

Isolation of Bacterial Uropathogens and Their Antibiotic Susceptibility Pattern in Brahmanbaria Medical College

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ABSTRACT

Background: Urinary Tract Infections (UTIs) are the most common bacterial infections in developing countries. Area-specific monitoring studies aim to comprehend the pathogen types and resistance patterns of urinary tract infections. The study aims to identify the type and pattern of antibiotic resistance in uropathogens from patients at Brahmanbaria Medical College Hospital.

Materials and methods: A hospital-based cross-sectional study was conducted from January to December 2023. Urine samples were collected using the mid-stream “clean catch” method from 5000 clinically suspected cases of urinary tract infections and tested bacteriologically using standard procedures. According to Clinical and Laboratory Standards Institute guidelines, an antimicrobial susceptibility test was performed for the isolated pathogens using the Kirby-Bauer disk diffusion method.

Results: A significant amount of uropathogens were detected in 50.9% of the total patients. UTIs were more common in females, and young people were more affected by uropathogens. The most common uropathogens isolated were *Escherichia coli* (E.coli) 1780 (69.94%), and *Staphylococcus saprophyticus* 326(12.81%). Very high frequencies of resistance have been ranging from 70-100 % to cefixime, cefuroxime, cotrimoxazole, azithromycin, and moderately high resistance to ceftriaxone, ciprofloxacin, and amoxiclav. *Staphylococcus saprophyticus* and *Enterococcus* spp showed moderate sensitivity to linezolid but Gram-negative uropathogen showed high resistance to linezolid. Amikacin, gentamicin, meropenem, nitrofurantoin, and piperacillin-tazobactam showed good sensitivity patterns against *E. coli*, *Enterobacter* spp, *Pseudomonas* spp, *Proteus* spp, *Klebsiella* spp, *Enterococcus* spp, *Staphylococcus saprophyticus*.

Conclusion: The study revealed *E. coli* isolates as the primary pathogens, causing high resistance to commonly prescribed drugs. Antibiotics became limited treatment options for UTIs. It is now essential to conduct routine, frequent studies and surveillance.

KEY WORDS

Antimicrobial agent; *E. coli*; MH agar plate; UTI; Uropathogen.

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INTRODUCTION

UTIs are bacterial infections characterized by clinical signs and symptoms, often linked to the detection of pathogenic microorganisms in the urine, bladder, urethra, kidney and prostate.¹ Common infections include bladder cystitis, pyelonephritis, urethritis and prostatitis, often causing asymptomatic bacteriuria in women and the elderly people. UTIs are the second most common bacterial infection globally, affecting 50% of women particularly those aged 16-64 years.² *E. coli* is the most common bacteria found in UTI patients, accounting for 80-90% of UTI.³⁻⁵ Though *E. coli* is the most common causative agent of UTI (70%-79% of cases) some Gram-negative Enterobacteriaceae, Gram-positive *Enterococcus faecalis*, *Proteus* spp, *Klebsiella* and *Staphylococcus saprophyticus* are also responsible for UTI.⁶⁻⁹ Risk factors for UTI include age, sex, marital status, social status and strains like Shiga toxin-producing *E. coli*. Shiga toxin-producing *E. coli* can cause hemolytic uremic syndrome, elevated white blood

cell count and C-reactive protein levels.¹⁰ The widespread use of antimicrobial agents in underdeveloped countries often leads to the development of resistant microorganisms, limiting the effectiveness of these agents in combating bacterial infections.¹¹ Empirical antimicrobial treatment often begins before urine culture results, potentially leading to antimicrobial resistance, a major cause of treatment failure in infectious diseases and a significant concern for UTIs. The study aimed to assess the bacterial uropathogens and their antibiotic susceptibility in UTI patients, to promote the selection of empirical antibiotics.

MATERIALS AND METHODS

A cross-sectional study was conducted from January to December 2023 on patients suspected of urinary tract infection at Brahmanbaria Medical College Hospital, using mid-stream urine samples and culture and sensitivity tests.

A total of 5000 urine samples were collected from the suspected cases of UTI patients and transported aseptically to the experimental laboratory by following thermostable conditions for analyzing the microbiological status.

