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Research Article

Isolation, Identification, and Antibiotic Susceptibility Assessment of Bacterial Isolates in Urinary Tract Infections at a Tertiary Care Hospital

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Abstract

Urinary tract infections (UTIs) cause health problems, especially in tertiary care settings where patients are at higher risk. The aim of this study was to isolate, characterize and evaluate the antimicrobial resistance patterns of bacteria isolated from patients with urinary tract infections in a tertiary hospital. The aim is to understand in detail the major pathogens that cause urinary tract infections and their resistance data; this will be important for the treatment of antibiotic disease and will improve patient outcomes. test samples of patients. Isolates were identified by biochemical tests and confirmed using molecular methods. Antibiotic resistance of these isolates was assessed using the Kirby Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Major diseases are the most isolated diseases. Antibiotics are important, especially antibiotics such as ampicillin and trimethoprim sulfamethoxazole. The fact that a majority of the isolates showed multiple resistance indicates that vaccination programs urgently need to be continued and improved. to guide empirical treatment and inform hospital antibiotic policy. This study highlights the evolving nature of urinary tract infections and the importance of developing appropriate treatment strategies to address the increasing problem of antibiotic-resistant urinary tract infections in these settings.

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Introduction

Urinary Tract Infections (UTIs) are among the most frequent infections encountered in clinical practice, significantly affecting patient morbidity and increasing healthcare costs. They constitute a considerable burden in tertiary care hospitals, where patients often present higher vulnerability due to underlying health conditions and invasive medical procedures. Effective management of UTIs heavily relies on the prompt and accurate identification of the causative pathogens and the determination of their antibiotic susceptibility patterns.

The rise of antibiotic resistance among uropathogens presents a severe challenge to UTI treatment. This problem is particularly acute in hospital settings, where the prevalent use of broad-spectrum antibiotics often leads to the selection and proliferation of resistant strains. Therefore, understanding the local epidemiology of UTI-causing bacteria and their resistance profiles is crucial for guiding empirical treatment and optimizing patient outcomes.

The primary objective of this study is to isolate and identify bacterial pathogens from patients diagnosed with UTIs in a tertiary care hospital. Additionally, we aim to assess the antibiotic susceptibility patterns of these isolates to provide insights into the current state of antibiotic resistance and to guide appropriate therapeutic strategies.

This research is especially pertinent to tertiary care hospitals, where the complexity of cases and the diversity of bacterial pathogens necessitate robust and detailed microbiological surveillance. By elucidating the resistance patterns of uropathogens, this study seeks to contribute to the development of more effective antibiotic stewardship programs and support the rational use of antibiotics in clinical practice.

In this study, clinical samples from patients with suspected UTIs were systematically collected and processed. Bacterial isolates were identified using conventional biochemical methods and advanced molecular techniques to ensure accuracy. The antibiotic susceptibility of these isolates was determined through the Kirby-Bauer disk diffusion method, adhering to the standards set by the Clinical and Laboratory Standards Institute (CLSI).

The findings from this research are expected to provide valuable data on the prevalence and resistance profiles of uropathogens in a tertiary care setting. This highlights the critical need for continuous monitoring and tailored interventions to combat antibiotic

resistance. This study aims to enhance our understanding of UTI management in hospital environments and support the implementation of effective infection control measures.

The isolation, identification, and assessment of antibiotic susceptibility patterns of bacterial isolates in UTIs is a vital endeavor in the ongoing battle against antibiotic resistance. The outcomes of this study will have significant implications for clinical practice, particularly in tertiary care hospitals, where the stakes are high and the need for precise and effective treatment strategies is paramount.[8, 9]

AIMS AND OBJECTIVES

AIM:-

Investigating the Isolation, Identification, and Antibiotic Susceptibility Profiles of Bacterial Isolates from Clinical Specimens in Urinary Tract Infections, and Elucidating Their Antibiogram Patterns.

OBJECTIVES:

1) To collect clinical samples from urinary tract infection patients.
2) To isolate and identify bacterial species present in the samples.
3) To assess antibiotic susceptibility of bacterial isolates.
4) To analyse resistance patterns and susceptibility trends.
5) To characterize antibiogram profiles for effective treatment strategies.

