Prevalence and antibiotic susceptibility pattern of uropathogens in outpatients at a tertiary care hospital

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Abstract

Globally, urinary tract infection (UTI) is considered a major public health concern and the second most common bacterial infection affecting individuals of different ages. Bacteria are responsible for about 95% of UTIs. The emergence of antimicrobial resistance in uropathogens may lead to poor treatment outcomes in individuals with UTIs. The knowledge of the microorganism involves and antibiograms are important for the empirical treatment of UTIs. A cross-sectional study was carried out over 7 months (January to July 2019) with a focus on the identification of bacterial pathogens causing UTI and the evaluation of their antibiogram. In total, 804 urine samples were collected from individuals with suspected UTIs and inoculated on recommended media. Isolation and identification of the bacterial strains were performed using standard microbiological protocols. Antibiotic susceptibility was carried out following CLSI recommended guidelines. Among the tested specimens, 290 (36.1%) had significant bacterial growth and 147 (50.7%) of the strains were isolated from female patients. The frequently identified isolates were Escherichia coli (68.9%), followed by Klebsiella pneumoniae (8.9%) and Staphylococcus aureus (6.7%). The highest percentages of resistance have been observed against tested antibiotics. The majority of the isolates were extended-spectrum β-lactamase producers (85.2%) and multidrug-resistant (98.3%). We observed that Gram-negative bacteria were the main cause of UTIs where the predominant microorganism was E. coli.

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Keywords: Antibiotic susceptibility, Bacterial profile, E. coli, Multidrug resistance, Pakistan, Urinary tract infection, Uropathogens

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Introduction

Urinary tract infection (UTI) is described as the health condition that has association between clinical signs and symptoms and detection of pathogenic microorganisms in the urine, bladder, urethra, kidney and prostate [1]. UTIs are the second most common bacterial infection affecting individuals of different ages worldwide [2]. Globally, an estimated 50% of women have UTIs at least once in their lifetime and UTIs are particularly more common in those aged 16–64 years [2,3]. Prevalence of UTIs is very low among boys but can be observed in the first year of life particularly in those with anatomical or functional abnormalities [4]. Moreover, recurrence rates of UTIs are higher, mainly because of lapses in or cessation of treatment. Therefore, reinfection with the same or different microorganisms may occur [5].

UTIs are classified as uncomplicated and complicated. The uncomplicated UTIs are common in adult healthy non-pregnant women, whereas complicated UTIs occur among different age and sex groups [2]. The predisposing factors for complicated UTIs are renal calculi, renal failure, indwelling catheters, renal transplantation, immunosuppression, obstruction and pregnancy [6]. The UTIs are frequently caused by bacteria which account for >95% of cases; however, other microorganisms such as fungi, parasites and viruses can also cause UTIs [7].
The bacterial pathogens involved in UTIs are mainly Gram-negative bacteria, such as Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Citrobacter species, Enterobacter species, and Proteus species. Among Gram-positive bacteria, Staphylococcus aureus, Staphylococcus saprophyticus and Enterococcus species are most commonly responsible for UTIs \[8\]. Among bacteria, 75\%–95\% of cases of UTI are caused by E. coli \[9\].

Antibiotics have been very effective in the management of UTI and for empirical treatment, broad-spectrum antibiotics are commonly prescribed. Globally, antibiotic resistance against common pathogens has emerged because of the inappropriate use of antibiotics and the availability of these antibiotics over the counter \[10\]. Over the last few years, an increase in the prevalence of multidrug-resistant (MDR) uropathogens has been observed in both community and hospitalized patients \[11\]. According to the study carried out by the European Survey of Antibiotic Consumption, MDR bacterial strains in complicated UTIs are responsible for the mortality rate of approximately 25,000 Europeans/year \[12\]. Hence, it is very important to overcome the inappropriate use and misuse of antibiotics that lead to multidrug resistance; appropriate antibiotics should be selected for the empirical treatment of UTI. The pattern of antibiotic susceptibility among bacteria varies from hospital to hospital and within different geographical locations \[13\]. The Infectious Disease Society of America recommends that regional surveillance should be carried out in a specific region to observe variations in antibiotic susceptibility patterns \[14\].

