

ANTIBIOGRAM OF UROPATHOGENS ISOLATED FROM SAMPLES OF ASYMTOMATIC UNDERGRADUATE STUDENT OF SARHAD INSTITUTE OF ALLIED HEALTH SCIENCES, PESHAWAR

Shameem Khan¹, Sayed Saqi Abbass², Hakeem Ullah³, Usman Ullah⁴,
Musadiq Khan⁵, Mr. Wahid Ullah^{*6}

¹Student of M.Phil. in Medical Laboratory Sciences at KMU Peshawar

^{2,3,4}Student of Medical Laboratory Technology at Sarhad institute of Allied Health Sciences, SUIT Peshawar.

⁵Academic Coordinator for Distance Education, Sarhad Institute of Allied Health Sciences, Sarhad University Peshawar.

⁶Senior Lecturer at Sarhad institute of Allied Health Sciences, Sarhad University of Science and Information Technology Peshawar

¹shameemjan0@gmail.com, ²syedsaqiabbass2@gmail.com, ³hakeemmlt123@gmail.com
⁴usman.ullah.khan.official@gmail.com, ⁵musadiq.siahs@suit.edu.pk, ⁶wahid.siahs@suit.edu.pk

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Corresponding Author: *

Mr. Wahid Ullah

Abstract

Urinary tract infections (UTIs) are among the most common bacterial infections worldwide and often caused by *Escherichia coli*, *Klebsiella*, *Staphylococcus*, *Enterococcus*, and *Enterobacter* species. They present a significant health burden, ranging from mild cystitis to severe pyelonephritis, and are complicated by the emergence of antimicrobial resistance due to the widespread and inappropriate use of antibiotics. A descriptive cross-sectional study was conducted at the Sarhad Institute of Allied Health Sciences, Peshawar, from April to July 2025. A total of 52 undergraduate students, selected through convenience sampling were enrolled. Midstream urine samples were collected and cultured on CLED and MacConkey agar for the isolation and identification of uropathogens using standard biochemical tests and Gram staining. Confirmed isolates were subjected to antibiotic susceptibility testing on MHA using the Kirby-Bauer disc diffusion method, and results were interpreted according to CLSI guidelines. Out of 52 enrolled students the male and female ratio were equal with the majority aged 18–22 years. Bacterial growth was detected in 4 (7.7%) urine samples, including *Enterobacter* (25%), *Escherichia coli* (25%), and *Staphylococcus aureus* (50%). Antibioqram revealed complete sensitivity of all isolates to Fosfomycin, Meropenem, Imipenem, Amikacin, Gentamycin, Cefoparazone-Sulbactam, and Ceftazidime, while partial resistance was observed against Ciprofloxacin and Flucloxacillin (25%), and high resistance against Amoxicillin (75%). Our finding suggests that the majority routinely used antibiotics are effective against uropathogens while the rising resistance against frequently prescribed drugs like Amoxicillin, Ciprofloxacin, and Flucloxacillin is a cause for concern.

INTRODUCTION

Urinary tract infections (UTIs) occur when microorganisms invade, multiply, and spread within the urinary system. In most cases, bacteria originating from the digestive tract travel upward into the urethra, where they begin to grow and cause infection. UTIs are among the most frequent bacterial infections encountered in clinical practice, particularly in developing countries (Mandrachia *et al.*, 2020). Globally, they are second only to respiratory and gastrointestinal infections among patients in outpatient care. A UTI is confirmed when pathogenic microorganisms are identified in urine, the urethra, bladder, kidneys, or prostate, regardless of whether symptoms are present (Shah *et al.*, 2021). Both Gram-negative and Gram-positive bacteria, as well as certain fungi, may cause UTIs. However, uropathogenic *Escherichia coli* (UPEC) remains the leading culprit, followed by *Klebsiella pneumoniae* (Behzadi *et al.*, 2023).

The type of symptoms depends on the location of the infection, the responsible microorganism, severity, and the patient's immune response (Foxman *et al.*, 2021). For example, bladder infections are known as cystitis, while infections of one or both kidneys are called pyelonephritis. Less commonly, the ureters—the tubes connecting the kidneys to the bladder—may be affected. Infection of the urethra, which drains urine from the bladder to the outside, is termed urethritis (Omeregie *et al.*, 2023). Intestinal Gram-negative bacteria are a particular concern as they frequently contribute to UTIs worldwide. In the United States alone, symptomatic UTIs account for roughly seven million emergency visits and about 100,000 hospital admissions each year (Mandrachia *et al.*, 2020). UTIs are also the most common hospital-acquired infections, responsible for around 35% of all nosocomial infections and ranking as the second leading cause of sepsis among hospitalized patients (Shaifali *et al.*, 2021).

