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ISOLATION AND IDENTIFICATION OF UROPATHOGENS FROM SAMPLES OF ASYMTOMATIC UNDERGRADUATE STUDENTS OF SARHAD INSTITUTE OF ALLIED HEALTH SCIENCES, PESHAWAR

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Abstract

Urinary tract infections (UTIs) are among the most common bacterial infections worldwideand 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. 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%). Biochemical characterization of the isolates was carried out following culture on CLED agar. The results demonstrated distinct profiles for each bacterial species. Enterobacter was identified as Gram-negative, catalase-negative, TSI-positive, coagulase-negative, indole-negative, and lactose fermenting. Escherichia coli also showed a Gram-negative reaction, with negative catalase and coagulase tests, but was positive for TSI, indole, and lactose fermentation. In contrast, Staphylococcus aureus was confirmed as Gram-positive, catalase-positive, coagulase-positive, TSI-negative, indole-negative, and non-lactose fermenting.*

INTRODUCTION

Among common bacterial infections Urinary tract infections (UTIs) are the most common bacterial infections globally which can be community acquired or from hospital settings (Mancuso *et al.*, 2023). Urinary tract infections are generally divided into lower and upper UTIs, and may be further classified as uncomplicated (uUTIs) or complicated (cUTIs). In upper UTIs, such as pyelonephritis including the kidneys and

ureters and in lower UTIsbladder is affected mainly and urethra (Kaur *et al.*, 2021). UTIs is mostly caused by bacteria including Uropathogenic Escherichia coli (UPEC) being the primitive causative agent for urinary tract infections. Other uropathogens which can be caused for urinary tract infections includes Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis, and different species of Staphylococcus. Beside bacteria fungi and some viruses are also responsible for urinary tract infections (Mancuso *et al.*, 2023).

Although UTIs can affect individuals of both sexes, they are especially prevalent among women due to anatomical and physiological factors in the female reproductive system (Vasudevan *et al.*, 2020). While the majority of infections are bacterial, fungi and viruses occasionally serve as causative agents (FloresMireles *et al.*, 2021). Gram-negative bacteria account for over 80% of cases, with Gram-positive organisms contributing to a smaller percentage. The presence of $\geq 10^5$ CFU/mL of bacteria in urine remains a key diagnostic criterion. Among the pathogens, E. coli is responsible for roughly 60–90% of cases, followed by Staphylococcus species, which cause 10–15% of infections (Ashur *et al.*, 2021).

Clinically, UTIs often present with symptoms such as frequent urination, urgency, painful or burning urination, dysuria, pyuria, suprapubic discomfort, and occasionally lower back pain. Host-related factorsincluding urinary composition, sexual activity, vaginal pH, osmolality, and mucosal secretionscan influence susceptibility (Nigussie & Amsalu., 2022). The severity of infection depends on both pathogen virulence and host vulnerability (Hannan *et al.*, 2020). Rising antimicrobial resistance, largely driven by misuse and overuse of antibiotics, has made management more difficult (Khan *et al.*, 2022). Accurate diagnosis should not rely solely on clinical signs but must be confirmed with laboratory testing to localize the infection and guide treatment, ultimately improving outcomes (Thergaonkar *et al.*, 2020). Failure to properly manage UTIs can lead to recurrent or chronic infections, with serious implications for health and quality of life (Eremenko *et al.*, 2020). Urine dipstick and biochemical testing are widely used due to their simplicity, rapid turnaround, affordability, and strong diagnostic accuracy, making them essential in routine clinical practice (Xie *et al.*, 2022).

Primarily, amoxicillin was used as first-line therapy for urinary tract infections, but the emergence of resistance in E. coli to different antibiotics has limited its use. Trimethoprim-sulfamethoxazole, fosfomycin, amikacin, meropenem, imipenem are now routinely preferred for its higher efficacy. Other antibiotics which are commonly prescribe for urinary tract infections including amoxicillin–clavulanate, cefixime, cefprozil, levofloxacin, nitrofurantoin and nalidixic acid (Kaufman *et al.*, 2020). Themild and uUTIs cases may resolve without antibiotics or hydration (Pulipati *et al.*, 2021).

METHODOLOGY

Study design

A descriptive cross-sectional study was conducted at Sarhad Institute of Allied Health Science, 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 studentsof 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 samples are excluded from this study.

Sample Size:

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

$$n = \frac{Z2.P.(1-p)}{d2}$$

Where:

Z=1.96 for a 95% confidence level

Based on a previously reported prevalence of UTI was reported as 8% in a five year 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)

$$n = \frac{(1.96)2\times0.04\times(1-0.04)}{(0.05)2}$$

$$n = \frac{3.84\times0.04\times0.96}{0.0025}$$

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

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.

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 collecting 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. 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 aged between 18 to 22 years. In first group falls 31 participants out of 52. In second group we included those participants with ages between 23 to 27 years fall 19 participants in this age group. In third group, participants with age between 28 to 32 years falls only 02 participants in this age group as shown in figure 4.1.