MATERIAL AND METHODS

STUDY DESIGN

This study was conducted at the Microbiology Laboratory of Sharda Hospital, affiliated with Sharda University in Greater Noida, Uttar Pradesh.

STUDY PERIOD –

5 Months (e.g. 1 Feb 2024 to 30 June 2024)

Sample Collection

Patient Selection: - Patients presenting with symptoms indicative of urinary tract infections (UTIs) were recruited from both the outpatient department and inpatient wards of the tertiary care hospital. Inclusion criteria encompassed individuals of all ages and genders who exhibited clinical symptoms such as dysuria, increased frequency of urination, urgency, suprapubic tenderness, or fever.[10]

Informed Consent: - Prior to sample collection, informed consent was obtained from each participant or their legal guardian. The purpose of the study, procedures

involved, and potential risks and benefits were explained in detail, ensuring voluntary participation.

Sample Collection Procedure

1. Patient Preparation: Patients were provided with sterile urine collection containers and instructed on proper hygiene procedures for urine collection to minimize contamination.[11]

2. Midstream Urine Collection: Patients were instructed to clean their genital area with soap and water. Midstream urine samples were collected into sterile containers to reduce the possibility of contamination from the skin or urethra.[11]

3. Labeling and Transport: Each urine sample container was labeled with a unique identifier, including the patient's initials, date, and time of collection, to ensure traceability. Samples were promptly transported to the microbiology laboratory for processing. In instances where immediate transportation was not feasible, samples were refrigerated at 4°C and processed within 24 hours.

Isolation of Bacterial Pathogens [12]

- ***Blood Agar***: Enriched medium that supports the growth of a wide variety of bacteria and helps in the identification of hemolytic activity (Figure No. – 2).

Culture Techniques: - Upon receiving the urine samples in the microbiology laboratory, the following procedures were followed to isolate and identify bacterial pathogens:

1. Initial Processing:

- Each urine sample was thoroughly mixed, and an aliquot was taken for culture.
- A calibrated loop (0.001 mL) was used to inoculate the urine onto various culture media.

2. Culture Media: [13]

- ***MacConkey Agar***: Selective and differential medium used to isolate Gram-negative bacteria and differentiate lactose fermenters (pink colonies) from non-lactose fermenters (colorless colonies) (Figure No. – 1).

Figure 1 colorless colonies on MacConkey Agar

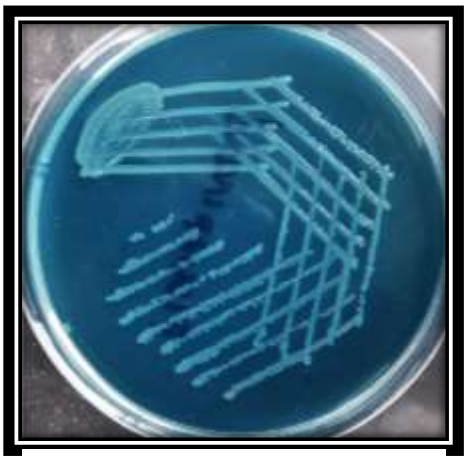


Figure 2Colonies on CLED Agar

Figure 3Colonies on Blood Agar

Cysteine Lactose Electrolyte-Deficient (CLED) Agar: Differential medium used for isolating and enumerating bacteria from urine samples, minimizing the swarming of Proteus species (Figure No. – 3).

3. Incubation:

- Inoculated plates were incubated aerobically at 37°C for 24-48 hours.
- Plates were examined for bacterial growth, colony morphology, and hemolysis patterns.

Morphological Identification[14]

1. Colony Morphology:

- Observations were made on the size, shape, color, and texture of the colonies on different media.
- Lactose fermenters on MacConkey agar appeared pink, whereas non-lactose fermenters remained colorless.

Hemolytic patterns on blood agar (alpha, beta, or gamma hemolysis) were noted.

2. Gram Staining:[15]

- Smears were prepared from isolated colonies and stained using the Gram staining technique.

Microscopic examination determined the Gram reaction (positive or negative), cell shape (cocci or bacilli), and arrangement.

