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Bacteriological Profile and Antimicrobial Resistance Pattern of Urinary Isolates in a Tertiary Care Hospital

Dr. Aparna Y Takpere[1] Dr. Chandan D Sabale[2] Dr. Hajra Tasneem Masali[3] Dr. Mohit Malge[4*]

[1]Professor & HOD, Department of Microbiology, S R Patil Medical College, Hospital & Research Centre Badagandi, Bilagi, Bagalkot, Karnataka
[2]Associate Professor, Department of Microbiology, S R Patil Medical College, Hospital & Research Centre, Badagandi, Bilagi, Bagalkot, Karnataka
[3]Assistant Professor, Department of Microbiology, S R Patil Medical College, Hospital & Research Centre Badagandi, Bilagi, Bagalkot, Karnataka
[4]Professor, Department of Physiology, FH Medical College and Hospital, Agra, Uttar Pradesh

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*Corresponding Author:

Dr. Mohit Malge
Professor, Department of Physiology, FH Medical College and Hospital, Agra, Uttar Pradesh

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ABSTRACT

Background: Urinary tract infections (UTIs) are among the most frequent bacterial infections, contributing substantially to patient morbidity and healthcare burden. Rising antimicrobial resistance has complicated management, necessitating continuous surveillance of local pathogen distribution and susceptibility profiles. The study was conducted to identify the spectrum of urinary isolates and determine their antibiotic susceptibility patterns in patients with clinically suspected UTIs attending a tertiary care hospital.

Materials and Methods: A hospital-based study was conducted in the Department of Microbiology over 12 months. A total of 216 urine samples from clinically suspected UTI patients were processed using standard microbiological methods. Significant isolates ($\geq 10^5$ CFU/mL) were identified by conventional biochemical tests, and antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar according to CLSI 2023 guidelines. Data were analyzed using descriptive statistics.

Results: Of 216 samples, 60 (28%) showed significant bacterial growth. Females and the 21–40 years age group were most affected. Gram-negative bacilli predominated (86.7%), with E. coli (56.7%) as the most frequent isolate, followed by Klebsiella pneumoniae (18.3%) and Pseudomonas aeruginosa (8.3%). Among Gram-positive isolates, Enterococcus spp. (11.7%) were most common. High resistance was noted to ampicillin (81%), cotrimoxazole (66%), and ciprofloxacin (59%), while amikacin (87%), nitrofurantoin (77%), and imipenem (100%) showed excellent activity. Enterococcus isolates exhibited resistance to penicillin (71%) and ciprofloxacin (57%) but retained 100% susceptibility to linezolid and vancomycin.

Conclusion: *E. coli* remains the most common uropathogen, although Gram-positive organisms such as *Enterococcus* and *Staphylococcus* are increasingly isolated. Resistance to first-line agents remains high, whereas nitrofurantoin, aminoglycosides, and carbapenems continue to be reliable treatment options. Periodic local antibiogram updates and judicious antibiotic use are critical to effective UTI management and antimicrobial resistance containment.

Keywords: Urinary tract infection, antimicrobial resistance, *Escherichia coli*, nitrofurantoin, antibiogram.

INTRODUCTION:

Urinary tract infections (UTIs) are among the most common bacterial infections affecting humans, second only to respiratory infections in frequency [1]. They account for nearly **150 million cases annually worldwide**, leading to substantial morbidity and economic burden [2]. In India, UTIs represent a significant proportion of community and hospital-acquired infections, particularly among women, elderly individuals, and catheterized patients [3].

The etiology of UTIs is diverse, but **Gram-negative bacilli predominate**, with *Escherichia coli* being the leading pathogen in both community and healthcare settings [4,5]. Other common uropathogens include *Klebsiella pneumoniae*, *Proteus* spp., *Pseudomonas aeruginosa*, and Gram-positive cocci such as *Enterococcus* spp. and *Staphylococcus saprophyticus* [6]. The spectrum of organisms can vary across populations, age groups, and geographical regions.

Antimicrobial resistance (AMR) among uropathogens has emerged as a major global public health problem. Increasing resistance to commonly used antibiotics such as **ampicillin, cotrimoxazole, and fluoroquinolones** has been reported widely [7]. The rise of **extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae** further complicates treatment by limiting oral antibiotic options [8]. In India, multidrug resistance is increasingly reported in *E. coli* and *K. pneumoniae*, which poses a challenge in both outpatient and inpatient management of UTIs [9].

