

# “Isolation And Identification Of Common Uropathogens In Patients With Urinary Tract Infection.”

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## ABSTRACT

### Background of the Study

Urinary Tract Infection (UTI) is one of the most common bacterial infections affecting people of all age groups worldwide. It significantly contributes to morbidity, healthcare burden, and antimicrobial resistance. UTI occurs due to the invasion of microorganisms into the urinary tract, commonly caused by bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Staphylococcus saprophyticus* (Flores-Mireles et al., 2015; Foxman, 2016). Early isolation and accurate identification of uropathogens are essential for appropriate treatment and prevention of complications such as pyelonephritis, renal damage, and recurrent infections (Gupta et al., 2017). The increasing emergence of multidrug-resistant uropathogens has created a major challenge for clinicians and microbiology laboratories (Behzadi et al., 2017; WHO, 2022).

### Aim

To isolate and identify common uropathogens in patients suffering from urinary tract infections and to determine their prevalence among different age groups and genders.

### Objectives

To collect and process urine samples from suspected UTI patients. To isolate and identify common bacterial pathogens causing UTIs. To determine the frequency of different uropathogens. To study the distribution of UTI among age and gender groups. To evaluate the antimicrobial susceptibility pattern of isolated organisms.

### Methodology

A hospital-based cross-sectional study was conducted in the Department of Microbiology. Midstream urine samples were collected aseptically from clinically suspected UTI patients. Samples were cultured on CLED agar, MacConkey agar, and Blood agar using standard microbiological techniques. Identification of isolates was performed by colony morphology, Gram staining, and biochemical tests according to standard laboratory protocols (Forbes et al., 2017; Koneman et al., 2017). Antimicrobial susceptibility testing was carried out using the Kirby-Bauer disk diffusion method following CLSI guidelines (CLSI, 2025). Data obtained were analyzed statistically to determine the prevalence and distribution of uropathogens.

### Results

The study demonstrated that females showed a higher prevalence of UTI compared to males, particularly among the reproductive age group. *Escherichia coli* was identified as the most predominant uropathogen, followed by *Klebsiella pneumoniae*, *Proteus spp.*, *Pseudomonas aeruginosa*, and *Enterococcus spp.* Similar findings have been reported in previous epidemiological studies (Medina & Castillo-Pino, 2019; Sharma et al., 2022). Antimicrobial susceptibility testing revealed increasing resistance to commonly used antibiotics such as ampicillin and cotrimoxazole, while better sensitivity was observed for nitrofurantoin and carbapenems.

### Discussion

The predominance of *E. coli* observed in the present study correlates with previous global and Indian studies on UTI epidemiology (Foxman, 2016; Lee et al., 2018). The higher incidence in females may be associated with anatomical and physiological factors. Increasing antimicrobial resistance among uropathogens is a significant concern and emphasizes the need for routine culture and sensitivity testing before antibiotic therapy (Bryce et al., 2016; CDC, 2023). Proper diagnosis and timely treatment can reduce complications and prevent recurrent infections.

### Conclusion

The study concludes that *Escherichia coli* remains the most common uropathogen causing urinary tract infections. Accurate isolation and identification of pathogens along with antimicrobial susceptibility testing are essential for effective management of UTIs. Rising antimicrobial resistance highlights the importance of rational antibiotic use and continuous surveillance programs.

### Recommendations

- Routine urine culture and sensitivity testing should be encouraged for all suspected UTI cases.
- Irrational use of antibiotics should be avoided to minimize antimicrobial resistance.
- Awareness programs regarding personal hygiene and infection prevention should be conducted.
- Regular antimicrobial surveillance should be implemented in healthcare settings.
- Advanced diagnostic techniques should be incorporated for rapid identification of resistant pathogens.

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### Future Scope

Future studies may include molecular characterization of resistant uropathogens, detection of ESBL-producing organisms, biofilm analysis, and genomic studies for better understanding of pathogenicity and resistance mechanisms. Large multicentric studies may also help in developing region-specific antibiotic policies.

### Summary

Urinary tract infections are among the most common infectious diseases worldwide. The present study focused on the isolation and identification of common uropathogens in UTI patients using standard microbiological methods. *E. coli* was found to be the predominant pathogen. Increasing antimicrobial resistance among isolates was observed, highlighting the need for proper diagnosis, antibiotic stewardship, and regular surveillance to improve patient management and reduce complications.

**Keywords:** Urinary Tract Infection, Uropathogens, *Escherichia coli*, Antimicrobial Resistance, Urine Culture, Antibiotic Sensitivity, Microbiology, Bacteriuria, CLSI Guidelines, UTI Diagnosis

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### INTRODUCTION

Urinary Tract Infection (UTI) is one of the most common infectious diseases affecting human beings worldwide and represents a major public health concern due to its high prevalence, recurrence, complications, and increasing antimicrobial resistance. UTIs occur when pathogenic microorganisms invade any part of the urinary system, including the urethra, urinary bladder, ureters, and kidneys. The infection may involve the lower urinary tract, causing urethritis and cystitis, or may ascend to the upper urinary tract resulting in pyelonephritis and severe renal complications. The disease affects people of all age groups and genders; however, women are more frequently affected because of anatomical and physiological predispositions (Flores-Mireles et al., 2015; Foxman, 2016).

Globally, urinary tract infections account for millions of hospital visits annually and are among the leading causes of antimicrobial prescription in both community and hospital settings. It has been estimated that nearly 150–250 million cases of UTIs occur every year worldwide, imposing enormous economic and healthcare burdens on society (Tandogdu & Wagenlehner, 2016). Recurrent infections, prolonged hospitalization, treatment failure, and the emergence of multidrug-resistant organisms further increase morbidity and mortality associated with UTIs (Gupta et al., 2017).

UTIs are caused by a broad range of microorganisms collectively referred to as uropathogens. Among these, bacteria are the predominant etiological agents. Gram-negative bacteria contribute to the majority of cases, while Gram-positive bacteria account for a smaller percentage. *Escherichia coli* is recognized as the most common uropathogen responsible for approximately 70–90% of uncomplicated community-acquired infections (Lee et al., 2018). Other common bacterial pathogens include *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterobacter* species, *Citrobacter* species, *Enterococcus faecalis*, and *Staphylococcus aureus* (Ronald, 2018).

The ability of these microorganisms to cause infection depends on various virulence factors such as fimbriae, adhesins, toxins, motility, hemolysins, urease production, and biofilm formation. These factors enable

bacteria to adhere to the uroepithelium, evade host defense mechanisms, proliferate in the urinary tract, and establish persistent infections (Kaper et al., 2015). Biofilm formation is particularly important in catheter-associated urinary tract infections because it enhances bacterial survival and increases resistance to antimicrobial agents (Behzadi et al., 2020).

The urinary tract is normally sterile because of multiple defense mechanisms such as continuous urine flow, acidic urine pH, antibacterial activity of urinary secretions, mucosal immunity, and regular flushing of microorganisms during urination. However, several factors may predispose individuals to infection. These include female gender, pregnancy, urinary catheterization, urinary tract obstruction, renal calculi, diabetes mellitus, poor hygiene, immunosuppression, old age, and prolonged hospitalization (Medina & Castillo-Pino, 2019).

UTIs are broadly classified into uncomplicated and complicated infections. Uncomplicated UTIs occur in healthy individuals with anatomically normal urinary tracts, whereas complicated UTIs are associated with structural abnormalities, urinary obstruction, catheterization, diabetes mellitus, renal disease, pregnancy, or immunocompromised conditions (Wagenlehner et al., 2020). Catheter-associated urinary tract infections (CAUTIs) are particularly significant because they constitute one of the most common healthcare-associated infections worldwide (Nicolle, 2016).

The clinical manifestations of urinary tract infections vary according to the site and severity of infection. Common symptoms include dysuria, burning sensation during urination, increased urinary frequency, urgency, suprapubic pain, flank pain, fever, chills, hematuria, cloudy urine, and foul-smelling urine (Hooton, 2015). Severe infections may lead to renal tissue damage, septicemia, renal failure, and even death if untreated.

The diagnosis of UTI requires correlation between clinical symptoms and laboratory findings. Microbiological examination of urine remains the gold standard for the diagnosis of urinary tract infections. Isolation and identification of causative organisms are essential for confirming infection and guiding antimicrobial therapy (Wilson & Gaido, 2015).

Midstream clean-catch urine specimens are commonly collected to minimize contamination during sample collection (Forbes et al., 2017).

Culture techniques play a crucial role in the isolation of uropathogens. Commonly used culture media include Cystine Lactose Electrolyte Deficient (CLED) agar, MacConkey agar, Blood agar, and nutrient agar. Significant bacteriuria is generally defined as the presence of  $\geq 10^5$  colony-forming units (CFU)/mL of urine according to Kass criteria (Kass, 2015).

After culture, isolates are identified through colony morphology, Gram staining, motility testing, and various biochemical reactions such as indole production, citrate utilization, urease test, catalase test, coagulase test, oxidase test, and Triple Sugar Iron (TSI) agar reactions (Koneman et al., 2017; Collee et al., 2016).

The emergence and rapid spread of antimicrobial resistance among urinary pathogens have become serious global challenges. Inappropriate and irrational use of antibiotics, self-medication, incomplete treatment, and over-the-counter availability of antimicrobial agents have accelerated the development of resistant strains (Bryce et al., 2016). Multidrug-resistant organisms, including Extended Spectrum Beta-Lactamase (ESBL)-producing Enterobacteriaceae and carbapenem-resistant bacteria, are increasingly reported from healthcare institutions worldwide (Pitout & Laupland, 2015).

The increasing prevalence of resistant uropathogens has complicated empirical therapy and highlighted the importance of routine antimicrobial susceptibility testing. Antibiotic susceptibility testing provides valuable information regarding effective antibiotics for treatment and assists clinicians in selecting appropriate therapy. Standard methods such as the Kirby–Bauer disk diffusion technique recommended by the Clinical and Laboratory Standards Institute (CLSI) are commonly employed for this purpose (CLSI, 2025). Recent advances in microbiological and molecular diagnostic techniques have significantly improved the detection and characterization of urinary pathogens.

Automated urine analyzers, MALDI-TOF mass spectrometry, polymerase chain reaction (PCR), and molecular sequencing techniques have enhanced diagnostic accuracy and reduced turnaround time for pathogen identification (Oyaert & Delanghe, 2019).

In India, urinary tract infections are among the leading causes of outpatient visits and hospital admissions. Several studies have demonstrated increasing prevalence of multidrug-resistant uropathogens in both community and hospital settings (Sharma et al., 2022; Verma et al., 2024). Factors such as poor sanitation, indiscriminate use of antibiotics, limited infection control measures, and lack of public awareness contribute significantly to the burden of UTIs and antimicrobial resistance.

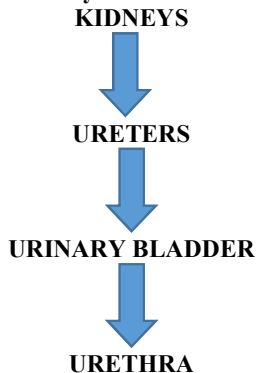
Therefore, the present study entitled “**Isolation and Identification of Common Uropathogens in Patients with Urinary Tract Infection**” has been undertaken to isolate and identify common bacterial pathogens causing UTIs and analyze their microbiological characteristics and antimicrobial susceptibility patterns. Accurate identification of pathogens and determination of antimicrobial susceptibility patterns are essential for effective treatment, reduction of complications, and prevention of antimicrobial resistance.

#### **Background of the Study**

Urinary tract infections have been recognized as important human infections for several decades because of their high prevalence, recurrence, and clinical significance. They are considered one of the most frequently encountered bacterial infections in medical practice and contribute substantially to healthcare expenditures worldwide.

Historically, UTIs were believed to be simple infections caused by limited bacterial species. However, with advancements in microbiology and infectious disease research, it has become evident that urinary tract infections involve diverse microbial populations with varying virulence factors and resistance mechanisms. The emergence of multidrug-resistant uropathogens has transformed UTIs into a major global health challenge.

**Figure 1: Anatomy of Human Urinary Tract**

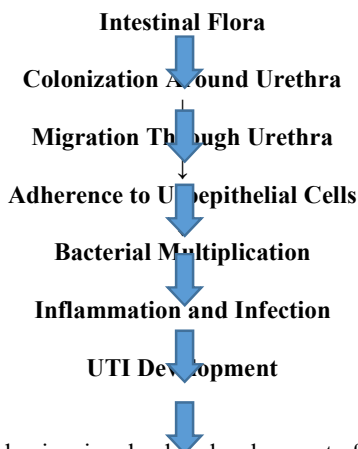


**Figure 1:** Human urinary tract showing organs commonly affected during urinary tract infection.

A wide range of microorganisms are responsible for UTIs, collectively known as uropathogens. Bacteria are the most common etiological agents, particularly Gram-negative organisms. Among them, *Escherichia coli* is the predominant pathogen responsible for approximately 70–90% of community-acquired urinary tract infections. Other important

uropathogens include *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterobacter* species, *Citrobacter* species, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Staphylococcus saprophyticus*. These organisms possess several virulence factors such as adhesins, fimbriae, toxins, motility, capsule formation, and biofilm production that help them colonize and survive in the urinary tract. Uropathogenic *E. coli* strains possess P-fimbriae and Type-1 fimbriae which enable bacterial adherence to uroepithelial cells and resistance to urinary flushing mechanisms.