Biochemical Identification

Isolated colonies were subjected to a series of biochemical tests to confirm their identity. The following tests were performed based on the preliminary Gram stain results:

1. for Gram-Negative Bacteria: (Figure No. – 6) [16]

- Oxidase Test: To determine the presence of cytochrome c oxidase. Positive results indicated organisms such as Pseudomonas spp.
- Indole Test: To detect the ability to convert tryptophan into indole. Positive results indicated organisms such as Escherichia coli.
- Methyl Red (MR) and Voges-Proskauer (VP) Tests: To differentiate between mixed acid fermenters (MR positive) and butylene glycol fermenters (VP positive).
- Citrate Utilization Test: To assess the ability to use citrate as the sole carbon source. Positive results indicated organisms such as Klebsiella and Enterobacter spp.
- Urease Test: To detect urease production. Positive results indicated organisms such as Proteus spp.
- Triple Sugar Iron (TSI) Agar Test: To determine carbohydrate fermentation and hydrogen sulfide production.

2. for Gram-Positive Bacteria: [17]

- Catalase Test: To differentiate catalase-positive staphylococci from catalase-negative streptococci.
- Coagulase Test: To identify Staphylococcus aureus (coagulase positive) from coagulase-negative staphylococci.
- Bile Esculin Test: To identify Enterococcus species.
- Mannitol Salt Agar (MSA): To differentiate Staphylococcus aureus (mannitol fermenters) from other staphylococci.

Confirmation of Identification[18]

1. Automated Systems: In some cases, automated systems such as the VITEK 2 or MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry) were used for more precise identification of bacterial isolates.

2. Quality Control: Known control strains were used to validate the accuracy and reliability of the biochemical tests and culture techniques.

Documentation and Reporting[19]

1. Data Recording: Results of the morphological, Gram staining, and biochemical tests were meticulously recorded in the laboratory data sheets.

2. Interim Reports: Preliminary identification results were communicated to the clinical team for immediate patient management, especially in cases of significant pathogens.

3. Final Reports: Comprehensive identification reports, including biochemical profiles and confirmed bacterial identities, were generated and integrated into the patient's medical records.

Antibiotic Susceptibility Testing [20]

Antibiotic susceptibility testing (AST) is crucial for determining the appropriate treatment regimen for bacterial infections. In this study, the Kirby-Bauer disk diffusion method was employed, following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) (Figure No. – 7).

Preparation of Inoculum [21]

1. Selection of Isolated Colonies: **-** Pure colonies from the primary culture plates were selected. These colonies were representative of the dominant morphology observed on the agar plates.

2. Preparation of Bacterial Suspension: [22]

- A few isolated colonies were emulsified in 0.85% sterile saline solution.
- The turbidity of the suspension was adjusted to match the 0.5 McFarland standard, which corresponds to approximately 1.5 x 10^8 CFU/mL.

Inoculation of Agar Plates

1. Media Preparation: **-** Mueller-Hinton agar (MHA) plates were used for the disk diffusion test. The plates were prepared according to the manufacturer's instructions and stored appropriately before use.

2. Inoculation:

- A sterile cotton swab was dipped into the standardized bacterial suspension.
- The surface of the MHA plate was evenly swabbed in three directions to ensure a uniform bacterial lawn.
- The plates were allowed to dry for 5-10 minutes before placing the antibiotic disks.

Placement of Antibiotic Disks [23]

1. Selection of Antibiotics:

- A panel of antibiotic disks was chosen based on commonly

prescribed antibiotics for UTIs and local antibiograms. The antibiotics included:

- Ampicillin
- Amoxicillin-clavulanic acid
- Ciprofloxacin
- Nitrofurantoin
- Trimethoprim-sulfamethoxazole
- Ceftriaxone
- Gentamicin
- Meropenem
- Piperacillin-tazobactam

2. Application of Disks:

- Antibiotic disks were dispensed onto the surface of the inoculated MHA plates using a sterile disk dispenser or sterile forceps.
- Each disk was gently pressed to ensure complete contact with the agar surface.

Incubation: - The inoculated and disk-applied plates were incubated at 37°C for 16-18 hours in an aerobic incubator.

Interpretation of Results[24]

1. Measurement of Inhibition Zones:

- After incubation, the diameter of the zones of inhibition around each antibiotic disk was measured using a calibrated ruler or digital caliper.
- The measurements were taken from the edge of the disk to the edge of the clear zone where no bacterial growth was observed.