The choice of empirical antibiotic therapy is heavily influenced by the **local prevalence of pathogens and their susceptibility patterns**, which can differ between institutions and even within regions [10]. Continuous monitoring of resistance trends is therefore crucial for guiding appropriate therapy and reducing the emergence of resistant strains. Despite several multicenter studies on UTIs in India, there remains a need for **institution-specific data** to inform antibiotic policies. Smaller, focused studies can provide valuable insights into local epidemiology and resistance trends, which are directly relevant for clinicians in day-to-day patient management.

With this background, the present study was undertaken to identify urinary isolates and analyze their antibiotic susceptibility pattern in patients attending a tertiary care center.

MATERIALS AND METHODS:

Study design and setting

This was a hospital-based study conducted in the Department of Microbiology at a tertiary care teaching hospital after Written informed consent was obtained from all participants or from guardians in case of minors. The study was carried out over a period of twelve months. The aim was to identify bacterial isolates from urine samples of clinically suspected urinary tract infection (UTI) cases and determine their antibiotic susceptibility patterns.

Study population and sample size

A total of 216 urine samples were collected from patients of all age groups and both sexes who were clinically suspected to have UTIs. Both outpatients and inpatients were included in the study. Based on expected prevalence of 64% and 10% allowable error, sample size was 216.

Inclusion criteria

- Patients of all ages and both sexes with clinical suspicion of UTI (symptoms such as fever, dysuria, urgency, frequency, suprapubic pain, or flank tenderness).
- Midstream clean-catch urine samples or catheter specimens collected under aseptic precautions.
  Exclusion criteria
- Patients who had received antibiotics within the previous 48 hours.
- Inadequately collected, leaking, or unlabeled urine samples.
- Samples showing mixed growth of more than two organisms (considered contaminated).

Ethical considerations: The study protocol was approved by the Institutional Ethics Committee. Written informed consent was obtained from all participants or from guardians in case of minors.

Sample collection and transport

- For adult patients, midstream clean-catch urine was collected after cleaning the periurethral area with sterile water.
- In catheterized patients, urine was collected aseptically from the catheter port using a sterile syringe.
- Approximately 10–20 mL of urine was collected in a sterile, wide-mouthed, leak-proof container.
- All specimens were transported to the microbiology laboratory within one hour of collection. If delay was anticipated, samples were refrigerated at 4°C and processed within two hours.

Microbiological processing

Culture and isolation of organisms

- Urine samples were inoculated using a calibrated loop (0.001 mL) onto:
  - Cystine Lactose Electrolyte Deficient (CLED) agar – for differentiation of lactose fermenters and colony counts.
  - MacConkey agar – for isolation of Gram-negative bacilli.
  - 5% Sheep Blood agar – for recovery of fastidious organisms**.**
- Plates were incubated aerobically at 37°C for 24–48 hours.

- Significant bacteriuria was defined as growth of a single organism at a concentration of $\geq 10^5$ colony -forming units (CFU)/mL.
- Isolates were identified based on colony morphology, Gram staining, and conventional biochemical tests (indole, methyl red, Voges–Proskauer, citrate, urease, oxidase, triple sugar iron, bile esculin, catalase, and coagulase tests, as appropriate).

Antimicrobial susceptibility testing (AST)

- AST was performed by the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, following the Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines.
- Screening for ESBL and carbapenemase was performed as per CLSI
- Inoculum was prepared by suspending 3–5 well-isolated colonies in sterile saline and adjusting turbidity to 0.5 McFarland standard.
- Plates were incubated at 37°C for 16–18 hours, and zones of inhibition were measured in millimeters using a digital caliper.
- Results were interpreted as Sensitive (S), Intermediate (I), or Resistant (R) according to CLSI breakpoints.

Antibiotics tested:
For Gram-negative isolates:

- Ampicillin (10 µg)
- Amoxicillin–clavulanate (20/10 µg)
- Ciprofloxacin (5 µg)
- Cotrimoxazole (1.25/23.75 µg)
- Gentamicin (10 µg)
- Amikacin (30 µg)
- Nitrofurantoin (300 µg)
- Piperacillin–tazobactam (100/10 µg)
- Imipenem (10 µg)

For Gram-positive isolates (Enterococcus and Staphylococcus):

- Penicillin (10 units)
- Ciprofloxacin (5 µg)
- Nitrofurantoin (300 µg)
- Linezolid (30 µg)
- Vancomycin (30 µg)

Data analysis: Data were entered into Microsoft Excel and analyzed using descriptive statistics. Results were expressed as frequencies and percentages. Organism distribution and antimicrobial susceptibility profiles were presented in tabular form.