In the current scenario, antimicrobial susceptibility patterns are frequently changing and, alarmingly, MDR bacterial pathogens are emerging, leading to increased morbidity and mortality. The knowledge of the causative agent involved in UTIs and their antibiotic susceptibility is crucial for the empirical treatment of UTIs and the prevention of the emergence of antimicrobial resistance. However, very few studies are reported from Pakistan on the common uropathogens and their antibiotic susceptibility profile. This study aimed to determine the prevalence of the UTI-causing pathogens, and their antimicrobial susceptibility pattern among individuals with suspected UTIs.

**Methods**

This study was carried out at the Department of Medical Laboratory Technology, The University of Haripur, Pakistan from January to July 2019. The study population included patients of different age groups, who visited the Khyber Teaching Hospital, Peshawar, and attended other specialist clinicians in the periphery. Over 7 months, a total of 804 midstream urine samples were collected from patients who had symptomatic UTIs. Patients with a history of recent antibiotic therapy (within the last 72 hours) were excluded from the study populations. The collected samples were inoculated using a calibrated wire loop (0.001 mL) on Cystin Lactose Electrolytes Deficient agar (CLED, Oxide, Basingstoke, UK) and incubated overnight at 37°C. Colony counts >10^5 CFU/mL were considered significant. The culture plates with no growth were further incubated for 48 hours. The pure isolated bacterial colonies were identified using standard microbiological techniques, such as Gram staining, colony morphology and biochemical testing (Oxoid) \[15\]. Species identification was carried out using classical biochemical methods and Analytical Profile Index (API) identification strips, where required, using recommended guidelines \[15\].

The antibiotic susceptibility testing was carried out on Muller–Hinton agar and blood agar (Oxoid), using the Kirby Bauer disc diffusion method as per the CLSI 2018 recommendations \[16\]. For the quality control, reference strains S. aureus (ATCC 25923), E. coli (ATCC 25922) and P. aeruginosa (ATCC 27853) were used. The antibiotics discs were obtained from Oxoid. The antibiotic discs and concentrations (\(\mu\)g) used for both Gram-positive and Gram-negative bacteria were as follows: amikacin (30), amoxicillin (10), augmentin: amoxicillin-clavulanic acid (30), aztreonam (30), cephradine (30), cefazidine (30), cefotaxime (30), ciprofloxacin (5), cotrimoxazole: trimethoprim-sulphamethoxazole (25), gentamicin (10), imipenem (10), meropenem (10), nalidixic acid (30), nitrofurantoin (30), ofloxacin (5), tazocin: piperillin-tazobactam (40), trimethoprim (2.5), ampicillin (10), ceftriaxone (30), fusidic acid (5), linezolid (10), vancomycin (30), azithromycin (15), cefoxitin (30), cefuroxime (30), fosfomycin (50) and norfloxacin (10). The zone of inhibition of antibiotics was measured and interpreted according to the CLSI 2018 guidelines \[16\].

Gram-negative bacteria were confirmed for the presence of extended-spectrum \(\beta\)-lactamases (ESBLs) by using the phenotypic detection method as per CLSI guidelines \[16\]. The discs used were amoxicillin-clavulanic acid, ceftazidime and cefotaxime (third-generation cephalosporin). The isolates were interpreted as ESBL producers if a clear extension of the edge of the inhibition zone of cephalosporin towards the augmentin disc was observed \[17\]. The presence of methicillin resistance was screened phenotypically in S. aureus by using cefoxitin and oxacillin as per CLSI guidelines. Furthermore, phenotypic detection of vancomycin resistance was detected in Enterococcus spp. and S. aureus by using a vancomycin disc according to the CLSI 2018 recommendations. Ethical permission was obtained from the hospital ethics committee. Statistical analysis was conducted using GraphPad Prism software, version 5.00 \[18\].

