Isolation, Identification, and Antibiotic Susceptibility Analysis of Bacterial Pathogens in Suspected Urinary Tract Infection Cases at a Tertiary Medical Center in Dhaka, Bangladesh

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Abstract

Objective: A urinary tract infection (UTI) is a bacterial infection of the urinary tract. The lower urinary tract, bladder, and urethra are the most often infected areas. Women are more likely than men to have a bacterial infection. The research aimed to determine the causative agent of UTI in patients and see how they responded to standard treatments. Methods: A total of 435 urine samples were examined using the culture technique. The samples were streaked evenly on blood agar and MacConkey for morphological characteristics of the colony on media to identify the presumptive bacterium. Gram staining and routine biochemical assays were also used to confirm the findings. The disk diffusion technique was employed to assess susceptibility to 12 different antibiotics. Results: The most prevalent uropathogenic in both genders and age groups was *E. coli* (44%), *Staphylococcus aureus* (21%), *Klebsiella spp.* (13%) and *Proteus spp.* (12%), *Enterobacter spp.* (10%). Overall, the uropathogenic were highest susceptible to Meropenem (82.2%), Amikacin (63.6%), and Cefixime (59.8%) were the most successful medications for the treatment of UTI, the highest resistance to Azithromycin (84.2%), Gentamycin (75.6%) and Nalidixic acid (64.4%) were the least effective. Conclusion: The present study can be helpful for clinicians in finding proper drugs in developing countries like Bangladesh, where the multi-drug resistance problem has just complicated the treatment of UTIs.

Keywords: Urinary tract infection, Causative agents, Antibiotic susceptibility, *Escherichia coli*, Treatment efficacy.

Significance | Identifies prevalent UTI pathogens and effective treatments, aiding clinicians in managing infections amidst rising antibiotic resistance challenges in Bangladesh.

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Introduction

Urinary tract infections (UTIs) represent a significant and prevalent health issue globally, contributing to considerable morbidity and healthcare costs. As one of the most common community-acquired bacterial infections, UTIs primarily affect the urinary tract, including the bladder, urethra, and occasionally the kidneys. The impact of UTIs is profound, not only due to the discomfort and health risks they pose but also due to their potential to lead to severe complications if left untreated (Akram et al., 2007; Stamm & Hooton, 1993; Warren et al., 1999).

UTIs occur when bacteria invade and proliferate in the urinary tract, overcoming the host's natural defenses. The infection is commonly categorized into lower urinary tract infections, such

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as cystitis, and upper urinary tract infections, such as pyelonephritis. Cystitis manifests with symptoms such as urinary urgency, dysuria, and pyuria, while pyelonephritis may present with fever, flank pain, and systemic symptoms (Lane & Takhar, 2011; Ferry et al., 1988). The primary causative agents of UTIs are Gram-negative bacteria, particularly *Escherichia coli* (E. coli), which is responsible for 75-90% of cases (Dromigny et al., 2005). Other notable pathogens include Pseudomonas species, Proteus species, *Klebsiella species*, and Citrobacter species, as well as Gram-positive bacteria like Group B Streptococci and *Staphylococcus aureus* (Hooton, 2012).

The pathogenesis of UTIs involves bacterial adhesion to the uroepithelium, a process facilitated by various virulence factors such as fimbriae and adhesins. This adhesion is often countered by the host's antimicrobial secretions and immune responses, yet persistent or recurrent infections can occur when these defenses are overwhelmed or compromised (Sobel, 1997; Wullt et al., 2000). Women are particularly susceptible to UTIs due to anatomical factors that facilitate bacterial entry and colonization (Tammana et al., 2016). However, UTIs are not exclusive to women; men can also develop infections, often linked to specific risk factors such as sexual practices or anatomical abnormalities (Barnes et al., 1986; Spach et al., 1992).