2. Classification of Susceptibility:

- The zone diameters were compared to the CLSI standard interpretative charts to classify the bacterial isolates as:
- Susceptible (S)
- Intermediate (I)
- Resistant (R)

Quality Control[25]

1. Control Strains:

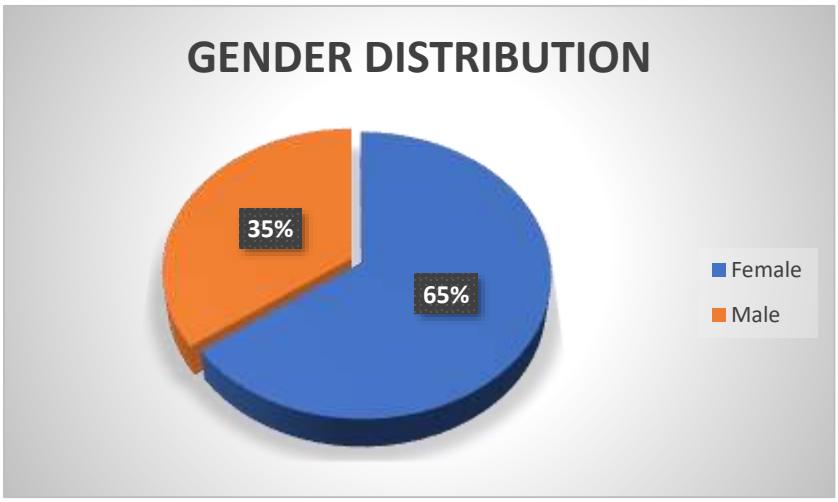
- Standard control strains (e.g., Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923) were tested concurrently to ensure the accuracy and reliability of the AST results.

2. Validation:

The control strains' inhibition zones were compared with the established CLSI reference ranges to validate the test conditions and reagents.

RESULT

The research encompassed an evaluation of 200 patients diagnosed with symptomatic urinary tract infections (UTIs) who sought medical care at Sharda Tertiary Care Hospital. During the investigation, comprehensive demographic information pertaining to the study population was meticulously collected and analyzed to gain insights into the epidemiological characteristics of UTIs in this clinical setting. This analysis aimed to provide a detailed understanding of the demographic composition of the patient cohort, facilitating a nuanced interpretation of the findings and informing tailored approaches to UTI management and prevention strategies.



Demographic analysis reveals a gender disparity in UTI prevalence, with females representing 65% (130 cases) of the 200-patient cohort, while males account for 35% (70 cases). This highlights the need for gender-specific approaches in UTI management and prevention strategies (Chart No. – 1).

Chart 1 Gender Distribution

A notable proportion of patients (35%) belong to the 31-40 years age group, indicating a heightened incidence of UTIs in this demographic segment. This underscores the significance of targeted interventions and heightened vigilance in managing UTIs

among individuals in their thirties and forties

(Table No. – 1).

S. No.	Age in Years	No. Of Patient	Percentage (%)	Female	Male
1.	18-30 years	60	30%	40	20
2.	31-40 years	70	35%	50	20
3.	41-50 years	30	15%	20	10
4.	51-60 years	20	10%	10	10
5.	61-75 years	20	10%	10	10

Table 1 Age Distribution and Gender Correlation

In the analysis of bacterial isolates from urinary tract infections (UTIs), *Escherichia coli* was the most prevalent pathogen, identified in 125 patients. This was followed by *Klebsiella* species, found in 35 patients, and *Proteus* species, detected in 20 patients. *Enterococcus* species and *Pseudomonas aeruginosa* were each isolated from 10 patients. These findings highlight the dominance of *E. coli* in UTI cases, with other bacteria such as *Klebsiella*, *Proteus*, *Enterococcus*, and *Pseudomonas* also contributing to the infection landscape (Chart No. – 2).

