

## Research Article

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# ISOLATION OF PATHOGENIC BACTERIA OF URINARY TRACT INFECTIONS AND THEIR ANTIBIOTIC SENSITIVITY IN CHILDREN

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Urinary tract infections (UTIs) remain the most common bacterial disease in children. This cross-sectional study investigated the distribution and antibiotic susceptibility of uropathogens isolated from 85 pediatric patients (aged 2–10 years) in the Swat District, Pakistan. *Escherichia coli* was the predominant isolate (71.21%), followed by *Enterococcus faecalis* (20.31%) and *Pseudomonas aeruginosa* (6.06%), with infections occurring more frequently in females. Disk-diffusion testing interpreted according to Clinical and Laboratory Standards Institute (CLSI 2023) guidelines showed that *E. coli* isolates were highly susceptible to Meropenem (100%) and Tazocin (93.61%), whereas complete resistance was observed to Doxycycline, Amoxicillin-clavulanate, and Trimethoprim-sulfamethoxazole. Similar resistance patterns were found for *E. faecalis* and *P. aeruginosa*. These findings highlight the growing problem of multidrug-resistant pediatric uropathogens and support the empirical use of Meropenem or Tazocin as effective first-line therapies in this region. Continuous regional surveillance is recommended to guide antibiotic stewardship and reduce resistance development.

**Keywords:** Antibiotic sensitivity, *Escherichia coli*, Meropenem, Pediatric UTI, Tazocin

## INTRODUCTION

Urinary tract infections (UTIs) are among the most prevalent bacterial infections, especially in the pediatric population (1). Globally, more than 400 million people are affected each year, resulting in significant morbidity and mortality (2). The risk of infection increases in females and individuals with underlying health conditions such as diabetes, kidney disease, and immunodeficiency (3, 4). Among causative agents, uropathogenic *Escherichia coli* (UPEC) accounts for approximately 80% of pediatric UTIs, followed by other pathogens such as *Enterococcus faecalis* and *Pseudomonas aeruginosa* (5). UPEC employs multiple strategies to evade the host's immune defenses, including adhesion, biofilm formation, and cellular invasion. The initial and crucial steps in establishing a persistent urinary tract infection involve primary adhesion to host cells and subsequent invasion (6). If left untreated, these infections can have serious complications, such as pyelonephritis, scarring of the kidneys, and sepsis (7).

Over the past decade, increasing antimicrobial resistance has posed a significant challenge to the effective treatment of bacterial infections. Patterns of resistance vary widely by region and are influenced by antibiotic misuse and limited diagnostic capacity (8, 9). Understanding the local microbiological spectrum and susceptibility profiles of uropathogens is therefore critical for optimizing pediatric treatment protocols (10).

Despite extensive national research, data on pediatric UTIs in northern Pakistan, particularly in the Swat District, remain scarce. Most available studies emphasize adult or hospital-acquired infections, leaving a major knowledge gap concerning community-acquired pediatric UTIs and local resistance trends. This study aims to identify the predominant uropathogens among children in Swat and evaluate their antibiotic susceptibility according to current CLSI standards. The findings provide region-specific evidence to inform empirical therapy and support antibiotic-resistance surveillance in pediatric care.



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## METHODOLOGY

### SAMPLES COLLECTION

A cross-sectional study was conducted between January and July 2024 across several clinical laboratories and hospitals in Saidu Sharif, Swat District, Pakistan, including Amreek Laboratory, Shah Hospital Laboratory, Dilaram Khan Hospital, and the Saidu Group of Teaching Hospitals. A total of 85 midstream clean-catch urine samples were collected from children presenting with symptoms suggestive of UTI. Sampling followed a convenience approach, restricted to children whose parents or guardians provided informed consent. Patients were grouped by age (<2 years, 2-6 years, and 7-10 years) and gender, with 31 samples from males and 54 from females. The age-specific distribution included 17 samples from children less than 2 years, 46 from those aged 2-6 years, and 22 from those aged 7-10 years.

### MICROSCOPIC AND CULTURE EXAMINATION

Urine samples were centrifuged at 4000 rpm for 5 minutes, and the sediment was examined microscopically for pyuria ( $\geq 10$  WBC mm $^3$ ). Each specimen was streaked on Cystine Lactose Electrolyte-Deficient (CLED) agar and incubated at 37 °C for 24 h. Colonies were sub-cultured on MacConkey and Blood agar plates for isolation and differentiation of Gram-positive and Gram-negative organisms.

