THE PREVALENCE AND ANTIBACTERIAL SUSCEPTIBILITY PATTERN OF UROPATHOGENIC BACTERIA AMONG PEOPLE OF ISLAMABAD

Sehrish Ajmal¹
Iqra Munir¹*\textsuperscript{,}

Muhammad Waseem Ashraf¹

¹University Institute of Medical Lab Technology, Faculty of Allied Health Sciences, The University of Lahore, Lahore, 54000, Pakistan

*Corresponding author: iqra.munir@mlt.uol.edu.pk

Abstract

Bacteria belonging to different genera are responsible for urinary tract infections. Antibacterial agents are routinely administered to treat these urinary tract infections. To counteract the effect of antibiotics, different bacteria develop resistance to these antibiotics. The present study aimed to check the prevalence of uropathogenic bacteria among the studied population. For this purpose, 150 urine samples were collected and were further processed for the isolation and identification of the disease-causing pathogen. The bacteria were identified by using API strips. Furthermore, antibiotic susceptibility testing was also performed for routinely administered antibiotics. From the present study, it was observed that urinary tract infections have affected more females as compared to males. The majority of the UTI patients belonged to the age group between 21 to 30 years. The isolated bacterial species belonged to different genera including \textit{E. coli}, \textit{Klebsiella} \textit{sp.}, \textit{Pseudomonas} \textit{sp.}, \textit{Proteus} \textit{sp.}, \textit{E. fecalis} and \textit{Citrobacter} \textit{sp.} The most prevalent UTI pathogen among the studied group was \textit{E. coli}. The antimicrobial susceptibility testing results have shown that the isolated bacteria from urine samples of patients with urinary tract infections resisted multiple tested antibiotics and were characterized as multi-drug resistant bacteria. From the present study, it has been concluded that \textit{E. coli} is the most commonly found pathogen among the UTI-causing pathogens and antibiotic resistance is rapidly increasing among these pathogenic bacteria. Further study must be carried out in
the clinical setups to evaluate the urinary tract infection complications so that their increasing prevalence can be minimized.

**Keywords:** Uropathogen, Urinary Tract Infection, Antibiotic, Resistance, Pathogen

**I. Introduction**

Urinary tract infections (UTI) are widespread microbial infections affecting individuals of both young and old ages. This infection generally involves one or more components of the urinary system and is mostly affecting the female population with an incidence of about one percent of school-going girls and four percent of females during childbearing age (John *et al.*, 2016). In developing countries, after gastrointestinal tract infections and respiratory tract infections, the 3rd most common infection in children is urinary tract infection. Urinary tract infections are severally occurring bacterial infections, affecting one hundred and fifty million humans every year throughout the world. Urinary tract infections are reported as the 2nd major reason for bacteremia in hospitalized patients, reported as thirty-four percent with nosocomial infection. According to WHO, urinary tract infection is the most epidemic microbial disease in Asia. The bacteria that mostly cause UTIs are also related to nosocomial infections (Barber *et al.*, 2013; Hrbacek *et al.*, 2020).

The urinary tract is usually germ-free; however, bacteria can arise from the perianal region, and cause infection. Symptoms like irritation and increased urination rates are due to the pathogens that stay silent in the bladder and eight percent of women might also have asymptomatic bacteriuria. If microorganism enters the blood circulation, they could cause serious complications, such as septicemia and sudden death (Shakya *et al.*, 2017).

There are two major clinical types of urinary tract infections, uncomplicated and complicated. Uncomplicated type mostly causes infection in those individuals who have no structural or neurological urinary abnormalities and these infections are further divided into lower urinary tract infections (cystitis) and upper urinary tract infections (Flores-Mireles *et al.*, 2015). The causes of uncomplicated UTIs are sexual activities, vaginal illness, diabetes, and genetic predisposition (Rahim *et al.*, 2017). Complicated urinary tract infections are described as factors that compromise the urinary tract, such as urinary blockage, urinary retention, neurological sickness, immune suppression, renal breakdown, multicystic transformation,
pregnancy, and the presence of foreign materials; for example, calculi, indwelling catheters or different drainage devices (Flores-Mireles et al., 2015). The causative agents for complicated infections are *Klebsiella pneumonia*, *Staphylococcus aureus*, *Candida*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* (Flores-Mireles et al., 2015; Al-Khikani, 2020).