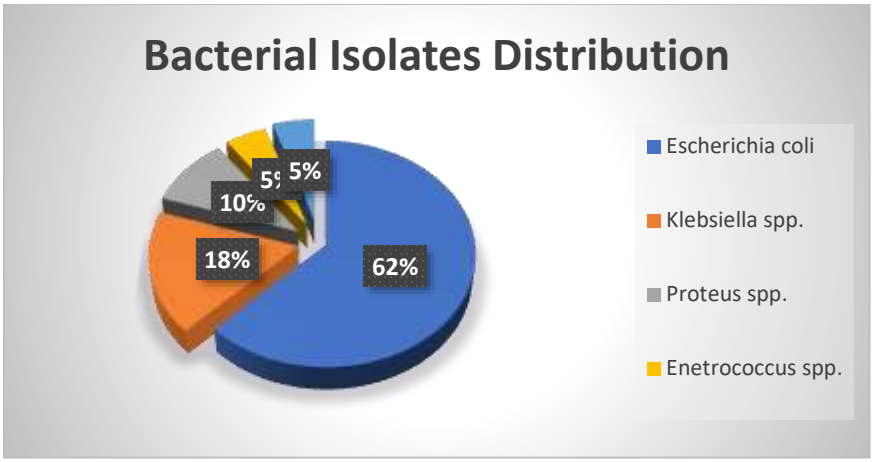


Chart 2 Bacterial Isolates Distribution

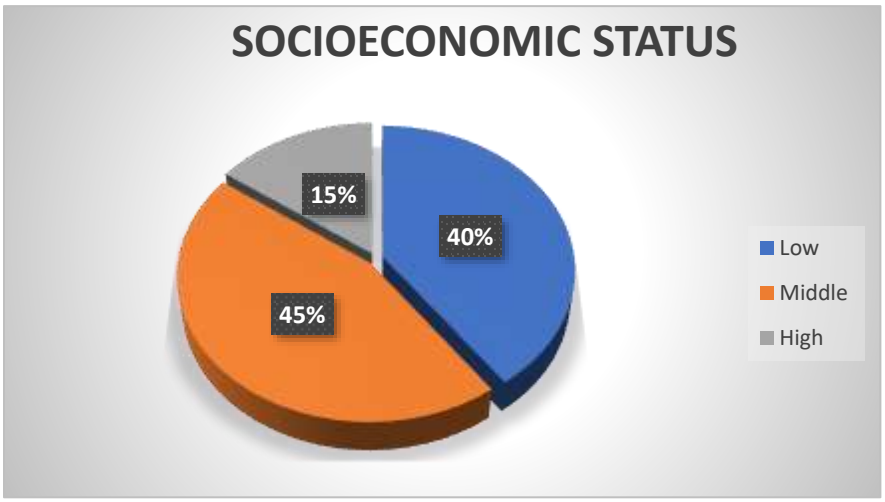
Escherichia coli emerged as the most frequently isolated pathogen, predominantly affecting females across all age groups. The occupational distribution indicated a higher prevalence of infections among office workers and students (table No. – 2).

Table 2 occupational distribution

S. No.	Occupation	No. of Patients	Percentage %
1.	Students	50	25
2.	Office Workers	70	35
3.	Manual Laborers	30	15
4.	Retired	20	10
5.	Others	30	15

Chart 3: Socioeconomic Status

the socioeconomic status data indicate that UTIs impact individuals across diverse economic backgrounds. Notably, there is a slightly higher prevalence of these infections within the middle-income group. This trend suggests that while UTIs are widespread and not confined to a specific socioeconomic middle-income demographic may contribute to a marginally elevated risk. Understanding these nuances can help tailor public health interventions and educational campaigns to more effectively address and mitigate UTI risks across all socioeconomic strata (Chart No. – 3).



The majority of patients (75%) hail from urban locales, underscoring the predominance of UTIs in urban settings. Additionally, nearly half of the cohort (45%) report a history of prior UTIs. These findings highlight the importance of tailored interventions and heightened awareness campaigns targeting urban populations (Chart No – 4).

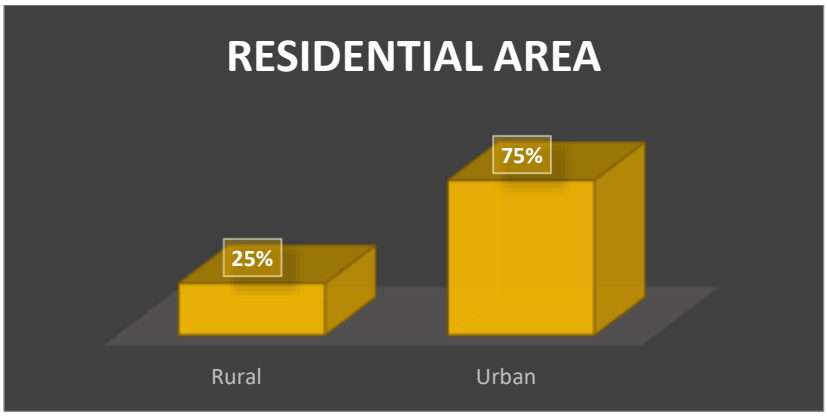


Chart 4 Residential Area

This demographic profile provides valuable insights into the population affected by UTIs and can inform targeted prevention and treatment strategies (Chart No. – 5).