Although bacteria are the primary cause, UTIs may occasionally be triggered by fungi or viruses (FloresMireles *et al.*, 2020). More than 80% are linked to Gram-negative organisms, with Gram-

positive strains accounting for a smaller proportion. *E. coli* is the dominant uropathogen (60–90%), followed by *Staphylococcus* species (10–15%). Other species, such as *Streptococcus*, *Proteus*, *Klebsiella*, *Neisseria*, *Corynebacterium*, and *Pseudomonas*, have also been isolated in clinical cases (Ashur *et al.*, 2021). Symptoms can vary but commonly include urgency, dysuria (painful urination), pyuria (pus in urine), lower abdominal or back pain, and a persistent urge to urinate. Host-related factors such as urine composition, sexual activity, vaginal pH, and local immune defenses also influence susceptibility (Nigussie & Amsalu., 2017). The severity of disease often reflects both bacterial virulence and the host's immune status (Hannan *et al.*, 2021). Widespread misuse of antibiotics has further complicated treatment, as resistance is now common across nearly all drug classes (Khan *et al.*, 2022).

METHODOLOGY

Study design

A descriptive cross-sectional study was conducted at Sarhad Institute of Allied Health Sciences, Sarhad University of Science and Information Technology, Peshawar.

Study Setting

The current study was conducted at lab of Sarhad Institute of Allied Health Sciences, Sarhad University of Science and Information Technology, Peshawar.

Study duration

The duration of this study was 4 months from 15 April 2025 to 15 July 2025.

Inclusion Criteria

1. In current study we included undergraduate students of Sarhad Institute of Allied Health Sciences of both sexes (male and female) and all age group.
2. Only those undergraduate students of Sarhad Institute of Allied Health Sciences who voluntarily agreed to provide samples were included in this study.

Exclusion Criteria

1. Those students who were on **antibiotic therapy** or completed an antibiotic course within last 7 days were excluded from this study.
2. Those students who were unable or unwilling to provide urine sample are excluded from this study.

Sample Size

The sample size for this study was calculated using Cochran's Sample Size formula:

$$n = \frac{Z^2 \cdot P \cdot (1 - P)}{d^2}$$

Where:

Z=1.96 for a 95% confidence level

Based on a previously reported prevalence of UTI was reported as 8% in a five years duration study conducted at Lahore, Pakistan (Safdar *et al.*, 2018). So, the prevalence for our four-month studies is 4%.d=0.05 (margin of error)

$$n = \frac{(1.96)^2 \times 0.04 \times (1 - 0.04)}{(0.05)^2}$$

$$n = \frac{3.84 \times 0.04 \times 0.96}{0.0025}$$

$$n = \frac{0.147}{0.0025}$$

$$n = 52$$

Thus, a total of 52 isolates were taken to achieve statistically significant results for this study.

Sampling technique

A non-probability convenience sampling technique was used in this study

Requirement

The materials required for antibiogram of uropathogens include urine samples collected in sterile bottle, petri dish for media, CLED (cysteine lactose electrolyte deficient) media for growth of urine pathogen, MacConkey media for differentiation, Muller Hinton Agar (MHA) for antibiotic sensitivity testing, routinely used antibiotic discs strips for sensitivity, forcep for handling antibiotics disc, ruler for measuring

zone of inhibition, CLSI (clinical laboratory standard Institute) guidelines for identifying sensitive, resistant and intermediate.

Sample Collection and Processing

To ensure proper sample collection, a standardized protocol was followed. Each participant's urine sample, approximately 50ml in volume, was collected using a sterilized bottle. After collection samples were inoculated at CLED media for the growth of Uropathogens at lab of Sarhad Institute of Allied Health Sciences, Peshawar. After 24 hours incubation the growth was confirmed by inoculating on MacConkey media for differentiation, gram staining for gram positive and negative, several biochemical tests were performed for conformation of species. After conformation 0.5 MacFarland standard was prepared and then spread on Muller Hinton Agar. After 10 minutes ten routinely used antibiotics for UTI were put on MHA inoculated media. After 24 hours the antibiotics sensitivity was determined by measuring the zone of inhibition of each drug as per CLSI guidelines. The procedures were conducted in accordance with established Standard Operating Procedures (SOPs) to ensure consistency and accuracy throughout the sample processing stage.

