have been recognized as uropathogens [4,5]. Common symptoms of urinary tract infections that patients frequently experience include dysuria, polyuria, and cystitis [6]. Neglecting to treat UTIs can lead to serious health issues, such as kidney damage, renal scarring, and renal failure [7]. This condition impacts a substantial percentage of adult females, with around 40–50% experiencing UTIs [6]. The heightened prevalence of urinary tract infections in adult females can be ascribed to factors such as sexual activity and pregnancy. The prevalence of UTIs in women increases by 5% over a decade, whereas the incidence of UTIs during pregnancy rises by approximately 7%. The incidence of urinary tract infections in males often increases with age, sometimes attributed to diminishing immunity. Moreover, prevalent causes contributing to urinary tract infections in males include urethral anomalies, bladder neck blockages, bladder calculi, bladder neoplasms, bladder diverticula, prostatic enlargement, and prostate carcinoma [9].

Urinary tract infections (UTIs) are prevalent among children and newborns under the age

of approximately two years and can be contracted in both community and hospital environments. By the age of 7, almost 5% of girls and 2% of boys had experienced at least one urinary tract infection event [11,12]. Diagnosing urinary tract infections in young children can be difficult, since they may lack the ability to express their symptoms. In contrast, older children may report urinary discomfort, such as a burning feeling during urination, lack of bladder control, increased frequency of urination, and foul-smelling urine, among other issues [13]. Significantly, a greater incidence of difficult urinary tract infections (UTIs) is noted in males, although uncomplicated UTIs are generally managed without culture testing and sensitivity analysis [14]. UTIs are frequently classified as re-infections due to their recurrence following several weeks of antibiotic therapy. The initial bacterial attachment is the major catalyst for these infections, subsequently resulting in biofilm formation that confers resistance to the host's immune responses [15]. Numerous investigations have documented various antibiotic resistances in uropathogens, with global concerns intensifying, especially about multidrug resistance (MDR) and extended-spectrum beta-lactamases (ESBLs) [6,15].

Thus, the efficient management of urinary tract infections and the formulation of antibiotic guidelines are crucial in addressing antibiotic resistance and multidrug resistance. In this context, clinical microbiologists are essential for identifying pathogenic organisms and working with physicians to provide tailored antibiotic regimens for individual patients, therefore minimizing inappropriate antibiotic use, dosing errors, and harmful drug interactions. This method improves the quality of care delivered to patients. The principal objective of this study was to evaluate the antibiotic susceptibility patterns in positive UTI samples obtained from male and female patients.

## Methods

### Study Population

The investigation was conducted from March to Sep 2025 at the Microbiology Department of Sanar International Hospitals in Gurgaon, India. A total of 266 urine samples were obtained, comprising 161 from male patients and 105 from female patients, sourced from both outpatient and inpatient departments.

### Sample Collection

Midstream urine samples were obtained from patients suspected of having a urinary tract infection using 20 mL sterile screw-capped containers (BD Urine Collection Kit, Franklin Lakes, NJ, USA). To inhibit bacterial proliferation, materials were transferred to a container to which boric acid (0.2 mg) was added. Patients were given instructions for the aseptic collection of urethral samples [16].

### Sample Processing

A 4 mm nichrome wire inoculating loop was employed to inoculate urine samples onto Cysteine–lactose electrolyte-deficient (C.L.E.D.) agar medium, utilizing an inoculum volume of 0.01 mL. The culture plates were subsequently incubated at 37 °C for 24 to 48 hours. Following incubation, the plates were examined for evident, discrete bacterial proliferation. If no colonies were observed, the incubation period was prolonged by 24 hours. We confirmed positive urine cultures by relying on colony counts exceeding  $10^3$  to  $10^5$  CFU/mL, indicating severe bacteriuria.