In Bangladesh, the incidence of UTIs is exacerbated by factors such as inadequate sanitation, prolonged catheter use, and uncontrolled contraceptive practices. The evolving resistance patterns of uropathogens, driven by the overuse and misuse of antibiotics, further complicate the management of these infections (Asma et al., 2019; Begum & Shamsuzzaman, n.d.). Despite the empirical use of antibiotics before culture results are available, the effectiveness of treatment is increasingly challenged by rising antibiotic resistance (Alam et al., 2017; Nahar et al., 2018).

The present study aims to identify the prevalent uropathogens responsible for UTIs and assess their susceptibility to commonly used antibiotics. By analyzing urine samples from a diverse patient population, this research seeks to provide valuable insights into the current landscape of UTI causative agents and their resistance profiles. This information is crucial for clinicians in Bangladesh, where the high burden of UTIs and the growing problem of multidrug resistance necessitate informed and targeted therapeutic strategies (Yasmeen et al., 2015). Through a comprehensive examination of bacterial isolates and their resistance patterns, the study aims to enhance the understanding of UTI management and contribute to better treatment protocols in the context of evolving resistance challenges.

Materials and Methods

Study Area and Population

This study was conducted at a tertiary care hospital in Dhaka city, Bangladesh, from January 2021 to October 2021. The study population comprised 435 patients suspected of having urinary tract infections (UTIs). Of these, 128 were male, and 307 were female, representing a diverse age range as shown in figure 1.

Preparation of Culture Media

For the isolation and identification of uropathogens, various culture media were prepared according to the manufacturer's instructions. These included CLED Agar, MacConkey Agar, Nutrient Agar, Blood Agar, Muller-Hinton Agar, Simmons' Citrate Agar, MR-VP Medium, and Peptone Water Medium. The media were sterilized using autoclaving at 121°C for 15 minutes to ensure they were free from contaminants as shown in table 1.

Isolation and Identification of Bacteria

Urine samples (100 μL) were inoculated onto sterile and solidified Blood Agar and MacConkey Agar plates. The plates were incubated aerobically at 37°C for 24 hours. After incubation, the colonies were examined for morphology, size, color, and growth characteristics. The number of colonies was recorded to confirm UTI diagnosis, with >10^5 CFU/mL considered significant for infection as shown in figures 3 and 4.

To identify the bacterial isolates, standard biochemical assays were performed. Gram staining was used for preliminary differentiation of Gram-positive and Gram-negative bacteria. Additional tests included catalase and mannitol fermentation for Staphylococcus aureus. Gram-negative bacteria were identified through standard biochemical profiles, including tests for urease, indole production, citrate utilization, and motility.

Antibiotic Susceptibility Testing

Antibiotic susceptibility was assessed using the Kirby-Bauer disk diffusion method, following the Clinical and Laboratory Standards Institute (CLSI) guidelines (2018). Twelve antibiotics were tested: Ciprofloxacin, Cefuroxime, Cefixime, Azithromycin, Sulphamethoxazole, Meropenem, Amoxicillin, Ceftriaxone, Nalidixic Acid, Amikacin, Gentamycin, and Nitrofurantoin.

For testing, the bacterial inoculum was adjusted to a 0.5 McFarland standard, and a sterile cotton swab was used to evenly spread the inoculum on Mueller-Hinton Agar plates. After allowing the plates to dry, antibiotic discs were placed on the agar surface using sterile forceps. The plates were incubated at 37°C for 24 hours. The zones of inhibition around the antibiotic discs were measured using millimetre callipers to determine the susceptibility of the isolates.

Statistical Analysis

Data were analyzed to determine the prevalence of different uropathogens and their resistance patterns. Statistical methods were used to compare antibiotic susceptibility among different bacterial species and to assess overall resistance trends.