RESULTS:
A total of **216 urine samples** were processed during the study period. Out of these, **60 (28%)** yielded significant bacterial growth, while the remaining 156 (72%) showed no growth. The relatively low culture positivity may reflect inclusion of both outpatient and inpatient cases, some with mild or atypical symptoms, as well as prior antibiotic expo sure.

Demographic distribution of culture-positive cases
Among the 60 culture-positive cases, **females were more commonly affected** than males, consistent with the known epidemiology of urinary tract infections. The highest number of positive cultures was observed in the **21–40 years age group (23; 38.3%)**, followed by 41–60 years (15; 25.0%), >60 years (15; 25.0%), and ≤20 years (7; 11.7%) (Table 1). This pattern indicates that UTIs predominantly affect young and middle-aged adults, which may be attributed to increased sexual activity, hormonal influences, and higher exposure to community-acquired infections in this age range.

Table 1. Age-wise distribution of culture-positive cases (n = 60)

Age group (years)	Number (n)	Percentage (%)
0–20	7	11.7%
21–40	23	38.3%
41–60	15	25.0%
>60	15	25.0%
Total	**60**	**100%**

Among the 60 culture-positive samples, **Gram-negative bacilli predominated (52; 86.7%)**, while **Gram-positive cocci accounted for 8 (13.3%)** isolates. The distribution of isolates is shown in Table 2.

- *Escherichia coli* was the most frequently isolated pathogen (**34/60; 56.7%**), followed by *Klebsiella pneumoniae* (**11; 18.3%**).
- Among Gram-positive isolates, *Enterococcus* spp. were the most common (**7/60; 11.7%**) and *Staphylococcus spp.* was isolated in **1 patient (1.7%)**.
- Other Gram-negative isolates included *Pseudomonas aeruginosa* (**5; 8.3%**) and *Proteus mirabilis* (**2; 3.3%**).

Table 2. Distribution of urinary isolates (n = 60)

Organism	**Number (n)**	**Percentage (%)**
Escherichia coli	34	56.7%
Klebsiella pneumoniae	11	18.3%
Enterococcus spp.	7	11.7%
Pseudomonas aeruginosa	5	8.3%
Proteus mirabilis	2	3.3%
Staphylococcus spp.	1	1.7%
Total	**60**	**100%**

A total of **52 Gram-negative isolates** were tested for susceptibility. High resistance was noted against commonly used oral antibiotics: **ampicillin (42/52; 81%)**, **cotrimoxazole (34/52; 66%)**, and **ciprofloxacin (31/52; 59%)**. On the other hand, **amikacin (45/52; 87%)**, **nitrofurantoin (40/52; 77%)**, and **imipenem (52/52; 100%)** showed excellent activity (Table 3).

These results highlight a concerning prevalence of resistance to first-line antibiotics but indicate that aminoglycosides, nitrofurantoin, and carbapenems remain reliable treatment options for Gram-negative uropathogens.

Table 3. Antibiotic resistance/sensitivity of Gram-negative isolates (n = 52)

Antibiotic	**Susceptibility**	**Number (n)**	**Percentage (%)**
Ampicillin	Resistant	42	81%
Cotrimoxazole	Resistant	34	66%
Ciprofloxacin	Resistant	31	59%
Amikacin	Sensitive	45	87%
Nitrofurantoin	Sensitive	40	77%
Imipenem	Sensitive	52	100%

Among the seven *Enterococcus* isolates, **5 (71%)** were resistant to penicillin, and **4 (57%)** were resistant to ciprofloxacin. **Five (71%)** isolates were sensitive to nitrofurantoin. All isolates showed **100% sensitivity to linezolid and vancomycin**, indicating these agents remain highly effective against Gram-positive uropathogens (Table 4).

Table 4. Antibiotic susceptibility of *Enterococcus* spp.

Antibiotic	**Sensitive (n, %)**	**Resistant (n, %)**
Penicillin	2 (29%)	5 (71%)
Ciprofloxacin	3 (43%)	4 (57%)
Nitrofurantoin	5 (71%)	2 (29%)
Linezolid	7 (100%)	0 (0%)
Vancomycin	7 (100%)	0 (0%)

The single *Staphylococcus* isolate was resistant to penicillin but **sensitive to vancomycin and linezolid**. Due to the low number of isolates, no definitive conclusions can be drawn regarding susceptibility trends for Staphylococcus in this population (Table 5)

Table 5. Antibiotic susceptibility of *Staphylococcus* spp.