### Identification and Sensitivity

Positive bacterial isolates were distinguished based on their colony shape, Gram staining, and biochemical properties. Gram-negative isolates were identified utilizing a VITEK 2 Compact machine with the GN ID-card, whilst Gram-positive isolates and yeast-like cells, such as *Candida*, were identified using the GP ID-card and YST ID-card, respectively, in the VITEK 2 Compact machine.

Antimicrobial susceptibility testing was performed with the esteemed VITEK 2 Compact

system. To ensure uniformity, we created a McFarland standard inoculum with an optical density of 0.5, evaluated using a Vitek Densicheck display base machine (Ref. No- 422220) in accordance with recognized protocols. The testing protocol adhered to the standards set forth by the Clinical and Laboratory Standards Institute (CLSI) in 2022. Vitek cards were employed for antimicrobial susceptibility testing, specifically AST-N405, AST-406, and AST-407 for Gram-negative bacteria; AST-P628 for Gram-positive bacteria; and AST-YS08 for *Candida*. The VITEK 2 Compact system was employed to identify ESBL production, which was validated via a synergistic assay including AMC and a third- or fourth-generation cephalosporin.

### Results

During a period of 7 months, 266 urine samples from persons suspected of urinary tract infections (UTIs) were analyzed. Of the samples, 161 (60.53%) were derived from male patients, whereas 105 (39.47%) were from female patients. The investigation identified 61 serious UTI cases, constituting 22.93%, across both male and female patients. In the analysis of positive bacterial isolates, a greater prevalence was noted among female patients, with 34 instances (55.74%), compared to 27 cases (44.26%) in male patients. The positive isolates were classified into four age brackets: 1–16, 17–35, 36–60, and over 60 years. The prevalence of urinary tract infections (UTIs) among female samples was highest (83.33%) in the 1–16 age group and lowest (37.50%) in the 36–60 age group, signifying a decline in UTI incidence with advancing age. In contrast, among positive samples from males, the incidence of UTI was highest (62.50%) in the 36–60 age group and lowest (16.67%) in the 1–16 age group. This indicates that the incidence of UTIs in male samples often rises with age, with the exception of the 1–16 age group, but declines in the over 60 age group (29.41%). A total of 61 uropathogenic microorganisms were discovered, including 48 (78.69%) Gram-negative bacteria, 7 (11.48%) Gram-positive bacteria, and 6 (9.84%) *Candida* species. *Escherichia coli* was the most prevalent of the isolated uropathogens, representing 37.70% of the cases. *Klebsiella pneumoniae* was the second most frequently isolated microorganism (26.22%), followed by *Pseudomonas aeruginosa* (11.47%), *Enterococcus faecalis* (4.91%), *Candida albicans* (4.91%), *Candida tropicalis* (4.91%), *Citrobacter koseri* (3.27%), *Staphylococcus haemolyticus* (3.27%), *Enterococcus gallinarum* (1.63%), and *Staphylococcus hominis* (1.63%), as depicted in Figure 1.

The antibiotic susceptibility results indicated that tetracycline had the highest resistance (88.89%) among all uropathogens, followed by ceftriaxone (77.14%). In contrast, colistin (97.30%) and amikacin (89.36%) had the highest sensitivity for both groups. Comprehensive antibiotic susceptibility statistics are illustrated in Figure 2.

*Escherichia coli* demonstrated significant resistance to ceftazidime (75.00%) and ciprofloxacin (73.91%), while exhibiting maximum sensitivity to tigecycline, colistin, and nitrofurantoin (100.00%) (Figure 3). In the instance of *Klebsiella pneumoniae*, resistance was discovered against ceftazidime (100.00%) and cefuroxime (83.33%), whereas susceptibility was noted for amikacin (93.33%), colistin (92.86%), and fosfomycin (71.43%). *Pseudomonas aeruginosa* exhibited significant susceptibility to nitrofurantoin, colistin, and aztreonam (100.00%), while demonstrating resistance to ceftazidime and levofloxacin (60.00%) (Figure 3). *Enterococcus faecalis* demonstrated significant resistance to tetracycline (100.00%) while exhibiting total susceptibility to nitrofurantoin, vancomycin, and linezolid (100.00%) (Figure 3).