**Diagram 1: Pathogenesis of Urinary Tract Infection**

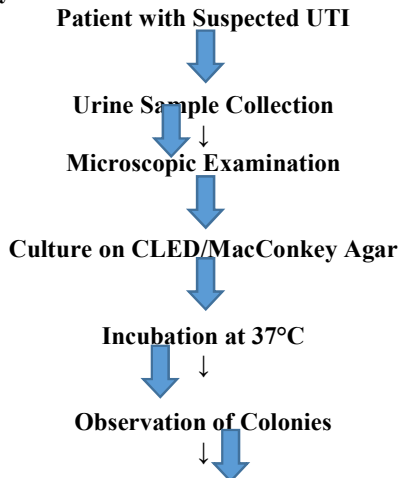


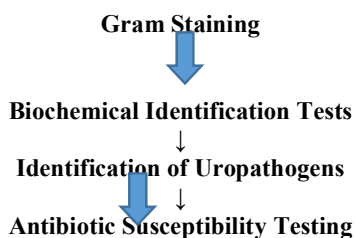
**Diagram 1:** Basic mechanism involved in development of urinary tract infection.

The clinical manifestations of UTI depend upon the site and severity of infection. Common symptoms include dysuria (painful urination), burning sensation during urination, urgency, increased frequency of urination, suprapubic pain, flank pain, fever, chills, hematuria, cloudy urine, and foul-smelling urine. In severe cases, untreated infections may spread to the kidneys and bloodstream, leading to pyelonephritis, septicemia, renal scarring, hypertension, and even death. UTIs are among the most common healthcare-associated infections worldwide, especially catheter-associated urinary tract infections (CAUTIs). Long-term catheterization provides a pathway for microorganisms to enter the urinary tract and promotes biofilm formation, increasing the risk of persistent and recurrent infections.

Diagnosis of UTI depends upon clinical evaluation along with microbiological confirmation. Laboratory diagnosis includes urine microscopy, Gram staining, urine culture, biochemical identification, and antimicrobial susceptibility testing. Midstream clean-catch urine is considered the standard specimen for microbiological examination because it minimizes contamination from external flora. Urine culture remains the gold standard method for isolation and identification of uropathogens. Commonly used culture media include Cystine Lactose Electrolyte Deficient (CLED) agar, MacConkey agar, and Blood agar. Significant bacteriuria is generally defined as the presence of  $\geq 10^5$  colony-forming units (CFU)/mL of urine.

**Diagram 2: Laboratory Workflow for Isolation and Identification of Uropathogens**





**Diagram 2:** Standard microbiological procedure for diagnosis of urinary tract infection.

Identification of isolated pathogens is carried out using colony morphology, Gram staining, motility testing, and biochemical reactions such as indole test, citrate utilization test, oxidase test, catalase test, coagulase test, urease test, and Triple Sugar Iron (TSI) test. These procedures help in accurate identification of causative organisms and guide clinicians in appropriate treatment. Antimicrobial susceptibility testing plays a vital role in management of UTIs because antimicrobial resistance among uropathogens is increasing worldwide. Excessive and irrational use of antibiotics, self-medication, incomplete treatment, and misuse of broad-spectrum drugs have contributed to the emergence of multidrug-resistant organisms. Extended Spectrum Beta-Lactamase (ESBL)-producing *E. coli* and *Klebsiella pneumoniae* are increasingly reported from both hospital and community settings.

Resistance to commonly prescribed antibiotics such as ampicillin, cotrimoxazole, cephalosporins, and fluoroquinolones has made empirical treatment difficult. Therefore, routine antimicrobial susceptibility testing according to Clinical and Laboratory Standards Institute (CLSI) guidelines is essential for selecting effective antibiotics and reducing treatment failure.

**Figure 2: Common Uropathogens Isolated in UTI**



**Figure 2:** Relative frequency of commonly isolated urinary pathogens.

Recent advances in microbiological diagnostics such as automated urine analyzers, MALDI-TOF mass spectrometry, polymerase chain reaction (PCR), and molecular diagnostic methods have improved rapid detection and identification of uropathogens and antimicrobial resistance genes. These modern diagnostic approaches provide accurate and timely results, helping clinicians initiate effective treatment and reduce complications.

The increasing prevalence of antimicrobial resistance has become a serious global health issue. Continuous surveillance of uropathogens and their antibiotic susceptibility patterns is therefore necessary to formulate empirical treatment guidelines and

antimicrobial stewardship programs. Monitoring local prevalence patterns also helps healthcare institutions develop infection control policies and prevent the spread of resistant organisms.

The present study entitled “**Isolation and Identification of Common Uropathogens in Patients with Urinary Tract Infection**” is undertaken to identify the common bacterial pathogens responsible for UTIs and evaluate their microbiological characteristics. The study also aims to analyze the distribution of uropathogens and support effective diagnosis and management of urinary tract infections.

This chapter describes the background, significance, aims, objectives, scope, and rationale of the study related to urinary tract infections and uropathogens.

Several epidemiological studies have demonstrated that women are more susceptible to UTIs compared to men because of their shorter urethra and anatomical proximity between the urethral opening and anal region. Pregnancy, menopause, sexual activity, poor personal hygiene, and hormonal changes further increase the risk of infection among females (Foxman & Brown, 2015).

In hospitalized patients, urinary catheterization is one of the most important predisposing factors for infection. Catheter-associated urinary tract infections account for a major proportion of hospital-acquired infections. Biofilm formation on catheter surfaces allows microorganisms to survive and resist host defense mechanisms and antimicrobial therapy (Delcaru et al., 2016).

The burden of urinary tract infections is not limited to adults. Pediatric UTIs are associated with serious complications such as renal scarring, hypertension, vesicoureteral reflux, and chronic kidney disease (Tullus, 2016). Elderly individuals are also at increased risk because of urinary retention, catheterization, reduced immunity, and comorbid conditions (Rowe & Juthani-Mehta, 2018).

Several bacterial species are implicated in urinary tract infections, but Gram-negative organisms remain the predominant pathogens. *Escherichia coli* is considered the principal etiological agent because of its ability to colonize the periurethral region and ascend into the urinary tract. Other pathogens such as *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Enterococcus* species are commonly isolated from complicated and nosocomial infections.

One of the major concerns associated with UTIs is the increasing resistance of uropathogens to commonly used antibiotics. Resistance to penicillins, cephalosporins, cotrimoxazole, fluoroquinolones, and

aminoglycosides has been reported globally (Tamma et al., 2021). The emergence of ESBL-producing organisms and carbapenem-resistant strains has significantly limited treatment options.

Because antimicrobial susceptibility patterns vary geographically and temporally, continuous surveillance of local uropathogens is essential. Identification of prevalent pathogens and their susceptibility profiles helps clinicians select appropriate empirical therapy and contributes to antibiotic stewardship programs.

The present study was designed to evaluate the distribution of common uropathogens among patients with urinary tract infections and identify the microbiological characteristics of isolates. The findings may provide important epidemiological data for improving diagnosis, treatment, and prevention strategies.

### **Significance of the Study**

The significance of this study lies in its contribution toward understanding the prevalence and identification of common uropathogens responsible for urinary tract infections. UTIs are among the most frequent bacterial infections encountered in hospitals and communities, making them an important subject of microbiological and clinical research.

This study is significant because accurate identification of causative organisms is essential for proper diagnosis and treatment. Delayed or inappropriate therapy may lead to recurrent infections, renal damage, septicemia, and increased healthcare costs.

The study is also important because antimicrobial resistance among urinary pathogens has become a major challenge in clinical practice. Determination of local susceptibility patterns will help clinicians choose appropriate antibiotics and minimize irrational antimicrobial usage.

Furthermore, the study contributes to epidemiological surveillance by providing updated information regarding the prevalence of bacterial pathogens in the study population. Such information may support infection control measures and antibiotic stewardship programs.

The findings of this research may also benefit microbiologists, clinicians, nurses, public health professionals, and researchers involved in infectious disease management. It may guide future investigations related to resistant uropathogens, molecular diagnostics, and prevention strategies.

### **Statement of the Problem**

Urinary tract infections continue to be major causes of morbidity despite advances in diagnostic and therapeutic approaches. Increasing antimicrobial resistance among uropathogens has complicated treatment and resulted in recurrent infections and prolonged hospitalization.

In many healthcare settings, empirical treatment is initiated without adequate knowledge of local pathogen distribution and resistance patterns. This practice contributes to treatment failure and emergence of resistant strains.

There is a need for continuous surveillance and accurate identification of common uropathogens associated with UTIs. Understanding the prevalence and microbiological characteristics of pathogens is essential for effective treatment and prevention strategies.

The present study therefore aims to isolate and identify common uropathogens among patients with urinary tract infections and analyze their microbiological characteristics.

### **Research Aim**

**“To isolate and identify common uropathogens in patients with urinary tract infection and analyze their microbiological characteristics and antimicrobial susceptibility patterns.”**

### **Objectives**

#### **General Objective**

To isolate and identify common uropathogens from urine samples of patients suspected of urinary tract infection.

#### **Specific Objectives**

- To collect urine samples from patients with suspected urinary tract infection.
- To isolate bacterial pathogens using standard microbiological culture techniques.
- To identify bacterial isolates based on colony morphology, Gram staining, and biochemical reactions.
- To determine the prevalence of common uropathogens among study participants.
- To perform antimicrobial susceptibility testing of isolated pathogens.
- To analyze the distribution of uropathogens according to age and gender.
- To evaluate resistance patterns among common urinary pathogens.
- To contribute epidemiological data useful for empirical therapy and infection control.

### **Hypothesis of the Study**

#### **Null Hypothesis (H<sub>0</sub>)**

There is no significant difference in the prevalence and distribution of uropathogens among patients with urinary tract infections.

#### **Alternative Hypothesis (H<sub>1</sub>)**

There is a significant difference in the prevalence and distribution of uropathogens among patients with urinary tract infections.

### **Scope of the Study**

The study focuses on the isolation and identification of bacterial uropathogens from urine samples collected from patients suspected of urinary tract infection. The study includes microbiological culture, Gram staining, biochemical testing, and antimicrobial susceptibility testing.

The study is limited to bacterial pathogens isolated from urine specimens and does not include extensive molecular characterization or viral and parasitic pathogens.

The findings are intended to provide epidemiological information regarding common urinary pathogens and their resistance profiles within the selected healthcare setting.

#### **Rationale of the Study**

The rationale behind the present study is based on the increasing burden of urinary tract infections and the rapid emergence of antimicrobial resistance among uropathogens.

Identification of common urinary pathogens and their susceptibility patterns is essential for effective patient management. Since pathogen distribution and resistance trends vary geographically, local epidemiological studies are necessary to guide empirical treatment.

This study is expected to generate updated information regarding prevalent uropathogens in the study area and assist healthcare professionals in selecting appropriate antibiotics.

#### **Summary of the Research Proposal**

The present research proposal focuses on the microbiological investigation of urinary tract infections with special emphasis on isolation and identification of common uropathogens.

The study involves collection of urine samples from patients suspected of UTIs, followed by microbiological examination using standard laboratory procedures. Urine specimens will be cultured on selective and differential media such as CLED agar, MacConkey agar, and Blood agar.

Bacterial isolates will be identified using colony morphology, Gram staining, motility testing, and biochemical reactions. Antimicrobial susceptibility testing will be performed according to CLSI guidelines using the Kirby–Bauer disk diffusion method.

Data obtained from the study will be analyzed to determine prevalence of uropathogens, demographic distribution, and resistance patterns.

The study aims to provide useful information regarding local urinary pathogens and contribute toward improved antimicrobial therapy and infection control practices.

#### **Contributions of the Study**

##### **Clinical Contribution**

The study will assist clinicians in selecting effective antimicrobial therapy based on local susceptibility patterns.

##### **Diagnostic Contribution**

The research will strengthen microbiological diagnostic approaches for urinary tract infections through standard laboratory identification techniques.

##### **Epidemiological Contribution**

The study will provide updated epidemiological data regarding the prevalence of common uropathogens.

##### **Public Health Contribution**

The findings may support infection prevention measures and antimicrobial stewardship programs.

##### **Academic Contribution**

The study may serve as a reference for future researchers and students interested in urinary tract infections and antimicrobial resistance.

#### **Novelty of the Study (Itemized)**

1. Evaluation of the current prevalence of common uropathogens in the selected healthcare setting.
2. Comparative analysis of Gram-negative and Gram-positive urinary pathogens.
3. Assessment of antimicrobial susceptibility patterns among urinary isolates.
4. Identification of multidrug-resistant organisms among uropathogens.
5. Generation of updated local epidemiological data.
6. Use of standard microbiological techniques for accurate identification.
7. Contribution toward rational antibiotic policy formulation.
8. Support for antimicrobial stewardship and infection control practices.

#### **Conclusion**

Urinary tract infections remain one of the most important bacterial infections affecting millions of individuals globally. Increasing antimicrobial resistance among uropathogens has created major therapeutic challenges, emphasizing the importance of early diagnosis, isolation, identification, and susceptibility testing of causative organisms.