Bacterial Isolation and Identification Procedures

The study involved transferring 0.01 ml urine samples to selective media like MacConkey agar plates to detect bacteria like *E. coli*, *Enterobacter*, *Pseudomonas*, *Proteus*, *Klebsiella* and Blood agar plate were used for *Staphylococcus* and *Streptococcus*. To find out the number of CFU/ml in the original sample = The number of colonies multiplied by the dilution factor divided by the amount of samples.^{8,12}

Biochemical Examination

To confirm the pathogens, the chosen colonies-which were chosen based on cultural, microscopic and microbiological examinations-were evaluated biochemically using the triple sugar iron agar test, oxidase test, catalase test, nitrate reduction test, indole production test, methyl red test, Voges-Proskauer test, citrate utilization test, and urease test.

Antimicrobial Sensitivity Testing (Kirby-Bauer Method)

The Kirby-Bauer Method was used to test the susceptibility of isolated organisms to various antibiotics commonly used to treat uropathogens. To test an organism, a Mueller-Hinton agar plate (MH agar plate) was brought at room temperature. The plates were labeled appropriately. To prepare an inoculum for testing, isolated colonies were touched, suspended in

sterile saline, vortexed, and the suspension was adjusted to a 0.5 McFarland standard turbidity. The suspension was used within 15 minutes of preparation. A sterile swab was dipped into the inoculum tube, rotated to remove excess fluid, and inoculated to a MH agar plate. The plate was streaked three times and rotated 60 degrees. The agar plate was allowed to dry at room temperature for 3 to 5 minutes, but no more than 15 minutes. Antimicrobial-impregnated disks were dispensed onto the agar surfaces, either using forceps or multidisc dispensers. The plates were then placed in a 35°C air incubator for 16–18 hours, ensuring the disks were no closer than 24 mm. Ordinarily, no more than 12 disks were placed on a 150-mm plate or more than 5 disks on a 100-mm plate.¹³ Antimicrobial susceptibility testing (AST) was done against a panel of 15 antibiotics – amikacin (30 mcg) gentamicin (30 mcg) meropenem (10 mcg) ceftriaxone (30 mcg) cotrimoxazole (25 mcg) doxycycline (10 mcg) amoxiclav (20/10 mcg) cefixime (5 mcg) cefuroxime (30 mcg) ciprofloxacin (30 mcg) azithromycin (15 mcg) nitrofurantoin (300 mcg) linezolid (30 mcg) colistin (10 mcg) piperacillin-tazobactam (100/10 mcg) as per the Clinical Laboratory Standard Institute (CLSI) guidelines susceptibility was noted as sensitive, Intermediate sensitive and resistant based on the diameter of zone of inhibition.¹⁴

Results

A total of 5000 people of different ages and sexes who were suspected of UTI were included in this study. Out of 5000 cases, 1500 (30 %) were male and 3500 (70 %) were female with a male-to-female ratio of 1: 2.33. The rate of isolation of uropathogens in males and females was 21.3% and 63.6 %, respectively (Table I). Most of the suspected were 21-40 age groups (Table II). And most positive come from 21-30 age group that was 32% and the 31-40 age group 29% (Figure I)

Table I Percentage (%) frequency of positive culture according to gender (n=5000)

Gender	No. of sample	Presence of significant uropathogens	No presence of significant uropathogens
Male	1500 (30%)	320 (21.3 %)	1180 (78.7%)
Female	3500 (70%)	2225 (63.6 %)	1275 (36.4%)
Total	5000 (100%)	2545 (50.9 %)	2455 (49.1%)

Table II Distribution of suspected persons of UTI according to age (n=2545)

Age groups	Frequency	Percent
10-20	280	5.6
21-30	1950	39
31-40	2060	41
41-50	472	9.44
>50	238	4.76
Total	5000	100