Antibiotic	**Sensitive (n, %)**	**Resistant (n, %)**
Penicillin	0 (0%)	1 (100%)

Antibiotic	Sensitive (n, %)	Resistant (n, %)
Ciprofloxacin	1 (100%)	0 (0%)
Nitrofurantoin	1 (100%)	0 (0%)
Linezolid	1 (100%)	0 (0%)
Vancomycin	1 (100%)	0 (0%)

DISCUSSION:

Urinary tract infections remain among the most prevalent bacterial infections worldwide, affecting both community and hospital populations. The present study demonstrated a **culture positivity rate of 28%**, which is lower compared to several previous Indian studies reporting 35–60% positivity in suspected UTI cases [11–13]. This lower isolation rate may be attributed to early empirical antibiotic use prior to sample collection, variations in study populations, or stringent exclusion criteria applied in the current work. Similar positivity rates (25–30%) have been reported from other tertiary care centers, highlighting regional differences in UTI epidemiology [14,15].

In our study, **females constituted the majority of culture-positive cases**, which is consistent with the well-established observation that women are more predisposed to UTIs due to anatomical and physiological factors, such as a shorter urethra and proximity to the anal opening [16]. The age group most commonly affected was adults between 21–40 years, correlating with previous studies that have emphasized higher susceptibility among sexually active females and individuals with increased healthcare exposure [17].

With regard to the spectrum of urinary isolates, **Gram-negative organisms predominated**, with *Escherichia coli* being the most common pathogen, followed by *Klebsiella spp.*, *Proteus spp.*, and *Pseudomonas spp.*. This finding is consistent with numerous Indian and international reports that continue to identify *E. coli* as the principal etiological agent of community- and hospital-acquired UTIs [18,19]. However, the isolation of **Gram-positive organisms such as *Enterococcus spp.* and *Staphylococcus spp.*** in our study underscores the emerging role of these pathogens in UTI cases, especially in catheterized and hospitalized patients. A similar trend has been noted in recent reports, suggesting an increasing prevalence of multidrug-resistant Gram-positive uropathogens [20,21].

Antibiotic susceptibility testing revealed **high resistance among Gram-negative isolates** to commonly prescribed agents such as ampicillin, amoxicillin–clavulanate, and cotrimoxazole. Fluoroquinolone resistance was also notable, in line with national surveillance data that document widespread misuse of these agents [22]. On the other hand, aminoglycosides (amikacin, gentamicin), carbapenems (imipenem), and nitrofurantoin retained good activity against the majority of isolates. The high efficacy of nitrofurantoin against *E. coli* and *Enterococcus* supports its continued role as an oral treatment option for uncomplicated UTIs [23].

Among **Enterococcus isolates**, high resistance to penicillin and ciprofloxacin was observed, though susceptibility to nitrofurantoin, linezolid, and vancomycin remained excellent. This pattern mirrors findings from other Indian studies, where *Enterococcus* strains show limited treatment options but maintain consistent sensitivity to linezolid and glycopeptides [24]. The single *Staphylococcus* isolate was penicillin-resistant, yet sensitive to vancomycin and linezolid, reflecting the global challenge of increasing beta-lactam resistance in *Staphylococcus aureus* and the importance of glycopeptides in treatment [25].

The relatively **low culture positivity rate (28%)** in this study also indicates that a significant proportion of symptomatic patients may have non-bacterial causes, viral infections, or lower colony counts below the diagnostic threshold. Additionally, empirical use of antibiotics prior to sample collection could have contributed to reduced growth rates. This highlights the importance of patient education regarding proper sample collection and the judicious use of antimicrobials.

CONCLUSION: This study highlights that urinary tract infections remain a significant clinical problem, with a culture positivity rate of 28%. Escherichia coli continues to be the predominant uropathogen, though the isolation of Enterococcus and Staphylococcus species indicates an emerging role of Gram-positive organisms in UTIs. High resistance was observed to commonly used antibiotics such as ampicillin, cotrimoxazole, and fluoroquinolones, whereas nitrofurantoin, aminoglycosides, carbapenems, linezolid, and vancomycin retained good efficacy. Our findings underscore the need for antimicrobial stewardship and routine surveillance of urinary pathogens. These findings emphasize the need for periodic local surveillance of antimicrobial resistance patterns to guide empirical therapy. Strengthening antimicrobial stewardship and rational antibiotic prescribing practices is essential to curb the growing problem of resistance in urinary pathogens.

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