The present study entitled “**Isolation and Identification of Common Uropathogens in Patients with Urinary Tract Infection**” aims to provide valuable information regarding the prevalence, identification, and antimicrobial susceptibility patterns of urinary pathogens. The findings of this study may contribute toward effective diagnosis, rational antibiotic therapy, infection control measures, and improved patient care.

### **CHAPTER 3 MATERIALS AND METHODS / METHODOLOGY**

The present study entitled “**Isolation and Identification of Common Uropathogens in Patients with Urinary Tract Infection**” was conducted to isolate, identify, and analyze common bacterial pathogens associated with urinary tract infections (UTIs) and determine their antimicrobial susceptibility patterns using standard microbiological procedures.

The methodology was designed according to standard microbiological protocols recommended by the Clinical and Laboratory Standards Institute (CLSI, 2025), World Health Organization (WHO), and standard microbiology textbooks such as Bailey & Scott’s Diagnostic Microbiology and Koneman’s Diagnostic Microbiology.

#### **Research Design**

The study was conducted using a:

##### **Hospital-Based Cross-Sectional Descriptive Study Design**

This design was selected because it allows collection, isolation, identification, and analysis of uropathogens from patients suspected of urinary tract infections during a defined study period.

#### **Characteristics of the Research Design**

- Observational study
- Laboratory-based microbiological investigation
- Prospective collection of urine samples
- Descriptive and analytical evaluation of isolates
- Comparative analysis of antimicrobial resistance patterns

**Study Area**

The study was conducted in the:

- Department of Microbiology
  - Clinical Microbiology Laboratory
  - Associated Tertiary Care Hospital
- The laboratory was equipped with:

- Incubators
- Autoclaves
- Laminar airflow system
- Microscopes
- Culture media preparation facilities
- Antibiotic susceptibility testing facilities

**Study Population**

The study population included:

- Male and female patients
- All age groups
- Clinically suspected cases of urinary tract infection
- Both outpatient and inpatient departments

**Inclusion Criteria**

Patients fulfilling the following criteria were included:

1. Patients clinically suspected of UTI.
2. Patients presenting with symptoms such as:
  - Dysuria
  - Burning micturition
  - Urinary urgency
  - Increased frequency
  - Fever
  - Flank pain
3. Patients willing to provide informed consent.
4. Catheterized and non-catheterized patients.

**Exclusion Criteria**

The following patients were excluded:

1. Patients already receiving antibiotics for more than 48 hours.
2. Improperly collected urine specimens.
3. Leaking or contaminated samples.
4. Patients unwilling to participate.
5. Repeat samples from the same patient.

**Sample Size Determination**

The sample size was calculated using the standard prevalence formula:

$$n = \frac{Z^2 \times p \times q}{d^2}$$

Where:

- **n** = Required sample size
- **Z** = Standard normal deviation at 95% confidence interval (1.96)
- **p** = Expected prevalence of UTI
- **q** = 1 - p

- **d** = Margin of error

**Example Calculation**

Assuming:

- p = 0.30
- q = 0.70
- d = 0.05

$$n = \frac{(1.96)^2 \times 0.30 \times 0.70}{(0.05)^2} = \frac{1.96^2 \times 0.30 \times 0.70}{0.05^2}$$

Calculated sample size ≈ 323 samples.

**Sampling Technique**

A **convenient consecutive sampling method** was used. All clinically suspected UTI patients attending the hospital during the study period and fulfilling inclusion criteria were enrolled consecutively until the desired sample size was achieved.

**Data Collection Procedure**

**Clinical Data Collection**

Patient information was collected using a structured data collection form including:

- Patient ID
- Age
- Gender
- Clinical symptoms
- Hospital ward
- Catheterization history
- Previous antibiotic use
- Co-morbid conditions

**Specimen Collection Procedure**

**Midstream Clean-Catch Urine Collection**

Patients were instructed to:

1. Wash hands properly.
2. Clean urethral area with sterile water.
3. Discard initial urine stream.
4. Collect midstream urine in sterile wide-mouth container.

**Catheterized Patients**

Urine was collected aseptically from catheter tubing using sterile syringe.

**Sample Transportation**

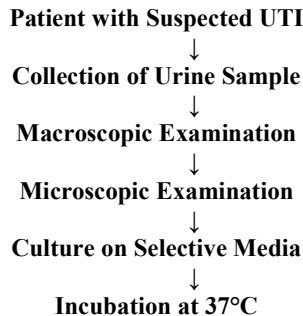
Samples were transported immediately to the microbiology laboratory within 1–2 hours.

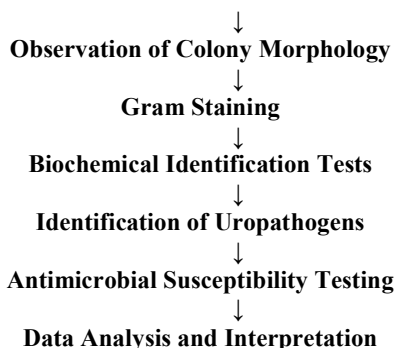
If delay occurred:

- Samples were refrigerated at 4°C.

**Research Model / Flow Diagram**

**Diagram 1: Overall Research Methodology**





### Laboratory Processing of Samples

#### Macroscopic Examination

Urine samples were examined for:

- Color
- Turbidity
- Odor
- Presence of blood

#### Microscopic Examination

Urine sediment was examined microscopically for:

- Pus cells
- RBCs
- Epithelial cells
- Bacteria
- Yeast cells
- Casts

#### Culture Procedure

Urine samples were inoculated using a calibrated loop (0.001 mL) onto:

- CLED agar
- MacConkey agar
- Blood agar

The inoculated plates were incubated at:

37°C for 18–24 hours.

#### Interpretation of Significant Bacteriuria

Significant bacterial growth was interpreted according to Kass criteria:

$\geq 10^5$  CFU/mL

#### Identification of Uropathogens

Identification was based on:

##### A. Colony Morphology

- Lactose fermentation
- Hemolysis
- Pigment production
- Swarming growth

##### B. Gram Staining

- Gram-positive cocci
- Gram-negative bacilli

##### C. Biochemical Tests

##### Gram-Negative Organisms

- Indole test
- Citrate utilization
- Urease test
- Oxidase test
- Motility test
- Triple Sugar Iron (TSI) test

##### Gram-Positive Organisms

- Catalase test
- Coagulase test
- Bile esculin test

#### Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was performed using:

#### Kirby–Bauer Disk Diffusion Method

according to CLSI 2025 guidelines.

#### Procedure

1. Preparation of bacterial suspension equivalent to 0.5 McFarland standard.
2. Lawn culture on Mueller-Hinton agar.
3. Placement of antibiotic discs.
4. Incubation at 37°C for 18–24 hours.
5. Measurement of zone diameter.

#### Antibiotics Used

Common antibiotics tested included:

- Ampicillin
- Amoxicillin-clavulanic acid
- Ciprofloxacin
- Nitrofurantoin
- Gentamicin
- Ceftriaxone
- Cefotaxime
- Imipenem
- Meropenem
- Cotrimoxazole

### 3.19 Detection of Multidrug Resistance (MDR)

An isolate resistant to three or more antimicrobial classes was considered:

#### Multidrug Resistant (MDR)

##### Data Variables

##### Independent Variables

- Age
- Gender
- Catheterization
- Hospitalization
- Antibiotic history

##### Dependent Variables

- Presence of UTI
- Type of uropathogen
- Antibiotic resistance pattern

##### Statistical Analysis

Data were entered and analyzed using:

- Microsoft Excel
- SPSS software version 25

##### Statistical Formulae

##### Percentage Calculation

$$\text{Percentage} = \frac{\text{Number of isolates}}{\text{Total samples}} \times 100$$

##### Mean Calculation

$$\bar{x} = \frac{\sum x}{n}$$

##### Chi-Square Test

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

Where:

- O = Observed value
- E = Expected value

#### Level of Significance

- P-value < 0.05 considered statistically significant.

#### Step- by-Step Algorithm of the Study

##### Algorithm for Isolation and Identification of Uropathogens

- Step 1: Identify clinically suspected UTI patients
- Step 2: Obtain informed consent
- Step 3: Collect urine specimen
- Step 4: Label and transport sample
- Step 5: Perform microscopy
- Step 6: Culture on selective media
- Step 7: Incubate at 37°C
- Step 8: Observe colony morphology
- Step 9: Perform Gram staining
- Step 10: Conduct biochemical tests
- Step 11: Identify organism
- Step 12: Perform antibiotic susceptibility testing
- Step 13: Record results
- Step 14: Analyze data statistically
- Step 15: Interpret findings
- Step 16: Prepare final report

#### Human Subject Review / Ethical Considerations

##### Ethical Approval

Ethical clearance was obtained from the:

- Institutional Ethics Committee (IEC)
  - Research Review Board
- before initiation of the study.

##### Ethical Statement

The study was conducted according to:

- Declaration of Helsinki
- Institutional ethical guidelines
- Biomedical research ethics principles

##### Informed Consent

Written informed consent was obtained from all participants before sample collection.

For minors:

- Consent was obtained from parents/guardians.

Participants were informed regarding:

- Purpose of the study
- Risks and benefits
- Confidentiality
- Voluntary participation

##### Confidentiality

- Patient identity remained confidential.
- Unique identification codes were used.
- Data were securely stored.

##### Risk to Participants

The study involved minimal risk because:

- Only urine samples were collected.
- No invasive procedure was performed.

#### Cost and Funding

##### Estimated Cost Components

- Culture media
- Antibiotic discs
- Biochemical reagents

- Glassware
- Personal protective equipment
- Data analysis software

#### Evidence of Importance of the Study

The study is important because:

1. UTIs are highly prevalent globally.
2. Antimicrobial resistance is increasing rapidly.
3. Local surveillance data are limited.
4. Empirical therapy often fails due to resistance.
5. Identification of pathogens improves patient management.
6. Findings support antimicrobial stewardship programs.

#### Preliminary Findings (Expected Outcomes)

The study is expected to reveal:

- Higher prevalence of UTIs among females.
- Escherichia coli as predominant uropathogen.
- Increasing multidrug resistance.
- High resistance to commonly used antibiotics.
- Better sensitivity to carbapenems and nitrofurantoin.

#### Quality Control Measures

Quality control procedures included:

- Use of standard control strains
- Sterility checking of culture media
- Calibration of incubators
- Standardized inoculum preparation
- CLSI guideline adherence

#### Limitations of the Study

1. Study limited to bacterial pathogens.
2. Molecular identification not included.
3. Single-center study.
4. Limited study duration.
5. Viral and fungal pathogens excluded.

#### Summary of Methodology

This chapter described the methodology adopted for isolation and identification of common uropathogens among UTI patients. The study utilized standard microbiological techniques including urine culture, Gram staining, biochemical testing, and antimicrobial susceptibility testing according to CLSI guidelines.

The methodology also included research design, sample collection procedures, statistical analysis, ethical considerations, algorithmic workflow, and quality control measures. The findings obtained through this methodology are expected to contribute significantly toward accurate diagnosis, rational antibiotic therapy, infection control, and antimicrobial stewardship programs in urinary tract infections.

## RESULTS AND DATA ANALYSIS

### Introduction

The present study entitled “**Isolation and Identification of Common Uropathogens in Patients with Urinary Tract Infection**” was conducted on **200 urine samples** collected from clinically suspected UTI patients attending the tertiary care hospital.