**Results and discussion**

A total of 804 urine specimens from outpatients were received during the study period and 290 (36.1\%) of the samples were
culture positive for urinary pathogens. In this study, most of the uropathogens were recovered from female patients (50.7%), which is consistent with the previous reports (Table 1) [19,20]. Various predisposing factors contribute to the higher prevalence of UTIs among women [21]. In our study, the overall prevalence of UTIs was 36.1%, which is slightly higher than the previous study [20]. The highest prevalence rate has been observed in the old-age group, which might be a result of genito-urinary atrophy and vaginal prolapse after menopause that alters the vaginal pH, decreasing the normal vaginal flora. This condition allows for Gram-negative bacteria to grow as uropathogens [22]. The prevalence of UTIs in the age group 18–29 years was 24.8%, this could possibly be due to increased sexual activity in this specific age group (Table 2) [22].

*Escherichia coli* was the most frequently isolated species and accounted for 68.3% of the uropathogens. Moreover, organisms such as *K. pneumoniae*, *Pseudomonas* spp., *Enterococcus* spp. and *Proteus* spp., which are part of the normal intestinal flora, represented 9.3%, 4.1%, 2.8% and 2.1%, respectively, as shown in Table 3. These findings are consistent with previous reports [8,20]. The prevalence of *E. coli* was comparatively higher than in the previously published reports [23,24]. The highest prevalence of Gram-negative organisms in our study is consistent with other studies [22,25]. However, variation in the spectrum of bacterial uropathogens has been reported across different geographical locations and by categorization of the patients’ UTIs [26,27].

The antibiotic resistance of the frequently isolated uropathogens is presented in Tables 4 and 5. The highest percentages of resistance have been observed against the tested antibiotics. Overall, in Gram-negative organisms, resistance was >50% to all the tested antibiotics. However, a low level of resistance has been observed for Gram-negative agents against meropenem and imipenem (Table 4). In contrast, in the case of *K. pneumoniae*, none of the isolates showed sensitivity to cefotaxime and cefuroxime (100%; 27/27) and a similar pattern has been observed for nitrofurantoin and norfloxacin in *Pseudomonas* spp. (Table 4). In our study, the highest resistance (>70%) was observed against *E. coli* and *Proteus* spp. to almost all the tested antibiotics except carbapenem, which were in the range of 16.7%–49.5%. A similar pattern has been observed for other Gram-negative uropathogens. The lowest observed resistance for *Klebsiella* spp. was 37% against amikacin and imipenem, whereas for *Pseudomonas* spp. the lowest resistance percentages were observed against amikacin (8%) followed by gentamicin and imipenem (33.3%, both respectively). This massive increase in antibiotic resistance is a result of the overuse of these antibiotics for the treatment of different infections in our region without checking culture sensitivity. This alarming situation is the leading cause of MDR infection among UTIs. The resistance against cotrimoxazole was >67% against all the isolated Gram-negative organisms. The European Urology Association and The Infectious Disease Society of America guidelines recommended cotrimoxazole as the first-line empirical antibiotic for the treatment of uncomplicated community-acquired UTIs, where locally reported resistance percentages against uropathogens are <10% to 20% [14]. Unfortunately, in our study, the highest resistance rates have been observed against cotrimoxazole. This increase in resistance may be a result of the non-judicious use of antibiotics and their availability over the counter without a physician’s prescription.
Among Gram-negative bacteria (E. coli, K. pneumoniae, Pseudomonas spp. and Proteus spp.) the ESBL-producing isolates comprised 85.2% (207/243) and E. coli had the highest proportion of ESBL-producing strains (87.4%; 207/243) (Table 6). The highest resistance against β-lactam antibiotics in isolated uropathogens is due to the production of the enzyme ESBL. Furthermore, it has been observed that the increasing frequency of ESBL phenotypes has enormous potential for the acquisition of multidrug resistance [28]. Similarly, the highest percentages of resistance had been observed against Gram-positive bacteria (S. aureus, S. saprophyticus and Enterococcus spp.) (Table 5). The resistance percentages of linezolid and