RESULTS

Age wise distribution:

All the patients that were included in this study were divided into three age groups. The first group includes those participants who were having age between 18 to 22 years. In first group fall 31 participants out of 52. In second group we included those participants with age between 23 to 27 years fall 19 participants in this age group. In third group includes participants with age between 28 to 32 years falls only 02 participants in this age group as shown in table 4.

Table 4.1 Age wise distribution of participants

		Frequency	Percent	Cumulative Percent
Age	18 to 22 Years	31	59%	59%
	23 to 27 Years	19	36%	95%

	28 to 32 Years	02	05%	100%
	Total	52	100.0	

Gender wise distribution

In this study total 52 undergraduate students were included are categorized into male and

female. Out of total 26 participants were male and 26 participants were female as shown in table

Table 4.2 Gender wise distribution of participants

		Frequency	Percent
Gender	Male	26	50%
	Female	26	50%
	Total	52	100%

Correlation of antibiotic sensitivity with Uropathogens

In this study total 52 asymptomatic undergraduate students were included and their samples were processed. Out of 52 samples only 4 samples show bacterial growth which is then isolated and identified by several biochemical tests. The identified species were Enterobacter 25%, Escherichia Coli 25% and Staphylococcus Aureus 50%. After identification the samples

were inoculated on MHA for antibiotic sensitivity of routinely used antibiotics for uropathogens. Amoxicillin was 25% sensitive 75% resistant, Ciprofloxacin and Flucloxacillin were 75 % sensitive and 25% resistant. Fosfomycin, Meropenem, Imipenem, Amikacin, Gentamycin, Cefoparazone Sulbactam and Ceftazidime were sensitive to all isolated species as shown in table 4.3 and figure 4.3.

Table 4.3 antibiotic sensitivity of uropathogens isolated from urine samples.

Antibiotics	Bacterial Species			
	<i>Enterobacter</i>	<i>Escherichia Coli</i>	<i>Staphylococcus Aureus</i>	<i>Staphylococcus Aureus</i>
Amoxicillin	Resistant	Resistant	Sensitive	Resistant
Fosfomycin	Sensitive	Sensitive	Sensitive	Sensitive
Ciprofloxacin	Sensitive	Sensitive	Sensitive	Resistant
Meropenem	Sensitive	Sensitive	Sensitive	Sensitive
Imipenem	Sensitive	Sensitive	Sensitive	Sensitive
Amikacin	Sensitive	Sensitive	Sensitive	Sensitive
Gentamycin	Sensitive	Sensitive	Sensitive	Sensitive
Flucloxacillin	Sensitive	Sensitive	Sensitive	Resistant
Cefoparazone Sulbactam	Sensitive	Sensitive	Sensitive	Sensitive
Ceftazidime	Sensitive	Sensitive	Sensitive	Sensitive