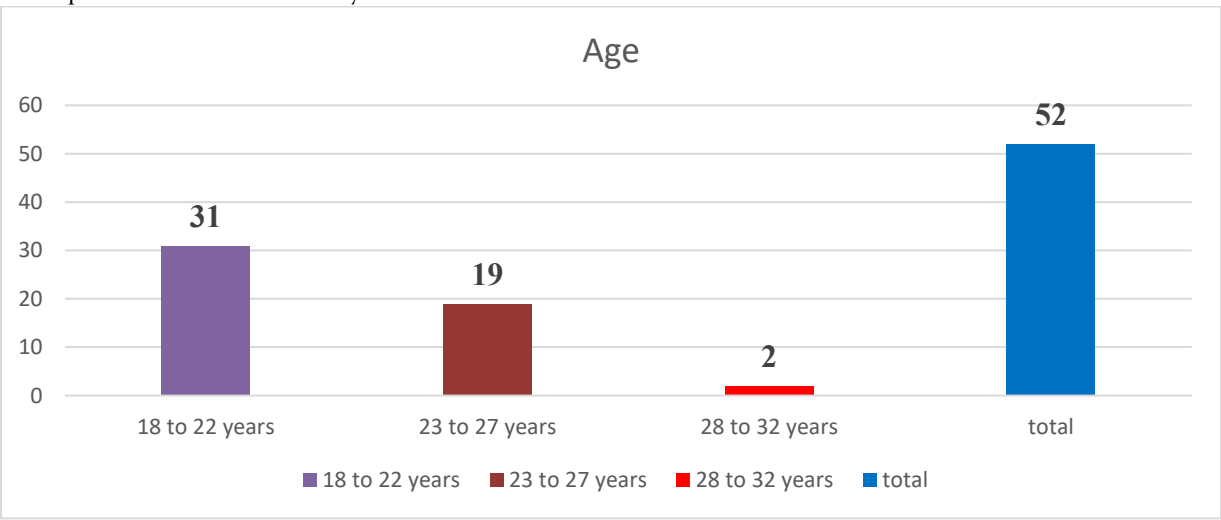

Figure 4.1 shows Age wise distribution of participants.

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 4.1.

Table 4.1 Gender wise distribution

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

Correlation of Uropathogens with gender:
In this study total 52 asymptomatic undergraduate students were included are categorized into male and female 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. All the isolated samples belong to female participants as shown in table 4.2.

Table 4.2 correlation of uropathogens with gender

		Gender	
		Male	Female
Bacteria	Negative	26	22
	Positive	0	4
Total		26	26

Bacterial distribution
Among total 52 samples 48 samples were negative for bacterial growth, only 4 samples show bacterial growth. The bacterial species of isolated samples were identified by several biochemical tests, which include Enterobacter 25%, Escherichia Coli 25% and Staphylococcus Aureus 50% as shown in table 4.3.

Table 4.3 bacterial distribution of samples

		Frequency	Percent	Cumulative Percent
Bacteria	Negative	48	92.3	92.3
	Enterobacter	1	1.9	94.2
	Escherichia Coli	1	1.9	96.2
	Staphylococcus Aureus	2	3.8	100.0
	Total	52	100.0	

Biochemical tests for species identification
After urine routine examination those samples in which bacteria were seen on microscope were then cultured on CLED (cysteine lactose electrolyte deficient) media. After 24 hours incubation at 37℃ the samples yield a growth which were further subjected to several biochemical tests for bacterial identification. The list of biochemical tests which were positive or negative for specific species are shown in figure 4.2.