Table 1. Presumptive Features of Uropathogenic Organisms

Features	***E. coli***	***Klebsiella spp.***	***S. aureus***	***Proteus spp.***	***Enterobacter spp.***
Colony on Blood agar	Non-hemolytic,large, gray colonies	Non-hemolytic, Mucoid colonies	β-hemolysis colonies	Non-hemolytic, swarming colonies	Non-hemolytic, Rough, white colonies
Colony on MacConkey agar	Smooth,Lactose, fermenter colonies	Mucoid, Lactose fermenter colonies	No growth	Smooth, non-lactose fermenter colonies	Small, Lactose fermenter colonies
Gram staining	Pink color, rod-shaped,	Pink color, rod-shaped,	Violet color, round shape	Pink color, rod-shaped,	Pink color, rod-shaped,
Indole	+	-	-	+	-
Methyl red	+	+	+	+	-
Voges-Proskauer	-	+	+	-	+
Citrate utilization	-	+	-	+	+
Motility	+	-	-	+	+
H_2S production	-	-	-	+	-
Gas production	+	+	-	+	+
Oxidase	-	-	-	--	-
Catalase	+	+	+	+	+
Urease	-	+	-	+	-

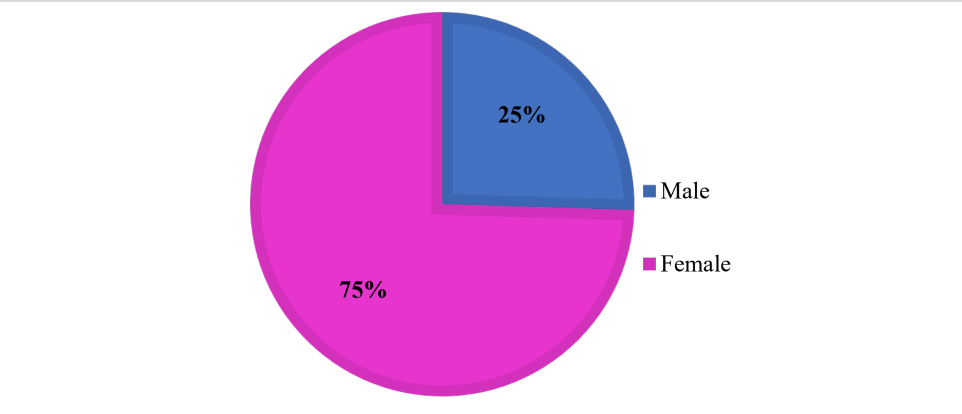


Figure 1: Total Sample distribution (Male and Female)