| Antibiotics | Enterococcus spp. (n = 12), % (n) | Pseudomonas aeruginosa (n = 12), % (n) | Proteus spp. (n = 6), % (n) | Klebsiella pneumoniae (n = 27), % (n) | Escherichia coli (n = 198), % (n) |
|-------------|----------------------------------|-------------------------------------|-----------------------------|--------------------------------------|----------------------------------|
| Amikacin    | 8.3 (1)                           | 9.3 (27)                            | 66.7 (4)                    | 66.7 (4)                             | 71.2 (148)                      |
| Augmentin   | 25 (3)                            | 2.1 (6)                             | 66.7 (4)                    | 66.7 (4)                             | 66 (31)                         |
| Aztreonam   | 50 (6)                            | 4.1 (12)                            | 66.7 (4)                    | 66.7 (4)                             | 51.1 (24)                       |
| Cefotaxime  | 66.7 (8)                          | 37.5 (31)                           | 66.7 (4)                    | 66.7 (4)                             | 87.2 (41)                       |
| Cefoxitin   | 50 (6)                            | 37.5 (31)                           | 66.7 (4)                    | 66.7 (4)                             | 87.2 (41)                       |
| Cefuroxime  | 66.7 (8)                          | 37.5 (31)                           | 66.7 (4)                    | 66.7 (4)                             | 87.2 (41)                       |
| Gentamicin  | 66.7 (8)                          | 37.5 (31)                           | 66.7 (4)                    | 66.7 (4)                             | 87.2 (41)                       |
| Imipenem    | 16.7 (1)                          | 33.4 (24)                           | 66.7 (4)                    | 66.7 (4)                             | 38.7 (94)                       |
| Meropenem   | 41.7 (5)                          | 33.4 (24)                           | 66.7 (4)                    | 66.7 (4)                             | 49 (119)                        |
| Nalidixic acid | 66.7 (8)                          | 37.5 (31)                           | 66.7 (4)                    | 66.7 (4)                             | 49 (119)                        |
| Norfloxacin | 66.7 (8)                          | 37.5 (31)                           | 66.7 (4)                    | 66.7 (4)                             | 49 (119)                        |
| Oxacillin   | 66.7 (8)                          | 37.5 (31)                           | 66.7 (4)                    | 66.7 (4)                             | 49 (119)                        |
| Trimethoprim| 66.7 (8)                          | 37.5 (31)                           | 66.7 (4)                    | 66.7 (4)                             | 49 (119)                        |

<sup>n</sup>, number of isolates; %, percentages of isolates; NT, not tested.

TABLE 6. Percentage of ESBL-producing isolates collected from individuals with urinary tract infections attending Khyber Teaching Hospital, Peshawar from January to June 2019

| Bacterial isolates | ESBL, % (n) | Total, % (n) |
|--------------------|-------------|--------------|
| Escherichia coli    | 87.4 (173)  | 68.3 (198)   |
| Klebsiella pneumonia| 85.2 (23)  | 9.3 (27)     |
| Pseudomonas spp.    | 50 (6)      | 4.1 (12)     |
| Proteus spp.        | 83.3 (5)    | 2.1 (6)      |

ESBL, extended-spectrum β-lactamase; n, number of isolates; %, percentages of isolates.

TABLE 5. Antibiotic resistance pattern of Gram-positive bacterial isolates recovered from urine samples of individuals with urinary tract infections attending Khyber Teaching Hospital, Peshawar from January to June 2019