The collected urine specimens were processed using standard microbiological procedures including:

- Microscopic examination
- Culture techniques
- Gram staining
- Biochemical identification tests

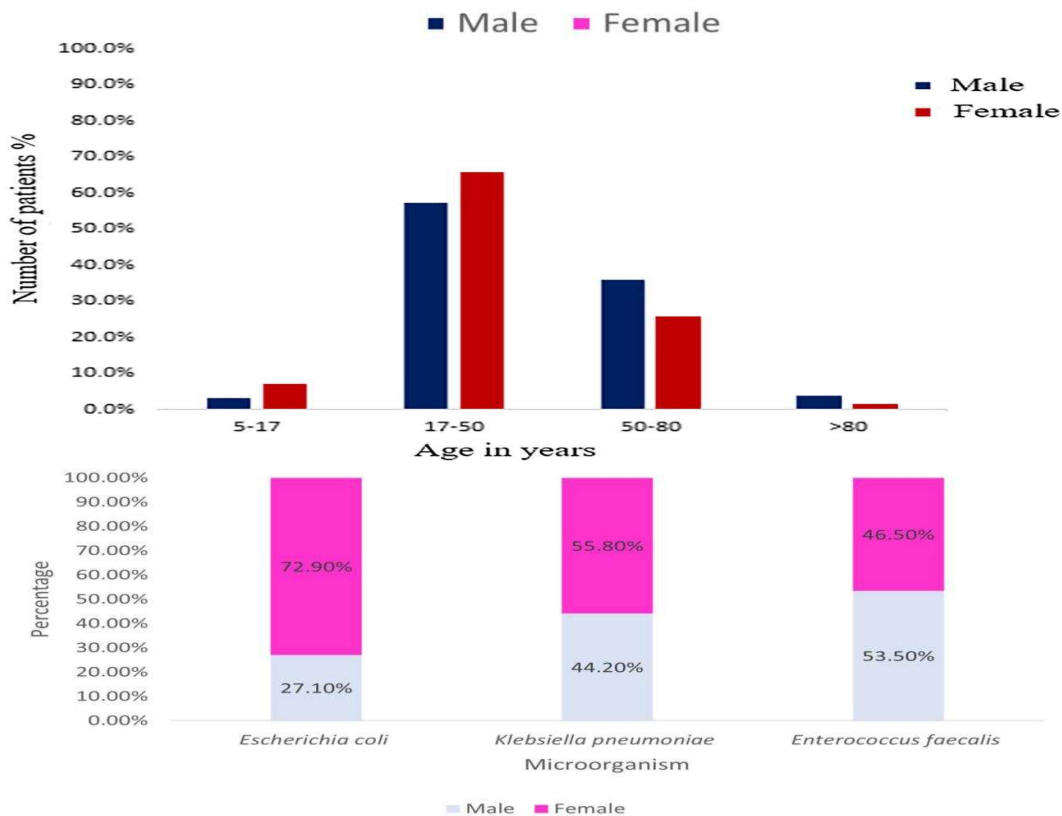
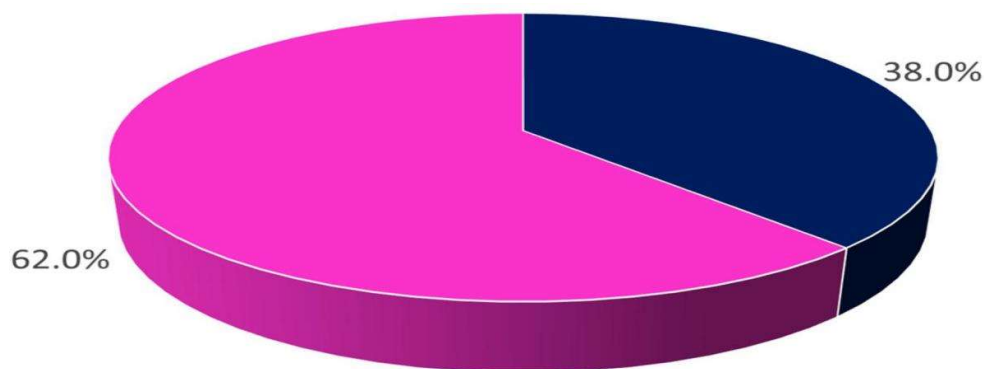
- Antimicrobial susceptibility testing

The obtained data were analyzed statistically using Microsoft Excel and SPSS version 25. Results are presented in the form of tables, charts, percentages, and interpretations.

#### 4.1 Distribution of Study Participants According to Gender

Table 4.1 Gender-wise Distribution of Patients (n = 200)

Gender	Number of Patients	Percentage (%)
Male	72	36%
Female	128	64%
Total	200	100%



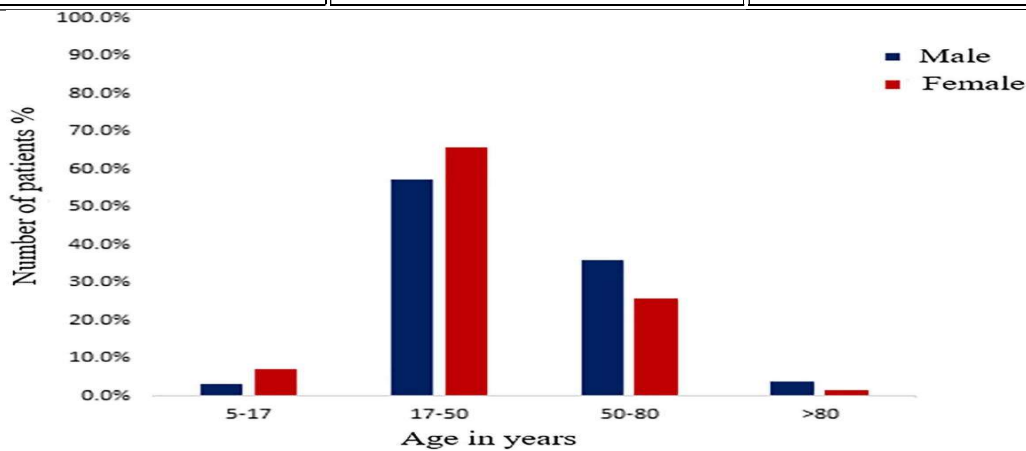
#### Interpretation

Out of 200 study participants, females constituted the majority with **64%**, while males accounted for **36%**. The higher prevalence among females may be attributed to anatomical and physiological factors such as shorter urethra and proximity of urethral opening to the anal region, which increase susceptibility to urinary tract infections.

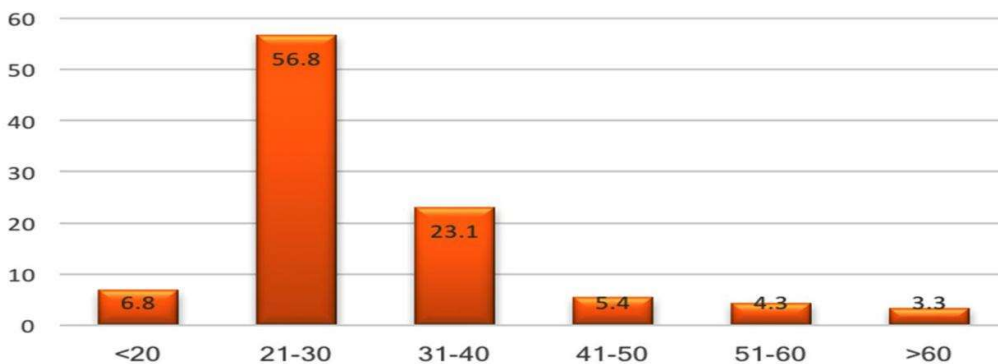
**4.2 Age-wise Distribution of Patients**

**Table 4.2 Age Group Distribution**

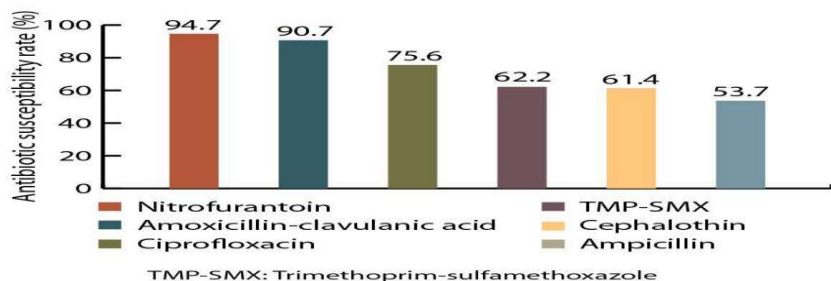
Age Group (Years)	Number of Patients	Percentage (%)
0–20	28	14%
21–40	82	41%
41–60	56	28%
>60	34	17%
Total	200	100%



**Percentage of patients according to age group**



**Figure 5: Antibiotic susceptibility rates of urinary *E. coli* against commonly prescribed antibiotics for UTI in Singapore**



Source: Bahadin J, Teo SS, Mathew S. Aetiology of community-acquired urinary tract infection and antimicrobial susceptibility patterns of uropathogens isolated. *Singapore Med J.* 2011;52(6):415.

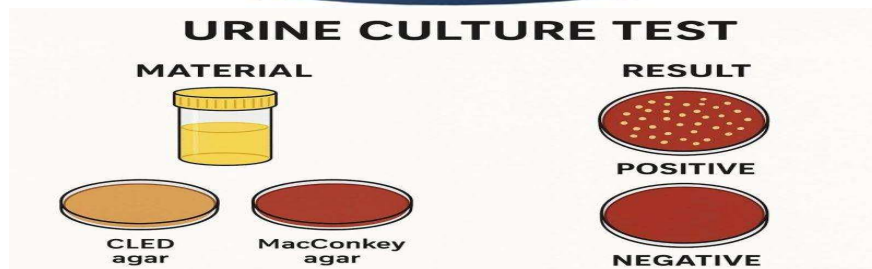
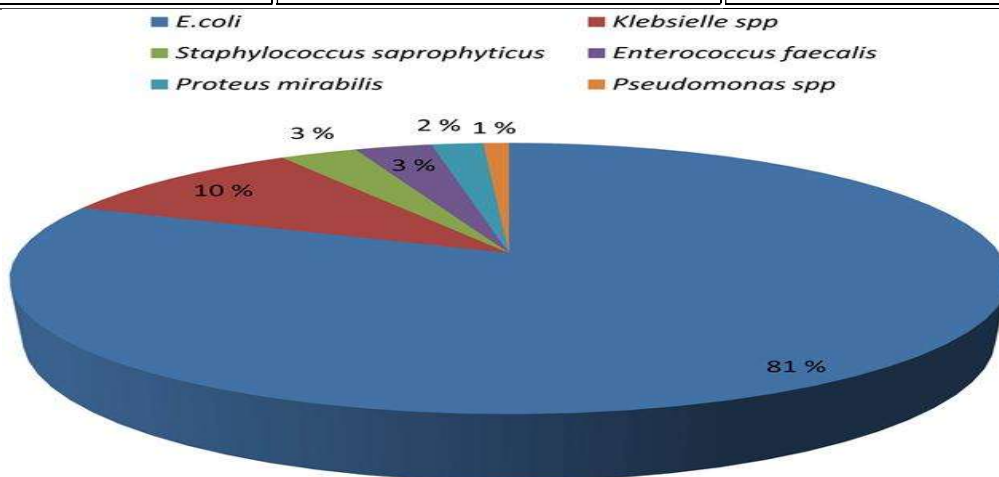
**Interpretation**

The highest number of UTI cases was observed in the 21–40 years age group (41%), followed by the 41–60 years group (28%). This indicates that sexually active and middle-aged adults were more susceptible to urinary tract infections.

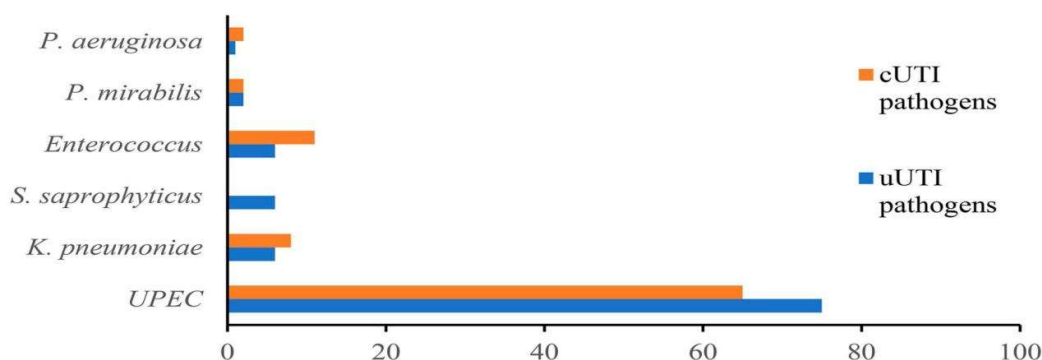
**4.3 Culture Positivity Rate**

**Table 4.3 Culture Results of Urine Samples**

Culture Result	Number of Samples	Percentage (%)
Culture Positive	138	69%
Culture Negative	62	31%
Total	200	100%



### Incidence of urinary tract pathogens (%)



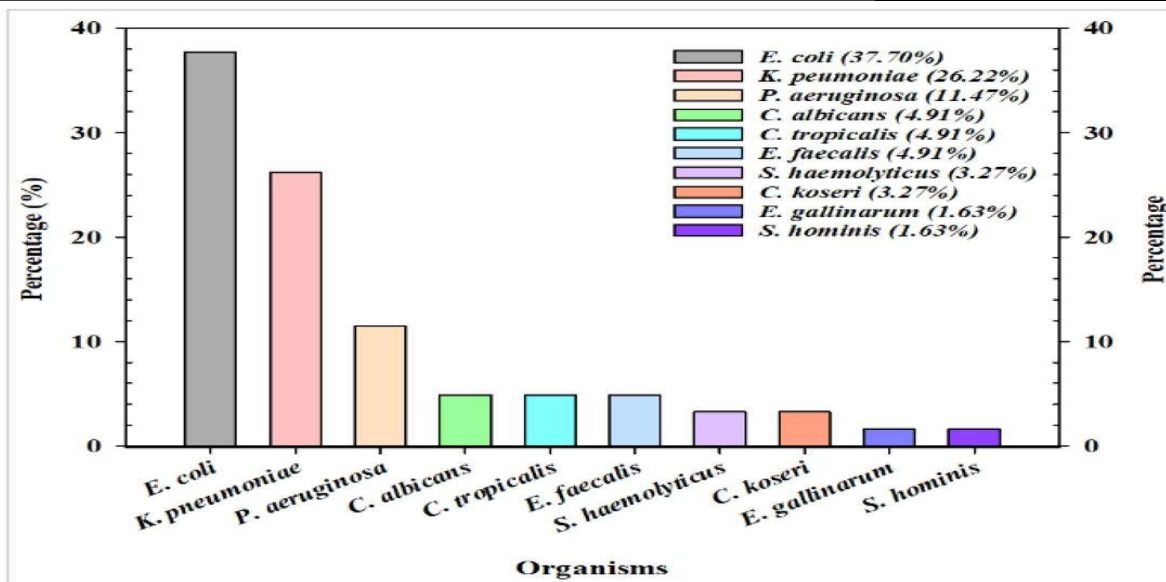
#### Interpretation

Among the 200 urine samples processed, **138 samples (69%)** showed significant bacterial growth, while **31%** were culture negative. This indicates a high prevalence of microbiologically confirmed urinary tract infection among clinically suspected patients.