### IDENTIFICATION OF BACTERIAL ISOLATES

Bacterial isolates were identified by standard morphological and biochemical tests following Clinical and Laboratory Standards Institute (CLSI, 2023) recommendations. Gram staining, oxidase, catalase, triple sugar iron (TSI), and IMViC assays were performed using established microbiological protocols (11, 12). Detailed procedural steps (e.g., individual staining reagents) were omitted here for brevity, but followed standard laboratory manuals, Cheesbrough, 2006 (13).

### QUALITY CONTROL AND REFERENCE STRAINS

Quality control for culture and biochemical testing was ensured using standard American Type Culture Collection (ATCC) reference strains: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *E. faecalis* ATCC 29212. All media and antibiotic disks were verified for performance according to CLSI (2023) guidelines before use.

### ANTIBIOTIC SUSCEPTIBILITY TESTING

Antibiotic susceptibility was determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, interpreted according to CLSI (2023) breakpoints. Tested antibiotics included Meropenem (10 µg), Tazocin (110 µg), Amoxicillin-clavulanate, Trimethoprim-sulfamethoxazole, Doxycycline (30 µg), Fosfomycin (50 µg), Amikacin (30 µg), Ceftriaxone (30 µg), Levofloxacin (5 µg), and others relevant to regional therapy. Minimum inhibitory concentrations (MICs) were not determined; however, the interpretation of resistant, intermediate, and sensitive categories strictly followed CLSI guidelines to ensure comparability with standardized reference data.

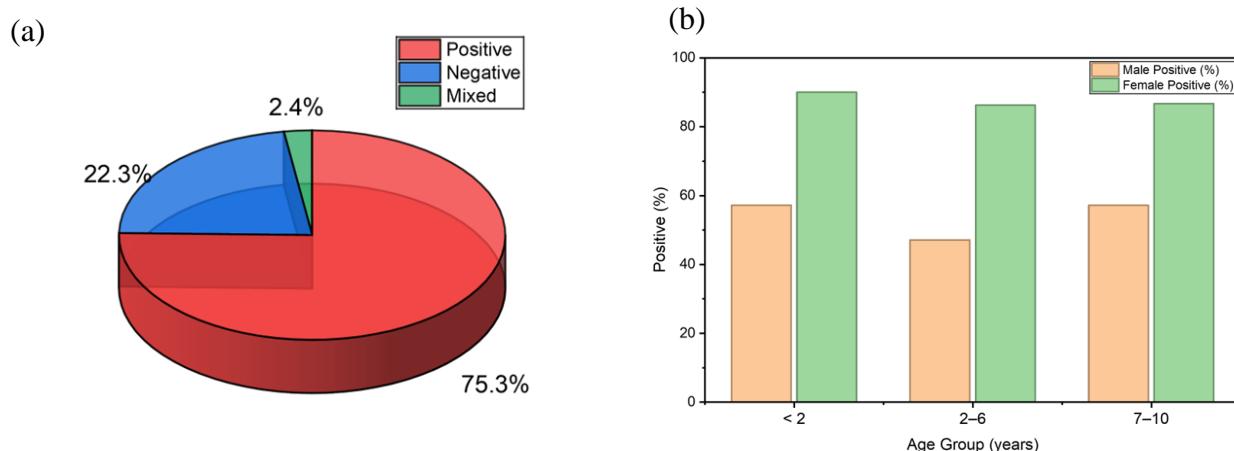
## RESULTS

### PREVALENCE OF UTIs

This study included 85 pediatric patients from Swat District, Pakistan, suspected of having urinary tract infections (UTIs). Of these samples, 63.53% were from females and 36.47% from males. Among the 85 samples, 75.3% (64) of the urine samples were culture positive for uropathogenic bacteria (Fig. 1). Negative cultures accounted for 22.3% of samples, while mixed bacterial growth was observed in 2.4% (Fig. 1a). Figure 2(b) illustrates the distribution of positive cultures by age and sex. The samples were classified into three age groups: under 2 years, 2 to 6 years, and 7 to 10 years. A higher prevalence of UTI-positive samples was observed among females across all age groups. The distribution of positive cultures revealed that 90% of females under 2 years had UTIs, compared to 57.14% of males in the same age group. In the 2 to 6 years age group, 86.2% of female samples and 47.05% of male samples tested positive for UTIs. For children aged



7 to 10 years, UTIs were present in 86.66% of female samples and 57.14% of male samples. *E. coli* was the most prevalent pathogen across all age groups, with *Enterococcus faecalis* more commonly isolated in males under two years. The higher female-to-male ratio of positive samples indicates greater susceptibility of girls to UTI infections.