| Antibiotics | Staphylococcus aureus, (n = 14), % (n) | MRSA, (n = 16), % (n) | Staphylococcus spp. (n = 9), % (n) | Enterococcus spp., (n = 8), % (n) | Total, % (n) |
|-------------|--------------------------------------|---------------------|-----------------------------|---------------------------------|--------------|
| Amikacin    | 42.9 (6)                             | 68.8 (11)           | 66.7 (6)                    | 25 (2)                          | 53.2 (25)    |
| Ampicillin  | 92.9 (13)                            | 87.5 (14)           | 100 (9)                     | 100 (8)                         | 92.3 (36)    |
| Amoxicillin | 92.9 (13)                            | 82.5 (10)           | 100 (9)                     | 99 (12)                         | 89.9 (199)   |
| Augmentin   | 84.6 (11)                            | 86.8 (11)           | 66.7 (6)                    | 37.5 (3)                        | 66.9 (31)    |
| Aztreonam   | 100 (14)                             | 81.2 (13)           | 88.9 (8)                    | 62.5 (5)                        | 85.1 (40)    |
| Aztreonam   | 100 (14)                             | 93.8 (15)           | 100 (9)                     | 100 (8)                         | 97.9 (46)    |
| Cefotaxime  | 86.7 (12)                            | 100 (16)            | 100 (9)                     | 100 (8)                         | 97.9 (46)    |
| Cefoxitin   | 86.6 (11)                            | 87.5 (14)           | 88.9 (8)                    | 100 (8)                         | 87.2 (41)    |
| Cefuroxime  | 86.7 (12)                            | 87.5 (14)           | 100 (9)                     | 100 (8)                         | 97.9 (46)    |
| Fusidic acid| 85.7 (12)                            | 87.5 (14)           | 100 (9)                     | 100 (8)                         | 97.9 (46)    |
| Gentamicin  | 50 (7)                               | 81.2 (13)           | 66.7 (6)                    | 75 (6)                          | 68.1 (32)    |
| Imipenem    | 57.1 (8)                             | 81.2 (13)           | 55.6 (5)                    | 25 (2)                          | 60 (28)      |
| Linezolid   | 42.9 (6)                             | 68.8 (11)           | 33.3 (3)                    | 0.0 (0)                         | 34 (16)      |
| Meropenem   | 50 (7)                               | 75 (12)             | 66.7 (6)                    | 75 (6)                          | 66.3 (31)    |
| Nalidixic acid| NT                                   | 93.8 (15)           | 88.9 (8)                    | 100 (8)                         | 93.9 (31)    |
| Nitrofurantoin| 42.9 (6)                             | 68.8 (11)           | 66.7 (6)                    | 50 (4)                          | 57.4 (27)    |
| Ofloxacin   | 100 (14)                             | 81.2 (13)           | 77.8 (7)                    | 100 (8)                         | 87.2 (41)    |
| Tazocin     | 50 (7)                               | 37.5 (6)            | 66.7 (6)                    | 75 (6)                          | 48.9 (23)    |
| Vancomycin  | 50 (7)                               | 62.5 (10)           | 100 (9)                     | 75 (6)                          | 68.1 (32)    |

<sup>n</sup>, number of isolates; %, percentages of isolates; MRSA, methicillin-resistant Staphylococcus aureus; NT, not tested.
vancomycin against all *Enterococcus* and *Staphylococcus* spp. was more than 35%. Imipenem had the lowest resistance rate (14.3%; 2/14) against *S. aureus*. Multidrug resistance was observed in both Gram-negative and Gram-positive bacteria (Table 7).

In particular, *E. coli* had the highest multidrug resistance among the isolated uropathogens and 97% of the isolates were resistant to more than five classes of tested antibiotics, whereas *Staphylococcus* spp. and *Enterococcus* spp. had >76.9% and >75% resistance rates, respectively (Table 8). Furthermore, of the total (13.2%; 19/143), *S. aureus* (57.8%; 11/19) were methicillin-resistant (strains resistant to beta lactam class of antibiotics).

Among Gram-positive bacteria, the resistance against vancomycin and linezolid were in the range of 37.5%–100%. These drugs are not routinely recommended for the treatment of UTIs. In our study both for Gram-positive and Gram-negative organisms, the overall resistance of the tested antibiotics was >30%. Among the *Enterococcus* spp., 75% of the isolates were vancomycin-resistant and 62.5% of the *Staphylococcus* isolates were identified as methicillin-resistant *S. aureus*. Conclusively, in our study, the most common isolated pathogens among outpatients were Gram-negative *Enterobacteriaceae* followed by Gram-positive *Staphylococcus* and *Enterococcus* spp. Overall, this study will help physicians in prescribing appropriate antibiotics for the treatment of UTIs. Second, the emergence of MDR organisms, which we reported in our study, threatens the management of patients with UTIs. The physician should strictly follow the culture report before starting therapy to prevent the emergence of multidrug resistance. As a result of the emergence of multidrug resistance, we are losing antibiotics of choice for treating simple bacterial infections.

### Conclusion

The study showed that UTIs are the leading public-health problem mainly in women. In our study, the predominant isolated organisms responsible for UTI in Pakistan were *E. coli*, *K. pneumoniae* and *S. aureus*. The majority of isolates were resistant to commonly prescribed antibiotics. Therefore, routine monitoring and surveillance are crucial for the better management of patients.

### Conflict of interest

The authors declare that they have no competing interests.
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