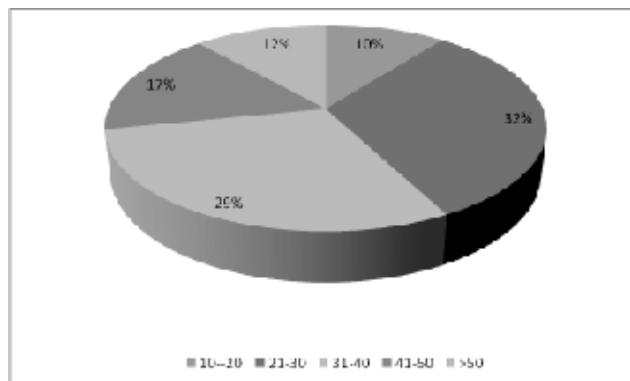


Figure 1 Distribution of UTI patients according to age group

After 5000 urine samples were cultured, 2545 (50.9%) of the samples had significant uropathogens. *E. coli* had the highest number of isolates 1780 (69.94%) followed by *Staphylococcus saprophyticus* 326 (12.81%) *Enterobacter* spp. 163 (6.40%) *Pseudomonas* spp. 126 (4.95%) *Proteus* spp. 44 (1.73%) *Klebsiella* spp. 48 (1.89%) and *Enterococcus* spp. 58 (2.28%). (Figure 2).

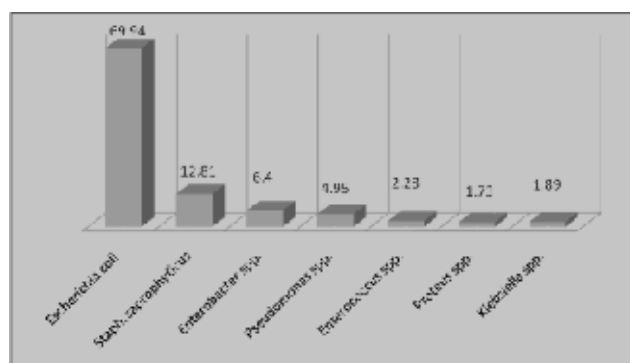


Figure 2 Different types of Isolation uropathogens

The antimicrobial sensitivity patterns of isolates are shown in Table III. *E. coli* was highly sensitive to amikacin, gentamicin, meropenem, nitrofurantoin and piperacillin-tazobactam (70%-85%). It was very low sensitive to linezolid 1.7% and cefixime 2.9%. It was 15%-30% sensitive to cefuroxime, azithromycin, ceftriaxone and amoxiclav. Doxycycline and colistin were 35%-60% sensitive. *Staph. saprophyticus* and *Enterococcus* spp. were highly sensitive to amikacin, gentamicin, meropenem, nitrofurantoin, amoxiclav and piperacillin-tazobactam (60-75%). Cefixime, cefuroxime, cotrimoxazole, ceftriaxone (20-30%) linezolid, ciprofloxacin (35-55%) and cefixime were very low in sensitivity. *Enterobacter* spp. were highly sensitive to amikacin, gentamicin, meropenem, nitrofurantoin, piperacillin-tazobactam (70-85%) ceftriaxone, amoxiclav, doxycycline, colistin and ciprofloxacin (30-60%). They were very low in sensitivity to linezolid 2.8% and cefixime 0.5%. *Pseudomonas* spp.

was highly sensitive to amikacin, gentamicin, meropenem, nitrofurantoin, piperacillin-tazobactam, ciprofloxacin (67-80%) ceftriaxone, amoxiclav, doxycycline, colistin (45-57.9%) cefuroxime and azithromycin (14-26%). It was very low in sensitivity to linezolid and cefixime at 3.9%.

Proteus spp. were highly sensitive to meropenem and nitrofurantoin at 87.1%. They were also sensitive to amikacin, gentamicin, Piperacillin-Tazobactam (61-75%) amoxiclav, doxycycline, colistin, ciprofloxacin, ceftriaxone (40-58%) cefuroxime, azithromycin and cotrimoxazole (20-29%). They were 9.7% sensitive to cefixime. *Klebsiella* spp. was highly sensitive to meropenem and nitrofurantoin 94.4%, amikacin 85%, piperacillin-tazobactam 77.8%, gentamicin, amoxiclav, doxycycline, cotrimoxazole, ciprofloxacin and colistin 50-60%, cefuroxime and azithromycin 11.1% and cefixime 100% resistant. Linezolid was 100% resistant to *Proteus* spp. and *Klebsiella* spp.