**Fig. 1 (a).** Culture results of urine samples, total samples 85; **(b).** Distribution of positive UTI cases among children by age group and gender

## BACTERIAL ISOLATES AND GRAM STAINING

Of all the positive bacterial cultures, gram-negative bacteria accounted for the majority of isolates (79.69%, 51/64 samples), while gram-positive bacteria made up 20.31% (13/64 samples). Table 1 illustrates that *E. coli* was the most common uropathogen, comprising 71.21% of positive samples, followed by *Enterococcus faecalis* (20.31%) and *Pseudomonas aeruginosa* (6.06%). Mixed cultures were observed in 3.03% of cases, with both *E. coli* and *Enterococcus faecalis* present. These results confirm that *E. coli* is the principal etiological agent in pediatric UTIs within this region.

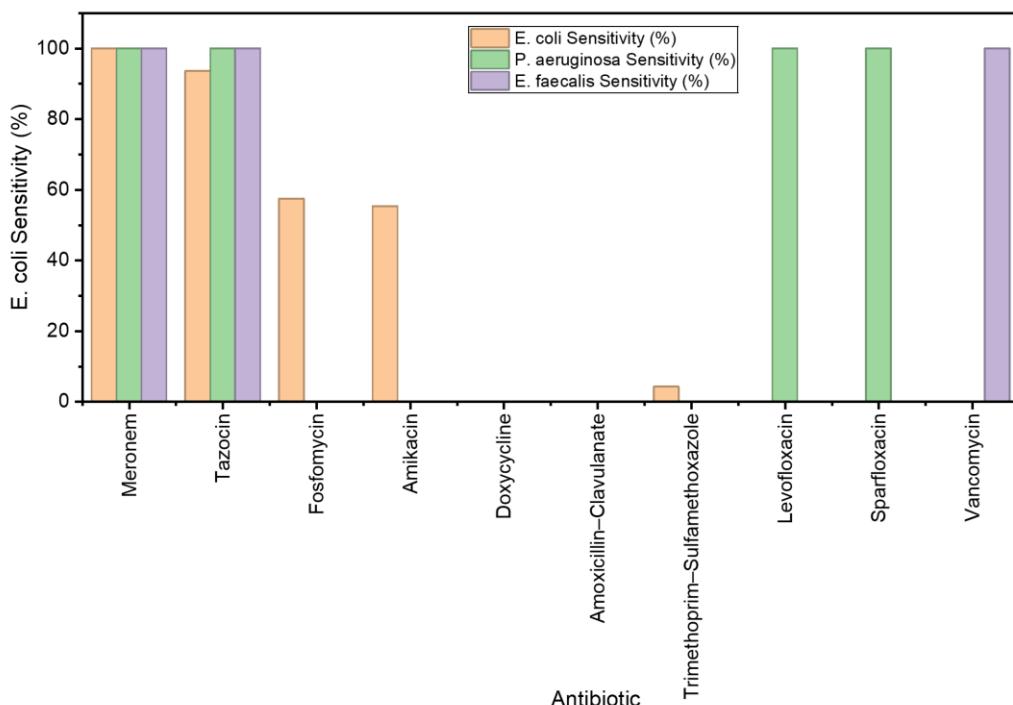
**Table I.** Frequency of pathogenic bacteria in UTI samples

Bacteria	Isolates (n)	Percentage (%)
<i>Escherichia coli</i>	47	71.21
<i>Enterococcus faecalis</i>	13	19.70
<i>Pseudomonas aeruginosa</i>	4	6.06
Mixed Growth ( <i>E. coli</i> + <i>E. faecalis</i> )	2	3.03
Total	66	100

## ANTIBIOTIC SUSCEPTIBILITY TESTING

Antibiotic susceptibility testing revealed varying sensitivity among the uropathogenic bacteria (Fig. 2). Meropenem (Meronem) and Piperacillin-Tazobactam (Tazocin) exhibited high efficacy against all tested pathogens, while certain antibiotics demonstrated limited effectiveness. All *E. coli* isolates were highly sensitive to Meronem (100%) and Tazocin (93.61%). Moderate sensitivity was observed for Fosfomycin (57.44%) and Amikacin (55.31%), while resistance was highest for Doxycycline, and Amoxicillin-clavulanate. *Enterococcus faecalis* isolates showed complete sensitivity to Meronem, Tazocin, and Vancomycin (100%). However, they exhibited resistance to Trimethoprim-sulfamethoxazole, Doxycycline, and Amoxicillin (100%), with variable intermediate responses to other antibiotics. All *Pseudomonas aeruginosa* isolates were sensitive to Tazocin, Meronem, Levofloxacin, and Sparfloxacin. This bacterium showed complete resistance to Trimethoprim-sulfamethoxazole and Doxycycline.