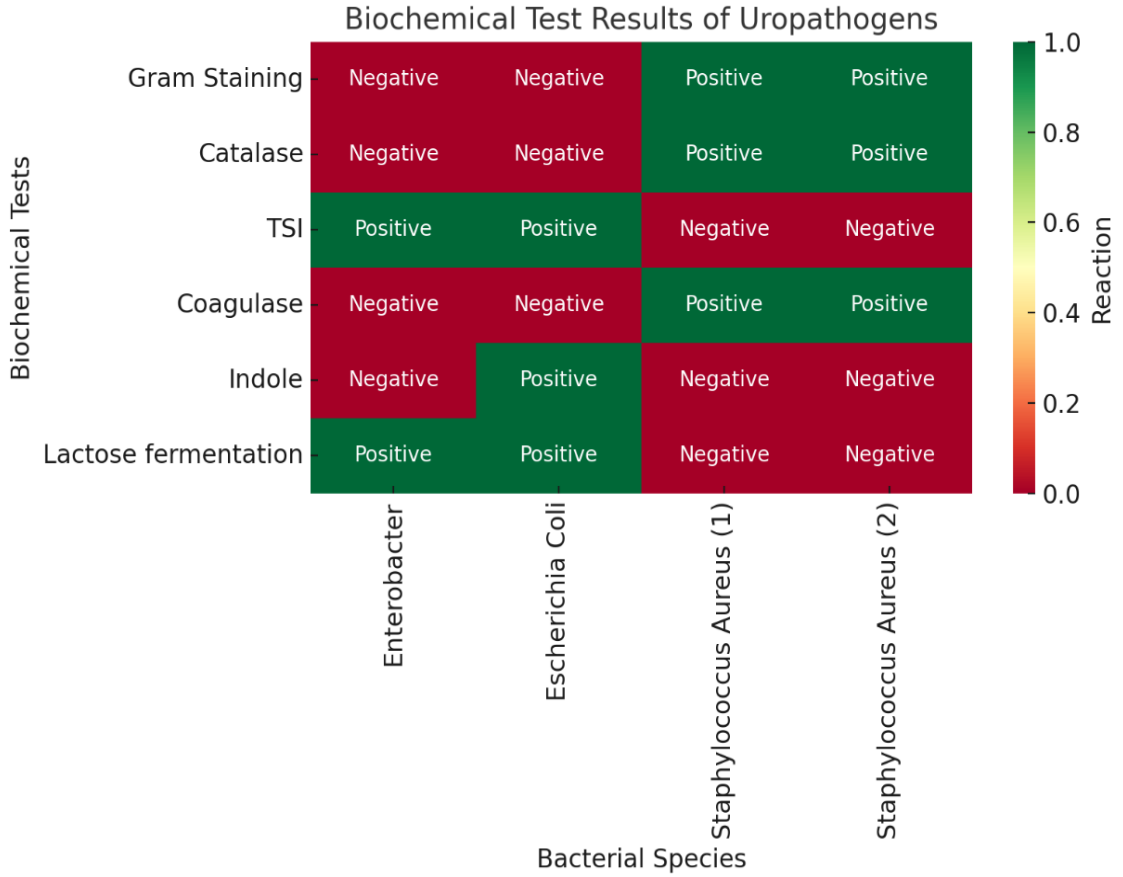

Figure 4.2 Biochemical tests of uropathogens are isolated from urine samples.

DISCUSSION

In this descriptive cross-sectional study conducted from April to July 2025 at Sarhad Institute of Allied Health Sciences, Peshawar, a total of 52 asymptomatic undergraduate students were screened for bacteriuria. Participants were divided into three age groups: 18–22 years (31 participants), 23–27 years (19 participants), and 28–32 years (2 participants). The study population was equally distributed by gender, with 26 males and 26 females. Among the 52 urine samples processed, bacterial growth was detected in 4 cases, all of which were isolated from female participants. The isolated bacteria were isolated and identified by standard biochemical tests. The isolated uropathogens included *Staphylococcus aureus* (50%), *Enterobacter* species (25%), and *Escherichia coli* (25%).

A study conducted in Haripur, Pakistan, demonstrated that *Escherichia coli* was the predominant uropathogens 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).

A study was conducted in the urology outpatient department of a tertiary care hospital in Pakistan to determine the frequency of uropathogens and their antimicrobial resistance patterns. A total of 1,000 clinically suspected UTI patients were enrolled, out of 1,000 specimens, 530 (53%) yielded positive cultures, with Escherichia coli being the most prevalent isolate (77.4%), followed by Klebsiella (6.4%), Enterobacter (6.0%), Pseudomonas (3.8%), Staphylococcus saprophyticus (3.4%), Citrobacter (1.1%), and Morganella (0.4%). Alarmingly high resistance rates were observed against commonly used antibiotics, underscoring the urgent need for continuous surveillance of antimicrobial susceptibility trends and strict adherence to prescribing guidelines to limit the rise of antimicrobial resistance in UTI pathogens (Rizvi *et al.,* 2020).

A study conducted to evaluate the susceptibility profiles of uropathogenic strains isolated from inpatient and outpatient departments. The identified pathogens included Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis, Acinetobacter baumannii, and Enterococcus faecalis. Results showed that first-generation cephalosporins, nitrofurantoin, and fluoroquinolones (norfloxacin/ciprofloxacin) were effective against outpatient isolates, whereas inpatient isolates associated with nosocomial UTIs required parenteral therapy with newer aminoglycosides and third-generation cephalosporins due to higher resistance levels (Gupta *et al.,* 2022).

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The findings of this study revealed that among 52 asymptomatic undergraduate students only 4 (7.7%) urine samples show bacterial growth, all of which were obtained from female participants. The isolated uropathogens included *Staphylococcus aureus* (50%), *Enterobacter* species (25%), and *Escherichia coli* (25%). Our finding indicates a higher frequency of asymptomatic bacteriuria in females compared to males within the studied population.

6.2. Recommendations

Larger Sample Size: Increasing the sample size will enhance the statistical power of the study, allowing for more reliable and generalizable results.

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 across a wider population.

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