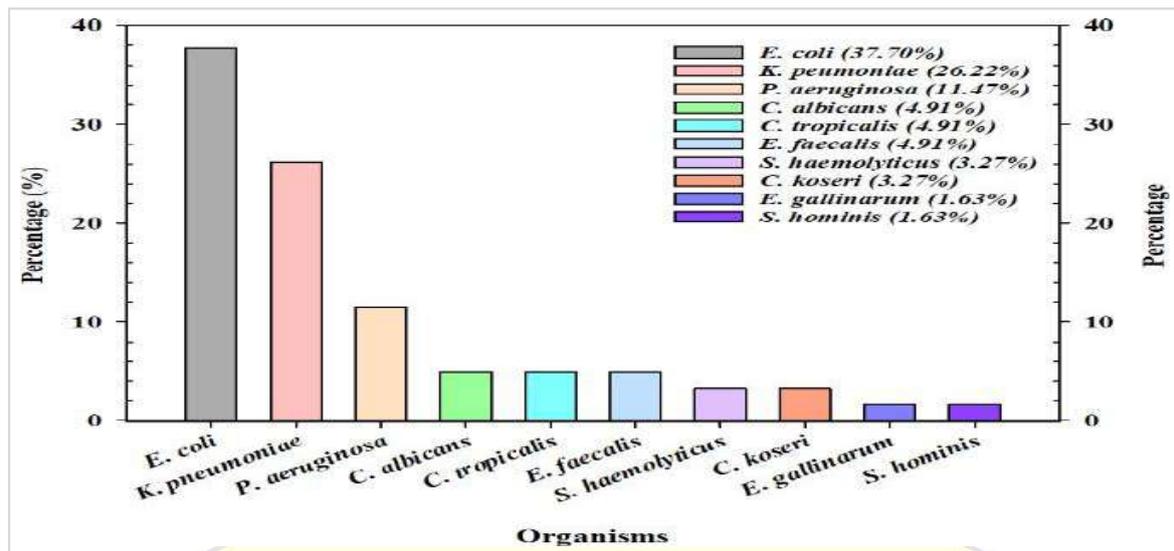


Figure 1. Occurrence of positive organisms in urine samples.