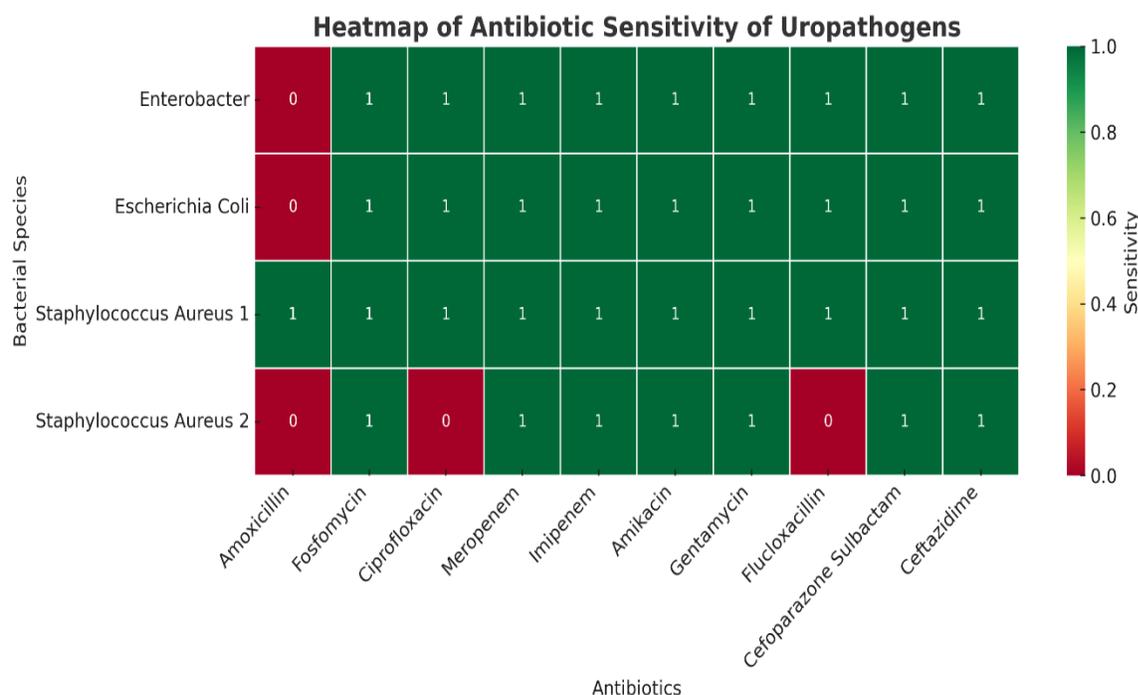


Figure 4.1 antibiotic sensitivity of uropathogens isolated from urine samples.

DISCUSSION

This study, focusing on asymptomatic undergraduate students at Sarhad Institute of Allied Health Sciences, Peshawar, identified that most uropathogens exhibited strong sensitivity to broad-spectrum antibiotics such as Fosfomycin, meropenem, imipenem, amikacin, gentamicin, Cefoparazonesulbactam, and ceftazidime. Conversely, notable resistance to amoxicillin, and moderate resistance to ciprofloxacin and flucloxacillin, was observed. These findings reflect and echo results from recent regional and international investigations, offering both reassurance and cause for continued vigilance.

In Swabi, Pakistan, similar patterns were seen; *E. coli* demonstrated high susceptibility to Cefoparazonesulbactam (over 90%), followed by notable sensitivity to Fosfomycin and meropenem, while resistance was markedly high against second and third-generation cephalosporins and fluoroquinolones (e.g., ciprofloxacin). This closely parallels our findings regarding the efficacy of broad-spectrum agents and growing resistance to older antibiotics (Khatoon *et al.*, 2023).

A study conducted in Haripur, Pakistan, demonstrated that *Escherichia coli* was the predominant uropathogen isolated from clinical samples, followed by *Klebsiella pneumoniae* and *Staphylococcus aureus*. The researchers further highlighted high levels of resistance to first-line antibiotics such as amoxicillin and cephalosporins, whereas carbapenems and aminoglycosides retained significant effectiveness. When compared with our findings, both studies identified *E. coli* and *S. aureus* as common urinary isolates; however, in our asymptomatic undergraduate population, *S. aureus* appeared with higher frequency (50%). Additionally, in contrast to the high resistance rates reported in Haripur, our study revealed that most isolates were still highly sensitive to broad-spectrum antibiotics such as Fosfomycin, meropenem, imipenem, gentamicin, and amikacin. This discrepancy may reflect differences in study populations, as hospital-based patients in Haripur are more likely to have prior antibiotic exposure and recurrent infections, while our participants represented a healthier, community-based cohort.

(Muhammad *et al.*, 2020).

CONCLUSION

The findings of our study revealed that antibiogram of most uropathogens shows high sensitivity to broad-spectrum antibiotics including Fosfomycin, Meropenem, Imipenem, Amikacin, Gentamycin, Cefoparazone-Sulbactam, and Ceftazidime. However, notable resistance was observed against Amoxicillin (75% resistant), while Ciprofloxacin and Flucloxacillin showed partial resistance (25%). Our finding suggests that the majority routinely used antibiotics are effective against uropathogens while the rising resistance against frequently prescribed drugs like Amoxicillin, Ciprofloxacin, and Flucloxacillin is a cause for concern and highlights the importance of careful antibiotic use.

RECOMMENDATION

1. **Larger Sample Size:** Increasing the sample size will enhance the statistical power of the study, allowing for more reliable and generalizable results.
2. **Broader Demographic Inclusion:** Future studies should aim to include a more diverse demographic, including students from different universities and regions, to gain a more comprehensive understanding of antibiogram of uropathogens distribution across a wider population.

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