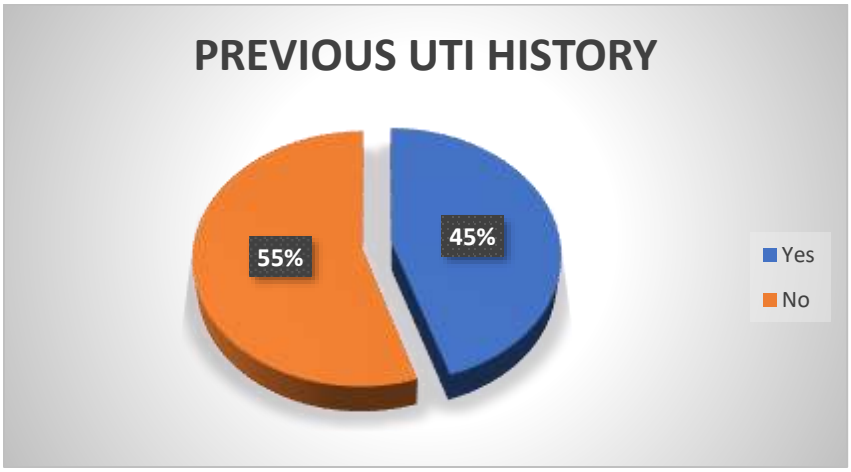


Chart 5 PREVIOUS UTI HISTORY

DISCUSSION

UTIs are a major health concern globally. A study at Sharda Tertiary Care Hospital analyzed 200 UTI patients to understand the disease better. It found a significant gender gap, with females being 65% of cases. The age group most affected was 31-40 years. E. coli was the most common bacteria causing UTIs. Socioeconomic factors and occupations like office work and student status also played a role. Urban areas saw a higher UTI prevalence. Tailored

interventions are needed, including gender-specific education, age-targeted awareness, and urban-focused public health campaigns. Collaboration among healthcare, public health, and community organizations is vital for effective prevention and treatment.

CONCLUSION

This study offers valuable insights into the epidemiology of urinary tract infections (UTIs) and the antibiotic susceptibility patterns of bacterial isolates in a tertiary care hospital setting. Through meticulous isolation, identification, and antibiotic susceptibility testing, we have generated critical data that can inform empirical therapy and support antimicrobial stewardship efforts.The predominant bacterial pathogens identified in our study were Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, and Staphylococcus aureus, aligning with findings from previous research. These results underscore the necessity for ongoing surveillance and monitoring of UTI-causing bacteria to effectively tailor treatment strategies.

Assessment of antibiotic susceptibility revealed alarming rates of resistance, particularly to commonly prescribed antibiotics such as ampicillin, amoxicillin-clavulanate, and ciprofloxacin. Notably, a significant proportion of isolates exhibited multidrug resistance, posing substantial challenges for UTI management and emphasizing the need for the prudent use of antibiotics.

Our study highlights the importance of adherence to antimicrobial stewardship guidelines and the promotion of rational antibiotic prescribing practices to combat the emergence of resistant strains. Additionally, efforts to promote alternative treatment options, such as nitrofurantoin and trimethoprim-sulfamethoxazole, should be emphasized based on their favorable susceptibility profiles in our study.

In conclusion, our findings underscore the dynamic nature of antimicrobial resistance in UTIs and emphasize the importance of ongoing surveillance and antibiotic stewardship initiatives. Future research should focus on molecular characterization of resistant strains to elucidate the mechanisms of resistance and inform the development of novel therapeutic strategies. By addressing these challenges collaboratively, we can mitigate the impact of antimicrobial resistance and ensure optimal patient outcomes in the management of UTIs.

Consent for Publication

All authors have approved the manuscript for publication.

Availability of Data and Material

All necessary data is available upon request.

Conflict of Interest

The authors declare that they have no conflict of interest.

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