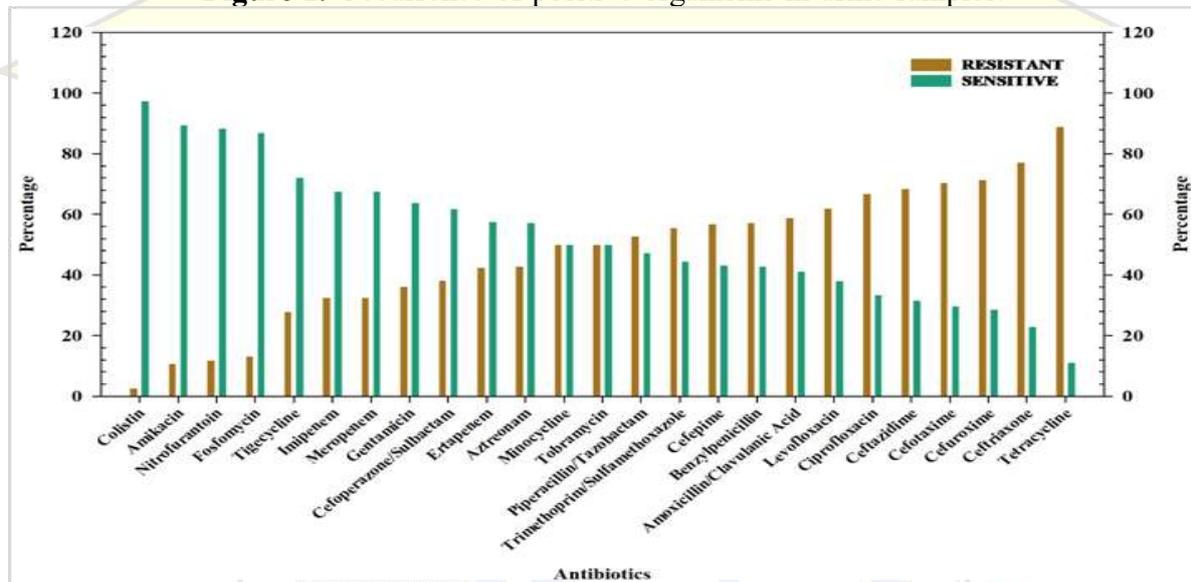


Figure 2. Overall, antibiotic susceptibility pattern observed in positive urine samples.

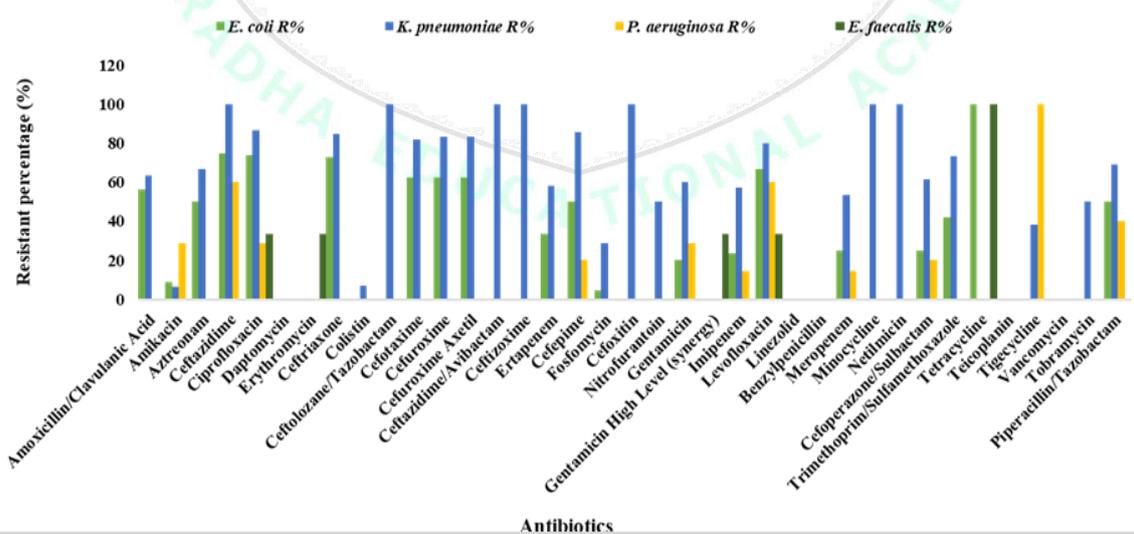


Figure 3. Antibiotic resistance pattern against organisms.

Both *Candida albicans* and *Candida tropicalis* exhibited full sensitivity to all evaluated antibiotics (amphotericin B, caspofungin, flucytosine, fluconazole, micafungin, and voriconazole).

Regarding resistance mechanisms, 30.43% of *Escherichia coli* specimens and 18.75% of *Klebsiella pneumoniae* specimens among the total isolates were identified as makers of extended-spectrum  $\beta$ -lactamases. Furthermore, 17.39% of the *Escherichia coli* specimens and 56.25% of the *Klebsiella pneumoniae* specimens were classified as carbapenemase producers.