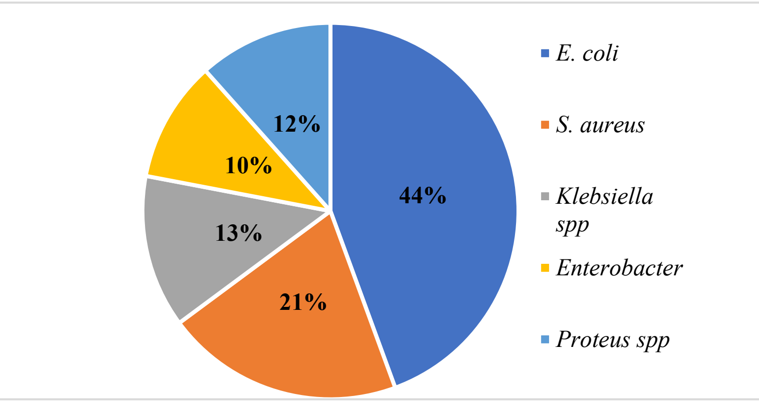
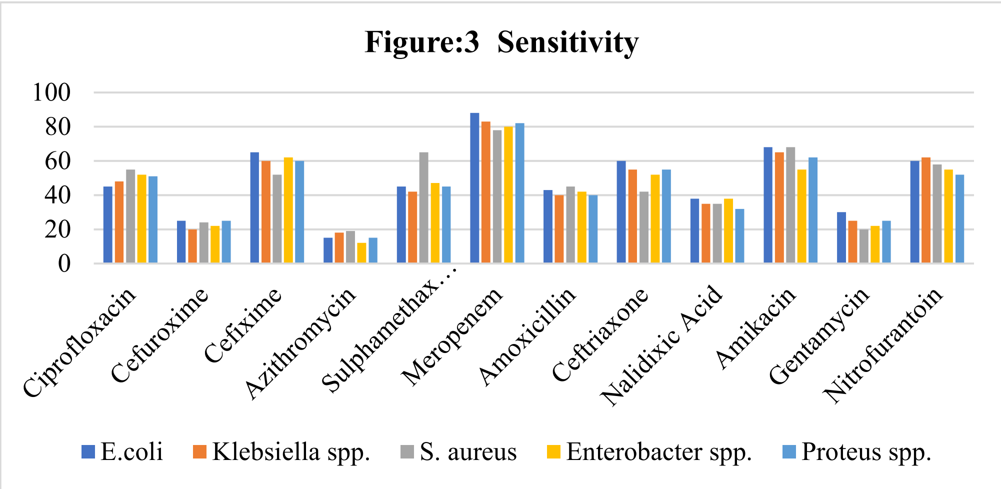
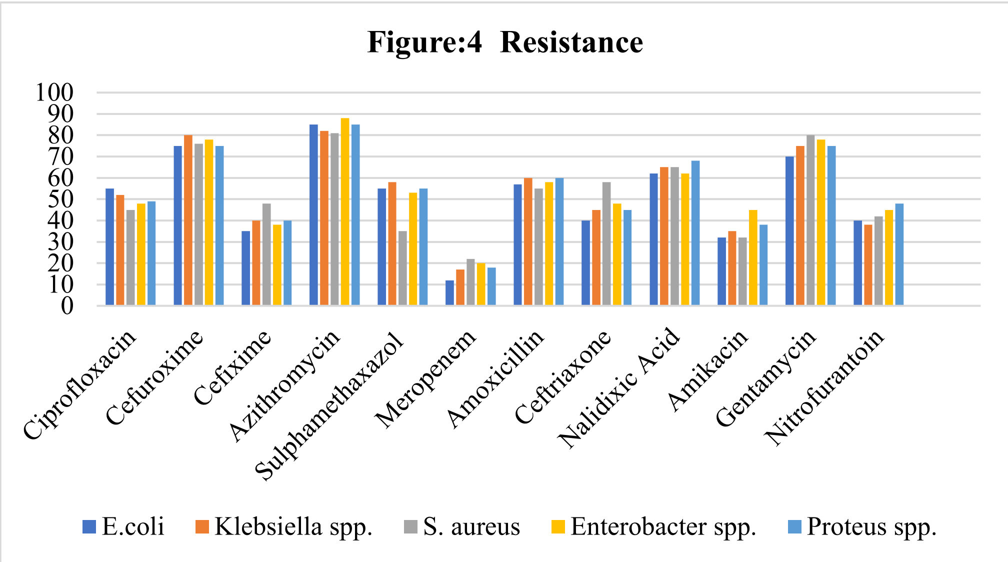


Figure 2. Distribution of isolated uropathogens





Figures 3 and 4: Sensitive and Resistance patterns of Antibiotics against isolated Microorganisms