Meropenem and Tazocin were the most effective antibiotics against the isolated uropathogens, contrasting sharply with the poor activity of older agents like Doxycycline, Amoxicillin-clavulanate, and Trimethoprim-sulfamethoxazole. The emergence of multidrug-resistant *E. coli* underlines the urgent need for regular resistance monitoring and revision of empirical therapy protocols in this region.



**Fig. 2.** Antibiotic susceptibility profiles of major uropathogens isolated from pediatric patients in Swat District (n = 64).

## DISCUSSION

This study demonstrates that urinary tract infections remain a significant pediatric health issue in the Swat District, with a culture positivity rate of 75.3 %. The predominance of infections in females aligns with established anatomical and behavioral risk factors, such as shorter urethral length and increased periurethral colonization (15, 18). Similar female predominance has been reported in pediatric UTI surveys from Peshawar and Lahore (16, 17), confirming that gender-associated vulnerability is consistent across Pakistan. The leading pathogen identified was *Escherichia coli* (71.2 %), consistent with global data that identifies *E. coli* as the leading cause of pediatric UTIs (13, 18, 19, 20). The dominance of *E. coli* underscores its adaptability in colonizing the pediatric urinary tract through adhesion, biofilm formation, and intracellular persistence mechanisms (21).

Antibiotic sensitivity testing revealed that Meropenem (Meronem) and Piperacillin-Tazobactam (Tazocin) were highly effective against *E. coli*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*. Their broad-spectrum activity supports their consideration as first-line treatments in this region with rising antibiotic resistance. Conversely, *E. coli* exhibited high resistance to commonly prescribed antibiotics such as Amoxicillin-clavulanate, Trimethoprim-sulfamethoxazole, and Doxycycline, suggesting that these agents may no longer be effective for empirical treatment of UTIs in this area. Similarly, *Pseudomonas aeruginosa* displayed high resistance to Trimethoprim-sulfamethoxazole and various cephalosporins, consistent with its inherent resistance mechanisms. The efficacy of Meropenem and Tazocin against *P. aeruginosa* underscores the necessity of prioritizing these antibiotics in cases of severe or complicated infections. However, the observed emergence of multidrug-resistant *E. coli* emphasizes that even carbapenems must be used judiciously to prevent further resistance escalation. The routine use of Doxycycline and Amoxicillin-clavulanate should be discouraged unless culture evidence supports their efficacy. Continued reliance on ineffective treatments will increase the likelihood of therapeutic failure and related complications. Therefore, regular surveillance at the district level is essential to detect shifts in resistance trends and update treatment protocols.

Although this study provides valuable insights into the prevalence and resistance patterns of pediatric UTIs, its limited sample size and single-district focus may restrict the generalizability of conclusions. Future studies should involve larger and more diverse populations across multiple healthcare centers to capture broader resistance trends. Moreover, molecular investigation of resistance mechanisms,

especially in *E. coli* and *P. aeruginosa*, could elucidate the genetic drivers of resistance and inform the development of targeted therapies for pediatric urinary infections.

## CONCLUSION

This study reveals a high prevalence of pediatric urinary tract infections in the Swat District, with *E. coli* as the dominant pathogen and a higher incidence among females. The isolates showed extensive resistance to amoxicillin-clavulanate and Doxycycline, while Meropenem and Tazocin remained highly effective. These results emphasize the importance of region-specific antibiotic policies and routine surveillance to guide empirical therapy and mitigate multidrug resistance. Although limited in sample size and geographic scope, this study provides critical baseline data for future large-scale investigations and supports the need for integrated antimicrobial management and responsible prescribing practices in pediatric healthcare.

### Conflict of interest:

Authors declare no conflict of interest.

### Authors' contribution

YA Sample collection, laboratory analysis and initial data interpretation; WK Supervised the study and designed the methodology; MNU Data analysis and statistical evaluation; NQ & IAS Assisted in laboratory work and culture identification and antibiotic sensitivity testing; SK Manuscript drafting, proofreading and final formatting of the article.

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