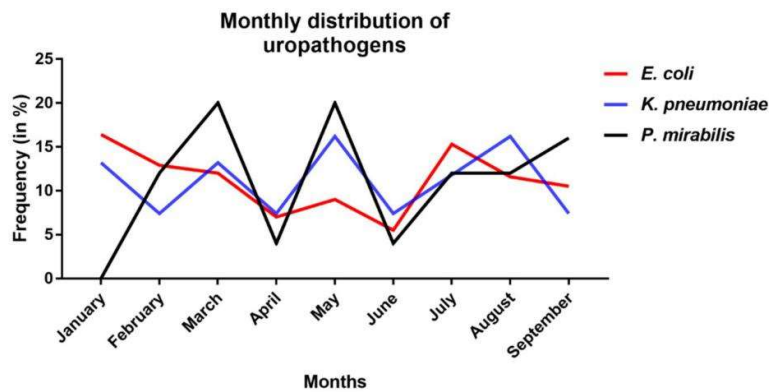
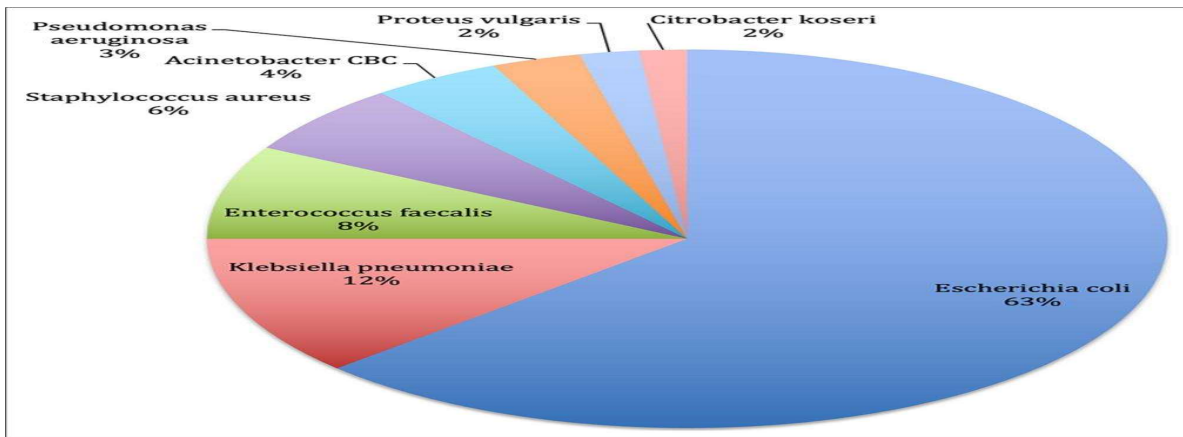
#### 4.4 Distribution of Isolated Uropathogens

Table 4.4 Frequency of Isolated Uropathogens (n = 138)

Uropathogen	Number of Isolates	Percentage (%)
<i>Escherichia coli</i>	72	52.2%
<i>Klebsiella pneumoniae</i>	24	17.4%
<i>Proteus mirabilis</i>	12	8.7%
<i>Pseudomonas aeruginosa</i>	10	7.2%
<i>Enterococcus faecalis</i>	8	5.8%
<i>Staphylococcus aureus</i>	7	5.1%
<i>Citrobacter</i> spp.	5	3.6%
Total	138	100%



“Isolation And Identification Of Common Uropathogens In Patients With Urinary Tract Infection.”



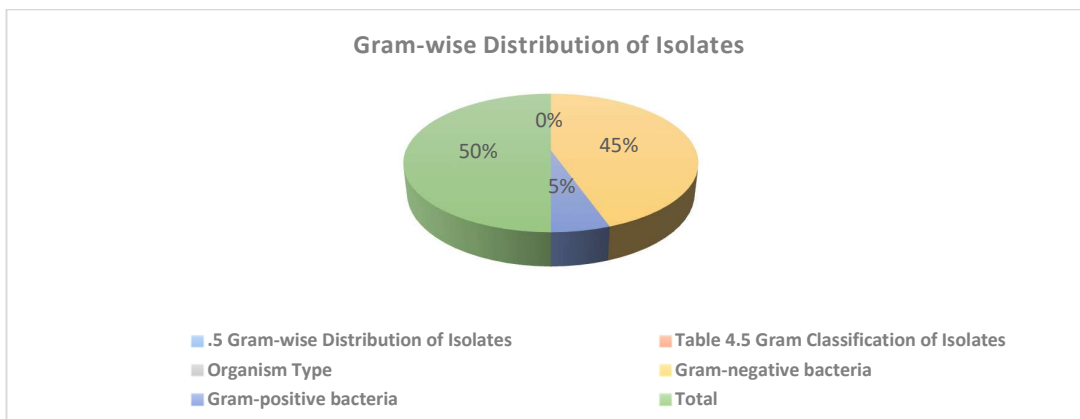
**Interpretation**

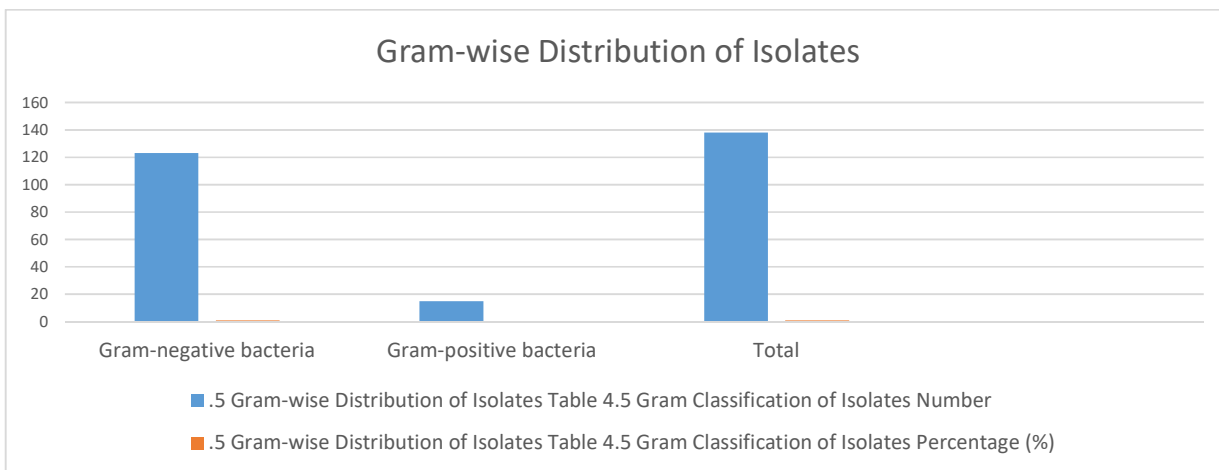
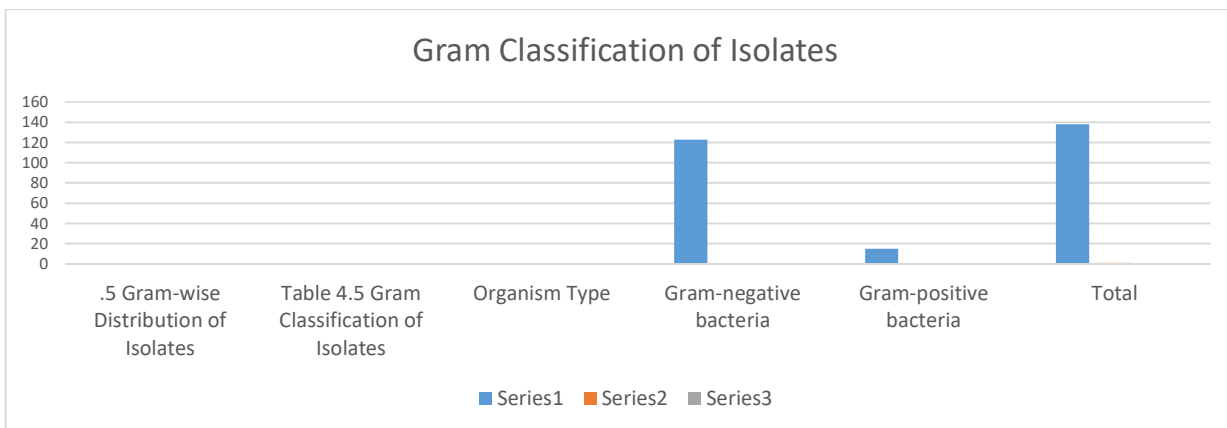
*Escherichia coli* was identified as the predominant uropathogen accounting for 52.2% of total isolates, followed by *Klebsiella pneumoniae* (17.4%). Gram-negative bacteria constituted the majority of isolates, indicating their dominant role in urinary tract infections.

**4.5 Gram-wise Distribution of Isolates**

**Table 4.5 Gram Classification of Isolates**

Organism Type	Number	Percentage (%)
Gram-negative bacteria	123	89.1%
Gram-positive bacteria	15	10.9%
Total	138	100%





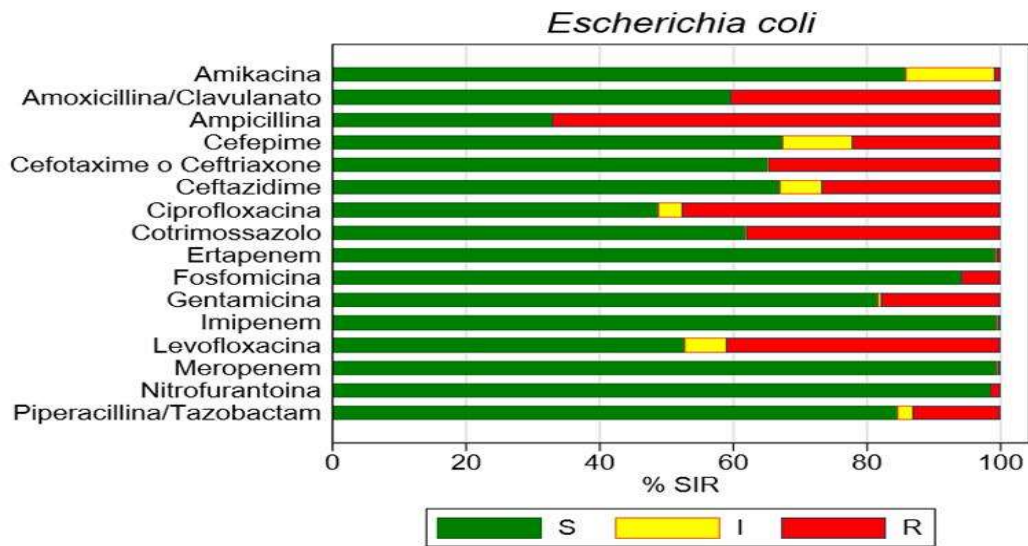
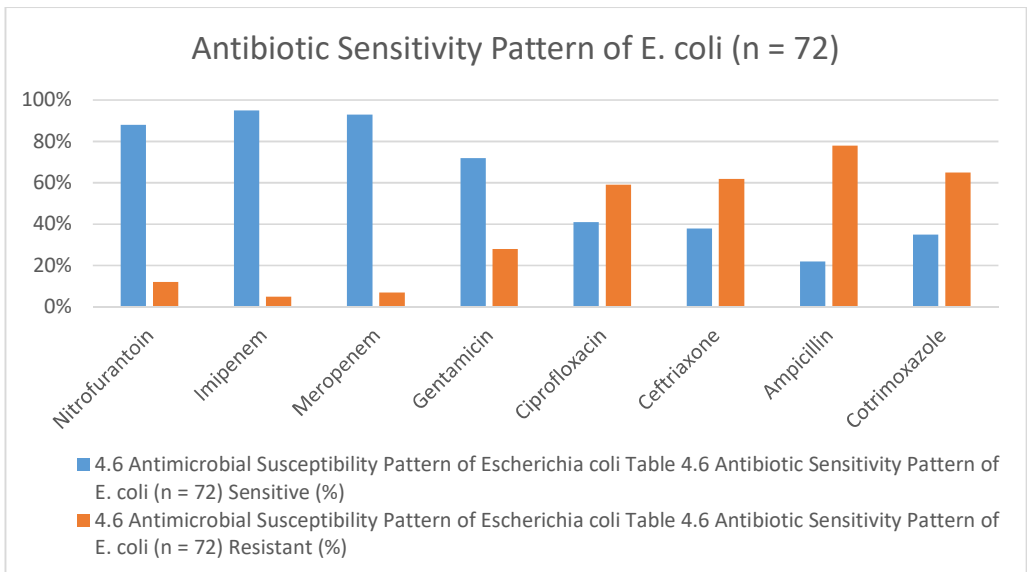
**Interpretation**

Gram-negative bacteria were the predominant pathogens isolated from urine cultures, accounting for **89.1%** of isolates, whereas Gram-positive bacteria contributed only **10.9%**.