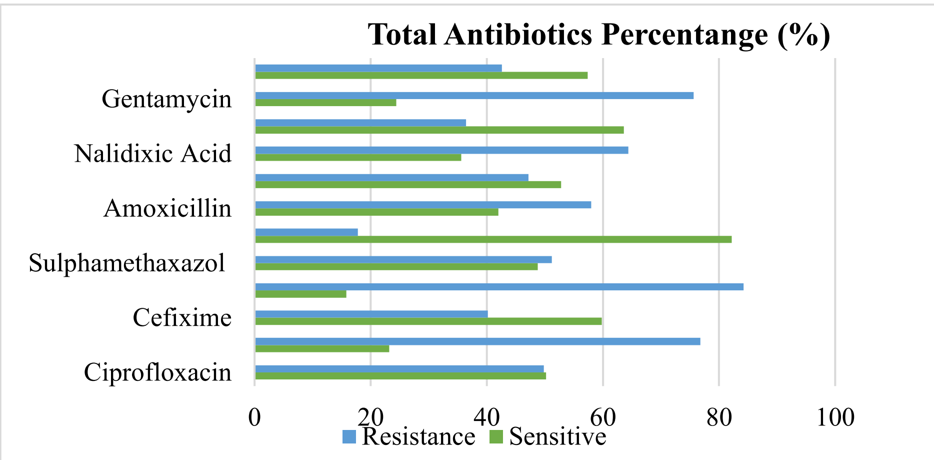


Figure 5. Total percentage of Antibiotics

Results

Out of 435 urine samples analyzed, 259 (59%) were positive for bacterial infections. Of these, 66 (25%) were from male patients and 193 (75%) from female patients. The predominant uropathogenic bacteria identified included *Escherichia coli* (44%), *Staphylococcus aureus* (21%), *Klebsiella spp.* (13%), *Proteus spp.* (12%), and *Enterobacter spp.* (10%). The data revealed that *E. coli* was the most frequent pathogen across all demographics, which aligns with similar findings in Dhaka and other regions (Akter et al., 2016; Alam et al., 2017; Asma et al., 2019). The prevalence of *E. coli* (44%) was notably higher compared to studies in India (31.5%; Akram, Shahid, & Khan, 2007) and Ethiopia (33.3%; Beyene & Tsegaye, 2011) but lower than reports from Russia (85.9%; Stratchounski & Rafalski, 2006) as shown in figure 2.

Antibiotic susceptibility testing highlighted several trends. The highest susceptibility among uropathogens was to Meropenem (82.2%), Amikacin (63.6%), and Cefixime (59.8%). Conversely, the greatest resistance was observed against Azithromycin (84.2%), Gentamycin (75.6%), and Nalidixic acid (64.4%). Specific findings included:

E. coli demonstrated susceptibility to Meropenem (88%), Amikacin (68%), and Cefixime (65%), but showed resistance to Azithromycin (15%) and Gentamycin (30%; Akter et al., 2016; Subramanian, Ganesapandian, Singh, & Kumaraguru, 2011). *Staphylococcus aureus* was most susceptible to Meropenem (78%) and Amikacin (68%), but resistant to Azithromycin (19%) and Cefuroxime (24%; Farajnia, Alikhani, Ghotaslou, Naghili, & Nakhlband, 2009). *Klebsiella spp.* exhibited high susceptibility to Meropenem (83%) and Amikacin (65%), with lower susceptibility to Azithromycin (18%) and Cefuroxime (22%; Andreu et al., 2005). *Proteus spp.* was responsive to Meropenem (82%) and Amikacin (62%), but resistant to Azithromycin (15%) and Nalidixic acid (32%; Hooton, 2012). *Enterobacter spp.* showed susceptibility to Meropenem (80%) and Cefixime (62%), but resistance to Azithromycin (12%) and Cefuroxime (22%; Dromigny, Nabeth, Juergens-Behr, & Perrier-Gros-Claude, 2005).

These findings underscore the critical need for careful antibiotic stewardship. The high resistance rates to commonly used antibiotics highlight the growing challenge of multidrug-resistant uropathogens. Clinicians should rely on culture and sensitivity tests to guide effective treatment strategies and combat the increasing threat of antibiotic resistance (Stamm & Hooton, 1993; Warren et al., 1999).

Discussion

The present study investigated the prevalence and antibiotic susceptibility of uropathogens in patients with urinary tract infections (UTIs) from Dhaka city, Bangladesh. Analyzing a total of 435 urine samples, we observed a high rate of bacterial infections, with 259 samples (59%) testing positive for pathogens. The predominant uropathogen was *Escherichia coli* (44%), followed by *Staphylococcus aureus* (21%), *Klebsiella spp.* (13%), *Proteus spp.* (12%), and *Enterobacter spp.* (10%). This distribution aligns with global trends where *E. coli* is the leading causative agent of UTIs but differs in proportions from studies conducted in other regions (Akter, Fatema, et al., 2016; Akter, Hossain, Khan, Sultana, Fatema, Al Sanjee, & Datta, 2016; Asma et al., 2019) as shown in figure 5.