### Discussion

This study aimed to evaluate the increasing trend of antibiotic resistance in uropathogens and improve treatment options. Antimicrobial resistance has escalated internationally. In our review, the total incidence of urinary tract infections (UTIs) was 22.93%, with certain studies indicating lower rates (e.g., 13.9%, 17%, and 17.26%) and others indicating higher rates (e.g., 23.7%, 32.7%, and 53.82%) [17–22]. We noted a greater incidence of UTIs in females (55.74%) than in males (44.26%), aligning with findings from other research [23,24]. In males, the incidence of urinary tract infections escalates with age, maybe attributable to diminished immunity, prostate complications, or prostatic hypertrophy [11,17]. The age distribution of sensitive UTI patients among females was as follows: above 60 years (35.29%), 17 to 35 years (32.35%), 36 to 60 years (17.64%), and 1 to 16 years (14.70%). The age distribution for males was as follows: 17–35 years (40.74%), 36–60 years (37.03%), above 60 years (18.51%), and 1–16 years (3.70%). A study in Ethiopia identified the highest prevalence of UTIs in the >45 age group (23.4%), whereas a Ugandan study indicated the highest incidence in the 20–29 age group (32.6), which contrasts with our findings [25,26]. A 2021 study indicated the highest prevalence in the 21–30 age demographic (22.8%) [27]. We observed a 78.69% prevalence of Gram-negative bacteria and a 11.48% prevalence of Gram-positive bacterial isolates, which contrasts with findings from earlier investigations [28,29]. *Escherichia coli* was the primary uropathogen (37.70%), succeeded by *Klebsiella pneumoniae* (26.22%) and *Pseudomonas aeruginosa* (11.47%) (Figure 1). Previous research has examined the prevalence of *E. coli* as a uropathogen, reporting rates of 50.09% [30], 50% [28], and 42.7% [21]. Notably, *E. coli* and *K. pneumoniae* together constitute over 80% of urinary tract infection cases worldwide, affecting both community-acquired and hospital-acquired infections [31,32]. Tetracycline exhibited the highest resistance rate at 88.89%, whereas colistin demonstrated the highest sensitivity at 97.30% [14,33,34]. The management of simple urinary tract infections without culture and susceptibility testing has significantly contributed to the rise of antibiotic resistance [35]. Seventy-five percent of *Escherichia coli* isolates exhibited resistance to ceftazidime, but nitrofurantoin, tigecycline, and colistin shown complete efficacy (100.00%). A study in Northeast Ethiopia by Aduugna and colleagues revealed that tetracycline exhibited the highest resistance rate at 65.70%, whereas colistin was determined to be the most effective antibiotic, achieving a 100.00% success rate against *E. coli* [21]. *Klebsiella pneumoniae* exhibited complete resistance to ceftazidime, although shown significant sensitivity to Amikacin (93.33%), colistin (92.86%), and fosfomycin (71.43%), findings that align with those from prior research [5,36]. *Pseudomonas aeruginosa* exhibited significant resistance to ceftazidime and levofloxacin (60.00%) while demonstrating complete sensitivity to nitrofurantoin, colistin, and aztreonam (100.00%). Linezolid and nitrofurantoin had 100.0% efficacy against *Enterococcus faecalis*, whereas tetracycline demonstrated complete resistance at 100.00%. The majority of the examined organisms exhibited resistance, particularly to beta-lactam antibiotics; nevertheless, contemporary agents such as amikacin, colistin, nitrofurantoin, and fosfomycin demonstrated efficacy against uropathogens.

## Conclusions

Our data illustrate a compelling overview of urinary tract infections, wherein Gram-negative organisms, predominantly *E. coli*, emerge as the most prevalent pathogens. Tetracycline constantly proves to be a formidable contender in the evolving realm of antibiotic susceptibility, although colistin and amikacin stand out as the most effective agents. The rising burden of antibiotic resistance originates from multiple sources, including the complex struggle against UTIs in cancer patients and the difficulties in managing complicated urinary tract infections. The practice of treating uncomplicated UTIs without the essential guidance of culture and susceptibility reports is as significant, unwittingly exacerbating the alarming increase in resistance. An array of risk factors contributes to increased susceptibility to urinary tract infections, including patient age, gender, diabetes, catheter utilization, immunological impairment, and previous antibiotic exposure. Within this complex array of elements, bacteriological culture serves as a vital diagnostic tool. It not only verifies the existence of illnesses but also serves a critical function in the identification of the causative bacteria.

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