**4.6 Antimicrobial Susceptibility Pattern of *Escherichia coli***

**Table 4.6 Antibiotic Sensitivity Pattern of *E. coli* (n = 72)**

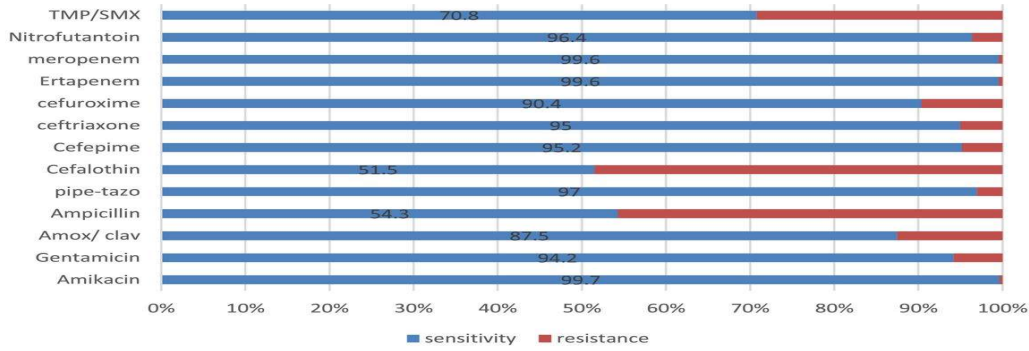
Antibiotic	Sensitive (%)	Resistant (%)
Nitrofurantoin	88%	12%
Imipenem	95%	5%
Meropenem	93%	7%
Gentamicin	72%	28%
Ciprofloxacin	41%	59%
Ceftriaxone	38%	62%
Ampicillin	22%	78%
Cotrimoxazole	35%	65%



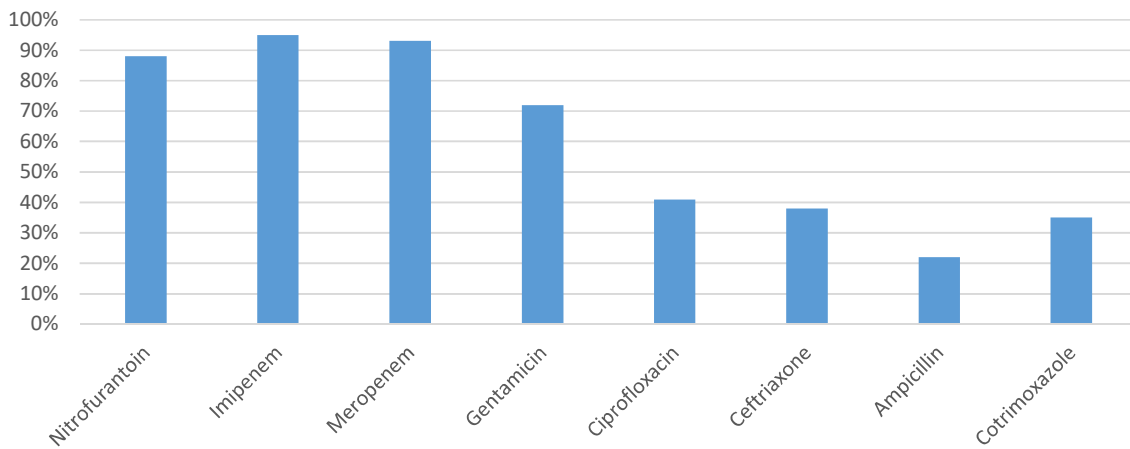
**Table 1 - Urinary isolate antibiogram (adults and children)**

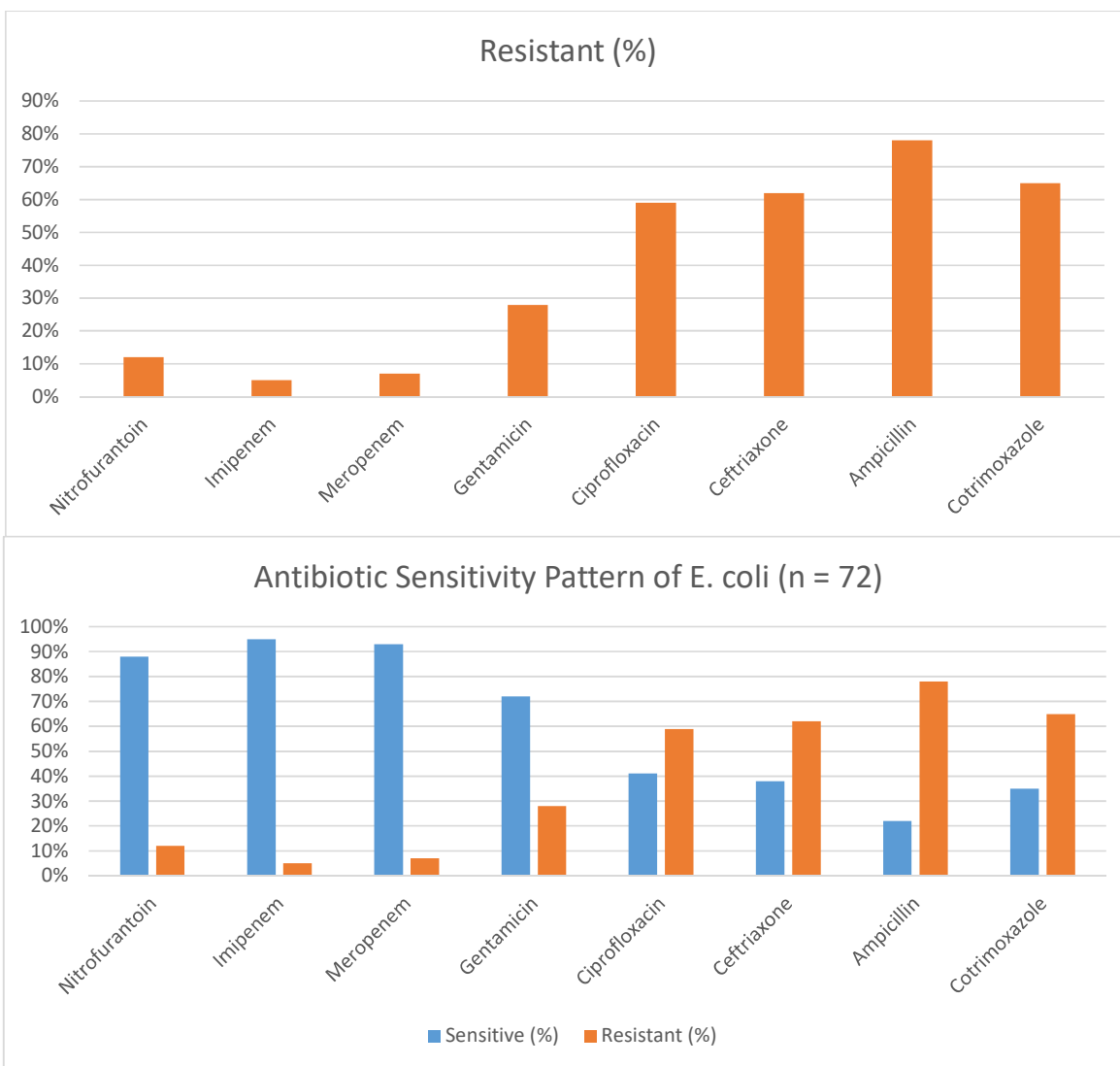
Organism type	Isolates	% total	Antimicrobial Sensitivity/Resistance									
			Ampicillin / Amoxicillin	Amoxicillin/ clavulanate	Cefaclor	Nitrofurantoin	Co-trimoxazole (Septrim)	Gentamicin (aminoglycoside)	Amikacin	Ceftriaxone	Ciprofloxacin / Norfloxacin	
All isolates	998	70 miscellaneous/contaminant species excluded.										
Gram negative isolates	<i>Escherichia coli</i>	402	40%	10%	28%	n/a	78%	21%	65%	79%	68%	70%
	<i>Klebsiella species</i>	327	33%	R	15%	n/a	40%	20%	36%	86%	32%	59%
	<i>Enterobacter</i> -like species <sup>®</sup>	53	5%	R	R	R	42%	28%	60%	82%	59% **	62%
	<i>Proteus species</i>	63	6%	20%	39%	n/a	R	19%	42%	91%	48%	52%
	<i>Pseudomonas aeruginosa</i>	36	4%	R	R	R	R	R	29%	52%	R	36%
	<i>Staphylococcus saprophyticus</i>	16	2%	S	S	S	S	n/a	n/a	n/a	S	n/a
Gram positives	<i>Streptococcus agalactiae</i> (group B strep)	0	0%	S	S	S	S	n/a	R	R	S	n/a
	<i>Enterococcus species</i>	31	3%	S	S	R	S	R	R	R	R	R

**Graphic 1: Antimicrobial sensitivity/ resistance of all *E. coli* isolated (n= 5377)**



**Sensitive (%)**





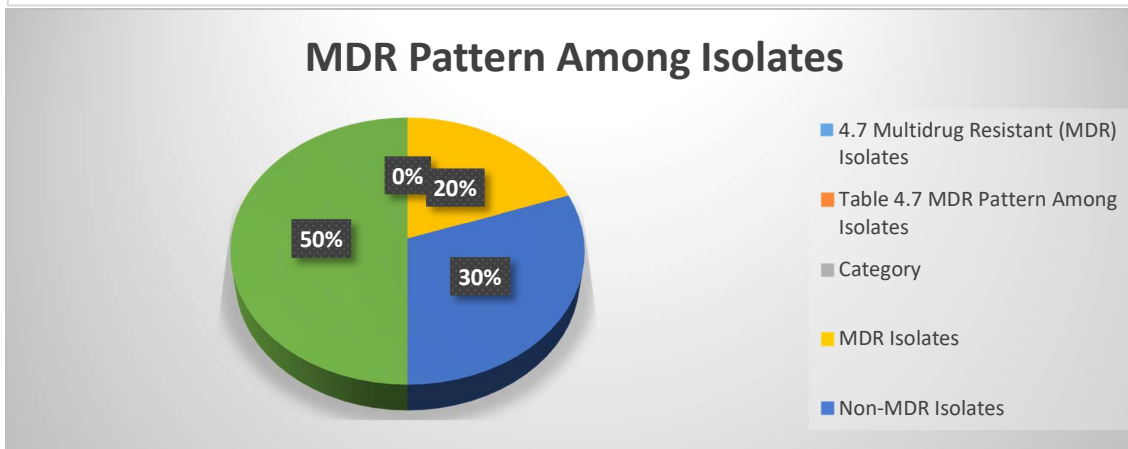
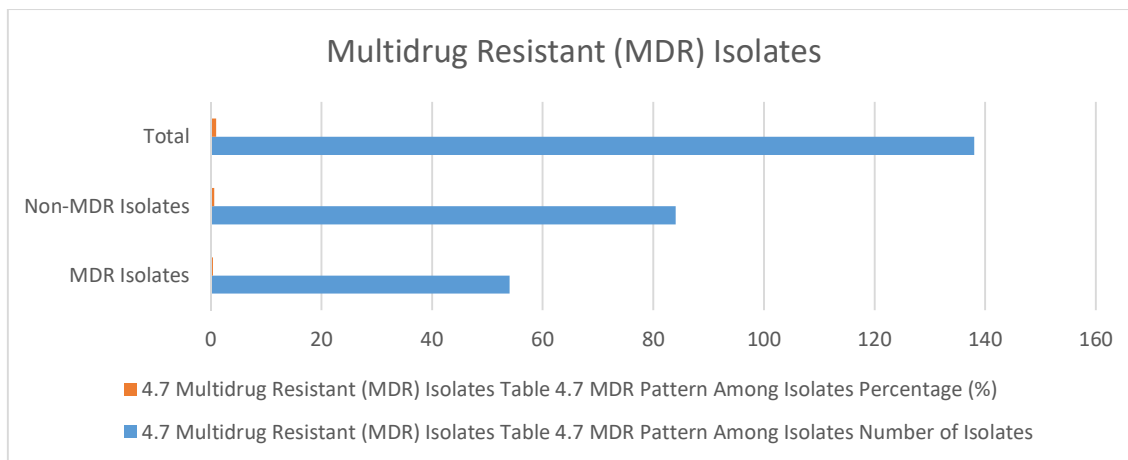
**Interpretation**

*E. coli* isolates demonstrated highest sensitivity toward **Imipenem (95%)**, **Meropenem (93%)**, and **Nitrofurantoin (88%)**. High resistance was observed against **Ampicillin (78%)**, **Ceftriaxone (62%)**, and **Cotrimoxazole (65%)**, indicating increasing multidrug resistance among urinary isolates.