Table III Antimicrobial sensitivity pattern of uropathogenes (Percentage)

Uropathogenes	AK	Gen	MEM	AMC	DO	CFM	CXM	COT	CTR
<i>Escherichia coli</i>	85.5	71	84.6	35.7	42.2	2.9	15.1	20.5	28.6
<i>Enterobacter</i> spp.	82.2	65.9	78	35.1	41.1	0.5	17.1	21.3	31.3
<i>Pseudomonas</i> spp.	80	77.6	77.3	45.3	49.3	3.9	14.5	26.3	36.8
<i>Proteus</i> spp.	61.3	67.7	87.1	48.4	45.2	9.7	22.6	29	41.9
<i>Klebsiella</i> spp.	85	50	94.4	50	55.6	0	11.1	50	38.9
<i>Staph. saprophyticus</i>	74	67.9	73.3	62.1	50.5	2.5	23.7	25.5	28
<i>Enterococcus</i> spp.	81.9	61.8	33.3	69.7	81.8	0	20.6	23.5	32.4

AK = Amikacin, GEN = Gentamicin, MEM = Meropenem, CTR = Ceftriaxone, COT = Cotrimoxazole, DO = Doxycycline, AMC = Amoxiclav, CFM = Cefixime, CXM = Cefuroxime, CIP = Ciprofloxacin, AZM = Azithromycin, NIT = Nitrofurantoin, LZ = Linezolid, CL = Colistin, TPZ = Piperacillin-Tazobactam.

DISCUSSION

Urinary Tract Infections (UTIs) can present as either simple or complex symptoms. Freshly voided urine from patients with symptomatic infections will contain more than 10^5 bacteria/ml and inflammatory cells. The prevalence of nosocomial and community-acquired bacterial urinary tract infections is high and antibiotic resistance is a global health concern.¹⁵⁻¹⁶ According to our research, uropathogenes were more common in people aged 21 to 40, which agrees with other studies.¹⁷ The findings of our research support the documented fact that UTIs are more common in women than in men, *E. coli* was the most common uropathogenes and they aligned with recent studies.^{9,17-19} *Staphylococcus*

saprophyticus, *Enterobacter* spp, *Pseudomonas* spp. *Proteus* spp, *Klebsiella* spp and *Enterococcus* spp. were also responsible for UTI. Growing drug resistance to common bacterial infections, that include UTIs, is a serious concern. Amoxicillin, cotrimoxazole, cefixime, ciprofloxacin and azithromycin are still used in many developing countries, including Bangladesh, to treat a variety of Gram-positive and Gram-negative bacterial infections, including urinary tract infections. Unfortunately, the antimicrobial activity of all these agents was found to be inadequate against the isolated uropathogens. One possible explanation for the high rates of oral antibiotic resistance in our study is the uncontrolled use of these medications.²⁰⁻²² *E.coli* was highly sensitive to amikacin, gentamicin, meropenem, nitrofurantoin and piperacillin-tazobactam, very low sensitive to linezolid and cefixime, moderate sensitive to cefuroxime, azithromycin, ceftriaxone and amoxiclav. Nitrofurantoin was found to be a reasonably high-efficacy agent among all antimicrobials used against almost all uropathogens in the current setting and similar results were also reported from other studies.²⁰ However, all pathogens were highly sensitive to amikacin, gentamicin, colistin, piperacillin-tazobactam and meropenem, which is probably due to less use of these medications, that similar results showed some study.^{18,20,23}

LIMITATION

Acknowledge that our work has certain limitations due to a lack of clinical data. The classification of UTI patients as symptomatic or asymptomatic, complicated or uncomplicated, was not provided because this study was solely based on retrospective laboratory analysis. Additionally, it was not possible to discuss the distribution of patients according to the sources of infection, such as nosocomial, community-acquired or catheter-associated.

CONCLUSION

Urinary tract infections are prevalent, primarily caused by Gram-negative bacteria, and resistance to commonly used antibiotics is high. Factors like inadequate treatment, patient adherence and uncontrolled drug distribution contribute to this issue.

RECOMMENDATION

Before prescribing antibiotics, urine culture and microorganism sensitivity testing should be performed whenever possible. Furthermore, the empirical therapy should be evaluated based on the most recent antibiogram of a specific geographic area.

To control the condition of rapidly occurring resistance traits, patients should be properly advised to take antibiotic medication and alternative drugs are needed developed for these kinds of ailments. All things considered, frequent educational events on this issue should be organized to promote awareness among the general public as well as among doctors.

DISCLOSURE

All the authors declared no competing interest.

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