The high prevalence of *E. coli* observed in this study corroborates findings from other research conducted in Bangladesh (Akter, Fatema, et al., 2016) and highlights the pathogen's dominance in UTI cases. The predominance of *E. coli* (44%) in our study was notably higher compared to reports from India (31.5%) (Akram, Shahid, & Khan, 2007) and Southwest Ethiopia (33.3%) (Beyene & Tsegaye, 2011). However, it was lower than the prevalence reported in Russia, where *E. coli* was found in 85.9% of UTI cases (Subramanian, Ganesapandian, Singh, & Kumaraguru, 2011). This variation in prevalence underscores the regional differences in UTI epidemiology and the need for localized data to guide treatment strategies (Stratchounski & Rafalski, 2006).

Antibiotic susceptibility testing revealed that Meropenem was the most effective antibiotic across all uropathogens, showing a susceptibility rate of 82.2%. Amikacin and Cefixime also demonstrated high efficacy, with susceptibility rates of 63.6% and 59.8%, respectively. These findings are consistent with the global view that Meropenem remains a critical agent for treating resistant bacterial infections (Yasmeen, Islam, Islam, Uddin, & Jahan, 2015). Conversely, high resistance rates were observed for Azithromycin (84.2%), Gentamycin (75.6%), and Nalidixic acid (64.4%). The substantial resistance to these antibiotics emphasizes the growing problem of multidrug-resistant (MDR) uropathogens, a significant concern in the context of emerging antibiotic resistance (Andreu, Alós, Gobernado, Marco, de la Rosa, & García-Rodríguez, 2005; Nahar, Hasnat, Akhter, & Begum, 2018). The results of our study are particularly relevant for Bangladesh, where antibiotic misuse and overuse are common. The high resistance rates to commonly used antibiotics such as Azithromycin and Gentamycin suggest that empirical treatment strategies may be inadequate, leading to treatment failures and increased healthcare costs. This aligns with global concerns about the rise of antibiotic-resistant infections and highlights the urgent need for effective antibiotic stewardship programs and adherence to culture and sensitivity testing before prescribing antibiotics (Warren et al., 1999; Weber, Plaisance, & Mancy, 1995).

Our study also identified gender differences in UTI prevalence, with a significantly higher rate in females (75%) compared to males (25%). This finding is consistent with other studies that show a higher incidence of UTIs in women due to anatomical and physiological factors (Hooton, 2012; Spach, Stapleton, & Stamm, 1992). The higher prevalence in females underscores the need for targeted preventive measures and interventions tailored to female patients (Sobel, 1997).

Conclusion

This study reveals the prevalence of E. coli, Staphylococcus aureus, Klebsiella spp., Proteus spp., and Enterobacter spp. as primary uropathogens in urinary tract infections (UTIs), with E. coli being the most common. It is evident that females are more frequently affected by UTIs compared to males. The antibiotic susceptibility testing reveals that Meropenem is the most effective treatment, showing high susceptibility across all isolates. Conversely, third and second-generation antibiotics exhibited significant resistance. This underscores the growing issue of multidrug resistance and the need for targeted antibiotic therapy. Physicians are advised to base antibiotic prescriptions on culture sensitivity tests to ensure effective treatment and mitigate resistance development. This approach is crucial for improving UTI management, particularly in regions like Bangladesh where resistance patterns are evolving rapidly.

Author contributions

S.D. conceptualized the project, developed the methodology, conducted a formal analysis, and drafted the original writing. M.R.I. contributed to the methodology. A.B. conducted investigations, provided resources, and visualized the data. D.K.R.M. contributed to the reviewing and editing of the writing.

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Competing financial interests

The authors have no conflict of interest.

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