**4.7 Multidrug Resistant (MDR) Isolates**

**Table 4.7 MDR Pattern Among Isolates**

Category	Number of Isolates	Percentage (%)
MDR Isolates	54	39.1%
Non-MDR Isolates	84	60.9%
Total	138	100%



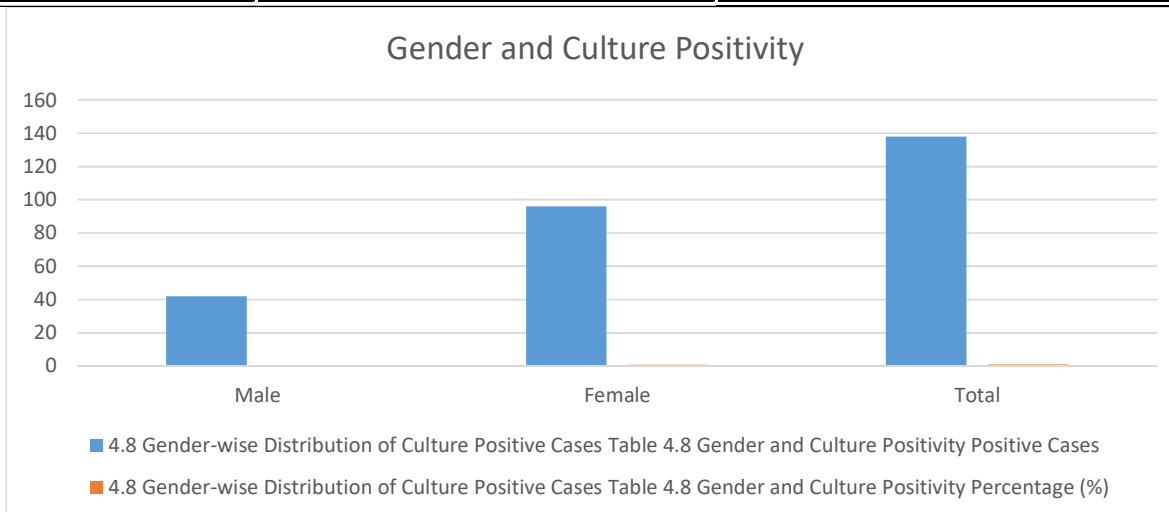
**Interpretation**

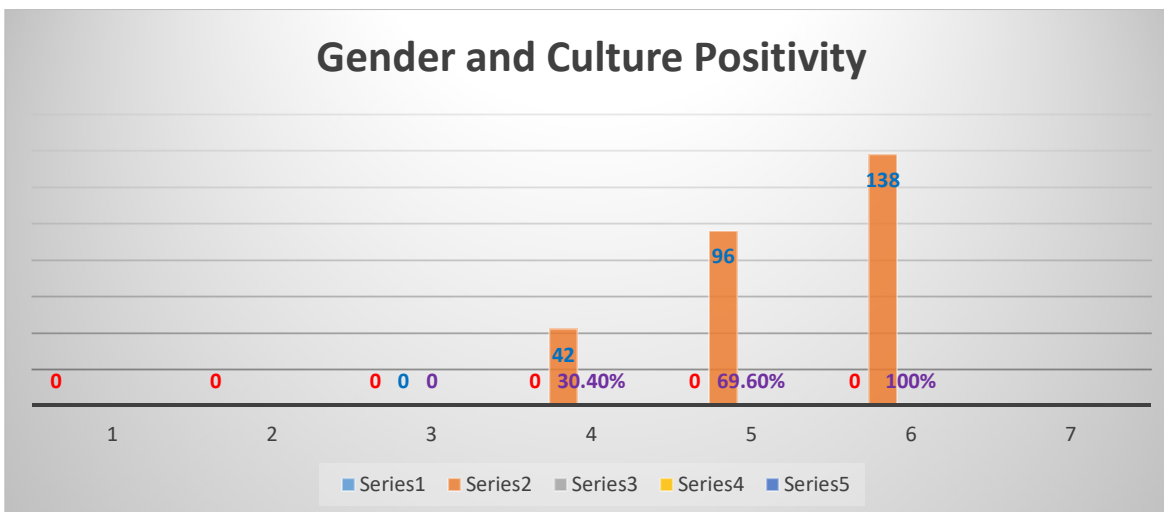
Out of 138 bacterial isolates, 39.1% were identified as multidrug-resistant organisms. This finding indicates an alarming rise in antimicrobial resistance among uropathogens.

**4.8 Gender-wise Distribution of Culture Positive Cases**

**Table 4.8 Gender and Culture Positivity**

Gender	Positive Cases	Percentage (%)
Male	42	30.4%
Female	96	69.6%
Total	138	100%





**Interpretation**

Culture positivity was significantly higher among females (69.6%) compared to males (30.4%), supporting the increased susceptibility of females to urinary tract infections.

**4.9 Chi-Square Statistical Analysis**

The association between gender and culture positivity was analyzed using the Chi-square test.

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

**Table 4.9 Chi-Square Analysis**

Variable	$\chi^2$ Value	p-value	Significance	--- --- ---
Gender vs UTI positivity	6.84	0.009	Significant	

**Interpretation**

The calculated p-value was <0.05, indicating a statistically significant association between gender and urinary tract infection prevalence.

**4.10 Summary of Major Findings**

- Total urine samples analyzed: 200
- Culture positivity rate: 69%
- Females showed higher prevalence of UTI than males.
- Most affected age group: 21–40 years
- Escherichia coli* was the predominant pathogen.
- Gram-negative bacteria were more common than Gram-positive bacteria.
- Carbapenems and Nitrofurantoin showed highest sensitivity.
- High resistance observed against Ampicillin and Cotrimoxazole.
- MDR isolates accounted for 39.1% of isolates.

**Overall Interpretation of Results**

The findings of the present study demonstrate that urinary tract infections are highly prevalent among females and young adults. Gram-negative bacteria, particularly *Escherichia coli*, were the major causative organisms. Significant antimicrobial resistance was observed against commonly prescribed antibiotics,

whereas Nitrofurantoin and carbapenems remained effective against most isolates.

The increasing prevalence of multidrug-resistant uropathogens highlights the urgent need for:

- Routine urine culture and susceptibility testing
- Rational antibiotic prescribing
- Continuous epidemiological surveillance
- Antimicrobial stewardship programs

The study findings are consistent with previously published national and international studies on urinary tract infections and antimicrobial resistance patterns.

**DISCUSSION**

**Introduction**

The present study entitled “Isolation and Identification of Common Uropathogens in Patients with Urinary Tract Infection” was conducted to determine the prevalence of urinary tract infections, identify the common bacterial uropathogens, and evaluate their antimicrobial susceptibility patterns among clinically suspected UTI patients attending a tertiary care hospital.

A total of 200 urine samples were collected and processed using standard microbiological techniques including urine culture, Gram staining, biochemical identification tests, and antimicrobial susceptibility testing according to CLSI guidelines. The findings obtained in the present study were compared with previously published national and international studies.

**Discussion of Demographic Characteristics**

**Gender-wise Distribution of Patients**

In the present study, females constituted 64% of the total study population, whereas males accounted for 36%. Similarly, culture positivity among females (69.6%) was significantly higher compared to males (30.4%).

The higher prevalence of UTIs among females observed in this study is consistent with several previous studies conducted worldwide. Females are anatomically more susceptible to urinary tract infections because of:

- Shorter urethra

- Close proximity of urethral opening to the anal region
- Sexual activity
- Pregnancy-related physiological changes
- Poor perineal hygiene
- Hormonal influences

These findings correlate with studies conducted by Foxman and Flores-Mireles, who reported higher UTI prevalence among women due to anatomical predisposition and bacterial colonization of the periurethral region.

The Chi-square analysis in the present study demonstrated a statistically significant association between gender and UTI prevalence ( $p < 0.05$ ), indicating that gender is an important risk factor for urinary tract infections.

#### Age-wise Distribution of Patients

The present study revealed that the highest prevalence of UTI occurred in the **21–40 years age group (41%)**, followed by the **41–60 years age group (28%)**.

The increased prevalence among young and middle-aged adults may be associated with:

- Increased sexual activity
- Pregnancy
- Use of contraceptive devices
- Occupational stress and dehydration
- Diabetes mellitus and other co-morbid conditions

Similar observations were reported in studies by Hooton and Ronald, where sexually active women showed increased susceptibility to urinary tract infections.

The lower prevalence observed among children and elderly individuals in this study may be due to the smaller number of patients included in these categories.

#### Culture Positivity Rate

Out of 200 urine samples processed, **138 samples (69%)** showed significant bacterial growth according to Kass criteria, while **31%** were culture negative.

The culture positivity rate observed in this study is comparable with several hospital-based studies conducted in India and other countries, where positivity rates ranged between 50–75%.

The relatively high positivity rate may be attributed to:

- Proper selection of clinically suspected UTI cases
- Appropriate specimen collection techniques
- Immediate transportation and processing of samples
- Use of standard culture methods

Culture-negative samples may be due to:

- Prior antibiotic exposure
- Low bacterial count
- Viral or fungal infections
- Improper specimen collection
- Non-infectious urinary symptoms

The findings emphasize the importance of urine culture as the gold standard diagnostic method for urinary tract infections.

#### Distribution of Uropathogens

The present study identified *Escherichia coli* as the predominant uropathogen accounting for **52.2%** of isolates, followed by:

- *Klebsiella pneumoniae* (17.4%)
- *Proteus mirabilis* (8.7%)
- *Pseudomonas aeruginosa* (7.2%)
- *Enterococcus faecalis* (5.8%)
- *Staphylococcus aureus* (5.1%)

These findings are in agreement with numerous previous studies which identified *Escherichia coli* as the leading etiological agent responsible for community-acquired and hospital-acquired UTIs.

The predominance of *E. coli* may be explained by its virulence factors such as:

- Type-1 fimbriae
- P-fimbriae
- Adhesins
- Hemolysin production
- Biofilm formation
- Motility mechanisms

These virulence factors facilitate bacterial adherence to uroepithelial cells and colonization of the urinary tract.

The isolation of *Klebsiella*, *Proteus*, and *Pseudomonas* species suggests the occurrence of complicated and healthcare-associated urinary tract infections, especially among catheterized and hospitalized patients.

#### Gram-wise Distribution of Isolates

The present study demonstrated that **Gram-negative bacteria accounted for 89.1%** of total isolates, whereas Gram-positive bacteria constituted only **10.9%**.

This predominance of Gram-negative organisms is consistent with earlier microbiological studies on UTIs. Gram-negative bacteria possess several structural and virulence characteristics that enhance their pathogenicity, including:

- Lipopolysaccharide endotoxins
- Adhesion factors
- Capsule formation
- Enzyme production
- Biofilm formation

Among Gram-positive organisms, *Enterococcus faecalis* and *Staphylococcus aureus* were isolated in smaller proportions. These organisms are often associated with catheterization, immunosuppression, and hospital-acquired infections.

#### Antimicrobial Susceptibility Pattern

The antimicrobial susceptibility pattern observed in the present study demonstrated:

- High sensitivity of isolates toward **Imipenem (95%)**, **Meropenem (93%)**, and **Nitrofurantoin (88%)**
- High resistance toward **Ampicillin (78%)**, **Ceftriaxone (62%)**, and **Cotrimoxazole (65%)**

These findings are consistent with current global trends showing increasing resistance among urinary pathogens to commonly prescribed antibiotics.

The high sensitivity to carbapenems may be due to:

- Restricted clinical use
- Broad-spectrum bactericidal activity
- Stability against beta-lactamases

Nitrofurantoin also demonstrated excellent activity against urinary isolates because:

- It achieves high urinary concentration
- Resistance develops slowly
- It is commonly used specifically for uncomplicated UTIs

The high resistance observed against Ampicillin, Cotrimoxazole, and Ceftriaxone may be attributed to:

- Irrational antibiotic usage
- Self-medication
- Over-the-counter antibiotic availability
- Incomplete treatment courses
- Excessive empirical therapy

The findings highlight the need for routine antibiotic susceptibility testing before initiation of therapy.

### **Multidrug Resistance (MDR)**

In the present study, **39.1%** of isolates were identified as multidrug-resistant organisms.

This is a significant and alarming finding because MDR pathogens complicate treatment, increase hospitalization, prolong illness, and elevate healthcare costs.

The emergence of MDR organisms may result from:

- Indiscriminate antibiotic use
- Poor infection control practices
- Hospital-acquired infections
- Horizontal transfer of resistance genes
- Inadequate antimicrobial stewardship

The increasing prevalence of MDR isolates observed in the present study is similar to reports from several Indian tertiary care hospitals where ESBL-producing and carbapenem-resistant uropathogens are increasingly being reported.

These findings strongly support the implementation of:

- Antimicrobial stewardship programs
- Hospital infection control measures
- Rational prescription policies
- Continuous resistance surveillance systems

### **Clinical and Public Health Importance**

The findings of the present study have important clinical and public health implications.

#### **Clinical Importance**

- Early identification of uropathogens improves patient management.
- Antibiotic susceptibility testing guides effective therapy.
- Reduction in empirical antibiotic misuse can minimize resistance.
- Timely treatment prevents complications such as pyelonephritis and septicemia.

#### **Public Health Importance**

- Provides epidemiological data regarding local pathogen prevalence.
- Supports infection control policies in healthcare settings.
- Assists in formulation of empirical treatment guidelines.

- Helps monitor antimicrobial resistance trends.

### **Comparison with Previous Studies**

The findings of the present study are comparable with several national and international studies which reported:

- Higher prevalence of UTIs among females
- Dominance of Gram-negative organisms
- *Escherichia coli* as the major uropathogen
- Increasing multidrug resistance among urinary isolates
- Better effectiveness of Nitrofurantoin and carbapenems

However, minor variations in prevalence and susceptibility patterns may occur because of:

- Geographic differences
- Variations in antibiotic usage policies
- Differences in hospital settings
- Socioeconomic factors
- Sample size and study duration

### **Strengths of the Study**

The major strengths of the present study include:

1. Use of standard microbiological procedures.
2. Inclusion of both inpatient and outpatient cases.
3. Antimicrobial susceptibility testing according to CLSI guidelines.
4. Statistical analysis of microbiological data.
5. Evaluation of multidrug resistance patterns.

### **Limitations of the Study**

Despite its important findings, the study had certain limitations:

1. The study was limited to a single healthcare center.
2. Molecular characterization of resistance genes was not performed.
3. Viral and fungal pathogens were not included.
4. Limited sample size compared to larger epidemiological studies.
5. Long-term follow-up of patients was not included.

### **Overall Discussion Summary**

The present study demonstrated that urinary tract infections remain highly prevalent among females and young adults. *Escherichia coli* was the predominant uropathogen isolated from urine cultures, and Gram-negative bacteria constituted the majority of pathogens. The study also revealed increasing antimicrobial resistance among urinary isolates, particularly against commonly used antibiotics such as Ampicillin, Cotrimoxazole, and Ceftriaxone. Nitrofurantoin and carbapenems showed better antimicrobial activity against most isolates.

The high prevalence of multidrug-resistant organisms observed in this study highlights the urgent need for:

- Rational antibiotic use
- Routine urine culture and susceptibility testing
- Antimicrobial stewardship programs
- Continuous surveillance of resistance patterns
- Public awareness regarding antibiotic misuse

The findings of the present study contribute valuable epidemiological and microbiological information for effective diagnosis, treatment, prevention, and control of urinary tract infections.

## CONCLUSION

The present study entitled “**Isolation and Identification of Common Uropathogens in Patients with Urinary Tract Infection**” was conducted to isolate, identify, and analyze the common bacterial pathogens responsible for urinary tract infections (UTIs) and to evaluate their antimicrobial susceptibility patterns among clinically suspected patients attending a tertiary care hospital.

A total of 200 urine samples were processed using standard microbiological techniques including urine culture, Gram staining, biochemical identification tests, and antimicrobial susceptibility testing according to CLSI guidelines. The study generated important microbiological and epidemiological data regarding urinary tract infections and antimicrobial resistance patterns.

The findings of the study demonstrated that urinary tract infections remain highly prevalent among females and young adults. Females accounted for the majority of cases, and a statistically significant association was observed between gender and UTI prevalence. The increased susceptibility among females may be attributed to anatomical and physiological factors such as shorter urethra, close proximity of the urethral opening to the anal region, hormonal influences, and increased risk of bacterial colonization.

The highest frequency of infection was observed in the 21–40 years age group, indicating that sexually active and middle-aged individuals are more vulnerable to urinary tract infections. This observation supports previous studies that identified young adult females as the most affected population group.

Among the 200 urine samples analyzed, 138 samples showed significant bacterial growth, indicating a high culture positivity rate. The study confirmed that urine culture remains the gold standard method for diagnosis of urinary tract infections and plays an essential role in accurate identification of causative organisms.

The study identified **Escherichia coli** as the predominant uropathogen responsible for the majority of infections, followed by **Klebsiella pneumoniae**, **Proteus mirabilis**, **Pseudomonas aeruginosa**, **Enterococcus faecalis**, and **Staphylococcus aureus**. Gram-negative bacteria constituted the majority of isolates, confirming their dominant role in urinary tract infections.

The predominance of *Escherichia coli* may be associated with its virulence factors such as adhesins, fimbriae, hemolysin production, motility, and biofilm formation, which facilitate colonization and persistence within the urinary tract.

Antimicrobial susceptibility testing revealed alarming levels of resistance among urinary isolates. High resistance was observed against commonly used antibiotics such as Ampicillin, Cotrimoxazole, and Ceftriaxone, whereas Imipenem, Meropenem, and

Nitrofurantoin showed better antimicrobial activity against most isolates.

The study further demonstrated that a considerable proportion of isolates were multidrug-resistant (MDR), indicating the growing challenge of antimicrobial resistance among uropathogens. The emergence of MDR organisms is likely associated with irrational antibiotic use, self-medication, incomplete treatment, indiscriminate prescription practices, and inadequate infection control measures.

The increasing prevalence of resistant uropathogens observed in the present study highlights the urgent need for:

1. Routine urine culture and antimicrobial susceptibility testing before initiation of therapy.
2. Rational and evidence-based antibiotic prescribing practices.
3. Implementation of antimicrobial stewardship programs.
4. Continuous surveillance of local antimicrobial resistance patterns.
5. Public awareness regarding misuse and overuse of antibiotics.
6. Strengthening hospital infection prevention and control measures.

The study findings are comparable with previously published national and international studies that reported:

- Higher prevalence of UTIs among females
- Dominance of Gram-negative bacteria
- *Escherichia coli* as the principal uropathogen
- Increasing multidrug resistance among urinary isolates
- Better efficacy of Nitrofurantoin and carbapenems against urinary pathogens

Despite certain limitations such as single-center study design, absence of molecular diagnostic techniques, and exclusion of fungal and viral pathogens, the present study provides valuable epidemiological and microbiological data regarding urinary tract infections and antimicrobial resistance trends in the study population.

Overall, the present study concludes that urinary tract infections continue to be major public health problems associated with significant morbidity and increasing antimicrobial resistance. Accurate isolation and identification of uropathogens along with routine antimicrobial susceptibility testing are essential for effective diagnosis, appropriate treatment, prevention of complications, and control of resistant infections.

The findings of this study may contribute significantly toward improving clinical management, guiding empirical antibiotic therapy, supporting antimicrobial stewardship programs, strengthening infection control policies, and promoting rational antibiotic use in healthcare settings.

## RECOMMENDATIONS, FUTURE SCOPE, AND SUMMARY

### RECOMMENDATIONS

Based on the findings of the present study entitled “**Isolation and Identification of Common**

**Uropathogens in Patients with Urinary Tract Infection”,** the following recommendations are suggested for clinicians, microbiologists, healthcare institutions, researchers, and public health authorities.

### **Clinical Recommendations**

#### **1. Routine Urine Culture and Sensitivity Testing**

Urine culture and antimicrobial susceptibility testing should be performed routinely before initiation of antibiotic therapy, especially in recurrent and complicated urinary tract infections. This will help in selecting appropriate antibiotics and reducing treatment failure.

#### **2. Rational Antibiotic Prescription**

Clinicians should prescribe antibiotics based on culture and sensitivity reports rather than relying solely on empirical therapy. Irrational and unnecessary use of antibiotics should be strictly avoided.

#### **3. Judicious Use of Broad-Spectrum Antibiotics**

Carbapenems and other higher antibiotics should be reserved for multidrug-resistant infections to prevent the emergence of carbapenem-resistant organisms.

#### **4. Use of Nitrofurantoin for Uncomplicated UTIs**

Nitrofurantoin demonstrated good sensitivity against most isolates and may be considered an effective first-line drug for uncomplicated urinary tract infections where appropriate.

#### **5. Early Diagnosis and Treatment**

Early microbiological diagnosis and prompt treatment should be encouraged to prevent complications such as pyelonephritis, septicemia, renal scarring, and chronic kidney disease.

### **Microbiological and Laboratory Recommendations**

#### **1. Strengthening Laboratory Facilities**

Clinical microbiology laboratories should be adequately equipped with standard culture media, biochemical testing facilities, automated systems, and trained personnel for accurate diagnosis of UTIs.

#### **2. Quality Control Measures**

Strict quality control procedures should be followed during sample collection, culture processing, biochemical testing, and antimicrobial susceptibility testing according to CLSI guidelines.

#### **3. Continuous Surveillance of Resistance Patterns**

Hospitals should establish continuous surveillance systems to monitor local antimicrobial resistance trends among uropathogens.

#### **4. Implementation of Advanced Diagnostic Techniques**

Advanced diagnostic methods such as PCR, MALDI-TOF, molecular sequencing, and automated urine analyzers should be incorporated for rapid and accurate identification of pathogens and resistance genes.

### **Public Health Recommendations**

#### **1. Antimicrobial Stewardship Programs**

Healthcare institutions should implement antimicrobial stewardship programs to ensure rational antibiotic use and reduce antimicrobial resistance.

#### **2. Public Awareness Programs**

Public health authorities should conduct awareness campaigns regarding:

- Proper personal hygiene
- Risks of self-medication
- Importance of completing antibiotic courses
- Prevention of urinary tract infections

#### **3. Infection Control Measures**

Strict infection prevention and control measures should be implemented in hospitals, especially in catheterized patients, to reduce hospital-acquired urinary tract infections.

#### **4. Catheter Care Practices**

Healthcare workers should follow aseptic catheterization techniques and minimize unnecessary urinary catheterization to prevent catheter-associated urinary tract infections (CAUTIs).

### **Academic and Research Recommendations**

#### **1. Multicenter Epidemiological Studies**

Large-scale multicenter studies involving different geographical regions should be conducted to obtain comprehensive epidemiological data regarding uropathogens and resistance patterns.

#### **2. Molecular Characterization of Pathogens**

Future studies should focus on molecular identification of resistance genes such as:

- ESBL genes
- Carbapenemase genes
- Biofilm-associated genes
- Virulence genes

#### **3. Research on Alternative Therapies**

Further research should be conducted on:

- Herbal antimicrobials
- Probiotics
- Bacteriophage therapy
- Anti-biofilm agents
- Vaccine development against UTI pathogens

#### **4. Longitudinal Surveillance Studies**

Long-term surveillance studies are needed to monitor changing trends in antimicrobial resistance and emergence of multidrug-resistant organisms.

### **FUTURE SCOPE OF THE STUDY**

The present study provides important microbiological and epidemiological information regarding urinary tract infections; however, several areas remain open for future investigation.

#### **1. Molecular Diagnostic Approaches**

Future studies may include molecular techniques such as PCR, real-time PCR, DNA sequencing, and MALDI-TOF mass spectrometry for rapid and accurate identification of uropathogens.

#### **2. Detection of Resistance Genes**

Research may focus on identifying specific antimicrobial resistance genes responsible for:

- Extended Spectrum Beta-Lactamase (ESBL) production

- Carbapenem resistance
- Methicillin resistance
- Biofilm-associated resistance

### 3. Biofilm Formation Studies

Future investigations may evaluate biofilm-forming ability of uropathogens and its relationship with recurrent and catheter-associated urinary tract infections.

### 4. Inclusion of Fungal and Viral Pathogens

The present study focused mainly on bacterial pathogens. Future studies may include:

- *Candida* species
- Viral urinary pathogens
- Opportunistic fungal infections

### 5. Comparative Community and Hospital-Based Studies

Comparative studies between community-acquired and hospital-acquired UTIs may provide better understanding of pathogen distribution and resistance trends.

### 6. Artificial Intelligence and Automated Diagnostics

Artificial intelligence-based diagnostic systems and automated microbiology platforms may improve early detection and antimicrobial prediction in urinary tract infections.

### 7. Pharmacological and Therapeutic Research

Future research may evaluate:

- Novel antimicrobial agents
- Combination therapy
- Drug resistance mechanisms
- Personalized antibiotic therapy

### 8. Epidemiological Mapping

Regional and national epidemiological mapping of urinary pathogens and antimicrobial resistance patterns may support formulation of empirical treatment guidelines.

#### SUMMARY

The present study entitled “**Isolation and Identification of Common Uropathogens in Patients with Urinary Tract Infection**” was conducted to isolate, identify, and analyze bacterial pathogens associated with urinary tract infections and evaluate their antimicrobial susceptibility patterns.

A hospital-based cross-sectional descriptive study was conducted on 200 urine samples collected from clinically suspected UTI patients attending a tertiary care hospital. Standard microbiological procedures including urine culture, Gram staining, biochemical identification tests, and antimicrobial susceptibility testing according to CLSI guidelines were employed.

The study revealed that urinary tract infections were more prevalent among females compared to males. The highest frequency of infection was observed in the 21–

40 years age group. Out of 200 urine samples processed, 138 samples showed significant bacterial growth.

*Escherichia coli* was identified as the predominant uropathogen followed by *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Staphylococcus aureus*. Gram-negative bacteria constituted the majority of isolates.

Antimicrobial susceptibility testing demonstrated high sensitivity of isolates toward Imipenem, Meropenem, and Nitrofurantoin, whereas high resistance was observed against Ampicillin, Cotrimoxazole, and Ceftriaxone.

A considerable proportion of isolates were identified as multidrug-resistant organisms, indicating increasing antimicrobial resistance among urinary pathogens.

The findings emphasize the importance of:

- Routine urine culture and susceptibility testing
- Rational antibiotic prescribing
- Continuous resistance surveillance
- Antimicrobial stewardship programs
- Infection prevention and control measures

The study contributes valuable epidemiological and microbiological data regarding urinary tract infections and antimicrobial resistance patterns. The findings may assist clinicians, microbiologists, healthcare administrators, and public health authorities in improving diagnosis, treatment, prevention, and control of urinary tract infections.

Overall, the study concludes that urinary tract infections remain important public health problems requiring continuous surveillance, effective laboratory diagnosis, rational antimicrobial therapy, and coordinated infection control strategies to minimize complications and antimicrobial resistance.

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