Upper urinary tract infection is termed pyelonephritis whereas; lower tract infection refers to cystitis (bladder) so the UTIs are divided into upper urinary tract infections and lower urinary tract infections. Uncomplicated infection in the person having a structural deficiency rather than functional abnormalities of the urinary tract interrupts the usual stream of urine. Complicated urinary tract infection consequently influences the damage of the urinary tract, for example, indwelling catheter, congenital abnormality either distortion of the urinary tract, stone, prostatic hypertrophy, blockage, and neurological deficiency which interfere with the routine urine stream. Hematuria, urgency, incontinence, dysuria, nocturia, supra-pubic pain, and frequent urination are the symptoms of urinary tract infections (Chooramani et al., 2018). Some symptoms are also related to the inflammatory response due to microorganism attack and can lead to the severity of the disease including bacteremia or sepsis (Shakya et al., 2017). Medical manifestations of urinary tract infections can lead to severe morbidity and mortality (Sabir et al., 2014). Occurrence and expansion of disease-causing bacteria somewhere in urinary tracts such as kidneys, ureters, bladder, and urethra are the reason for urinary tract infections. Fifty to sixty percent of females in their life span have an incidence of infection related to the urinary system (Dereje et al., 2017).

Mainly, Gram-positive or Gram-negative bacteria are the causative agents of urinary tract infections, sometimes fungi are also the cause of UTIs. The disease-causing agent for both uncomplicated and complicated urinary tract infections is uropathogenic *Escherichia coli*. However, the most common pathogen causing uncomplicated UTIs includes *Enterococcus faecalis*, *Klebsiella*, *Staphylococcus*, group B *Streptococcus*, *Candida* species, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida* species (Shakya et al., 2017). *Escherichia coli*, a facultative anaerobe, and Gram-negative *Bacillus* have been stated as the most common microorganism among UTI pathogens, the second most disease-causing bacteria is *Klebsiella pneumoniae* and then the other bacterial species belonging to the genus *Staphylococcus*, *Proteus*, *Pseudomonas*, *Enterococcus*, and *Enterobacter* (Mirsoleymani et al.,
2014; Petca et al., 2020). Critical illnesses caused by some strains of E. coli worldwide include urinary tract infections, bloodstream infections, skin infections, otitis media, and diarrhea (Tuem et al., 2018).

Antibacterial drugs are typically used for the control of bacterial infections all over the world. For the improvement of human and animal health, antibacterial drugs are used for more than fifty years. The main origin of antibiotics is microbes including Actinomycetes, Streptomyces, Bacilli, and Penicillium. Globally, the invention of antibiotics and antibacterial agents has changed the treatment method for infectious bacterial diseases that have killed millions of people in the pre-antibiotic era (Bbosa et al., 2014).

Resistance to antibiotics is the ability of bacterial strains to defend themselves against the antibiotics, it occurs due to the changes in the genetics of bacteria at a level that eliminates or decreases the efficiency of chemicals, drugs, or other agents that are used for the treatment of infections. Antibiotics resistance helps in the survival and continuous reproduction of bacteria to destroy the human body (Tang et al., 2016; Behzadi et al., 2020). Bacterial resistance to drugs is mediated by the inactivation of commonly used drugs, β-lactamase drugs; for example, penicillin, cephalosporin etc. Modified drug targets in bacteria also initiate antibiotic resistance such as a mutation in penicillin-binding proteins, the mutation in protein in 30S ribosomal subunit, the mutation in DNA gyrase, and mutation in RNA polymerase. Reduction in the permeability of drugs and export of drugs from bacteria also mediate antibiotic resistance (Yasmeen et al., 2015). The objective of this study was to determine the prevalence of uropathogenic bacteria among people in Islamabad and the determination of antibiotic resistance among the pathogens isolated bacteria from urinary tract infections (UTIs).

II. Materials and methods

Study Duration and Sampling

The duration of the study was from October 2018 to December 2018. The sample size was 150 patients. Urine samples were collected from the patients having clinical symptoms of urinary tract infection in a diagnostic center in Islamabad, Pakistan. In an appropriate sterile wide-mouth container, the mid-stream urine samples were collected. The collected samples were preceded immediately for further testing. The pathogenic organisms from the samples were
identified and characterized morphologically and biochemically. For bacterial identification, API 20E strips were used.

**Antimicrobial Susceptibility Testing**

For the determination of the antibacterial susceptibility of the identified bacteria, the disk diffusion method was used. For this purpose, Muller-Hinton agar plates were prepared and inoculated with standardized bacterial suspension. Selected antibiotic discs were applied aseptically onto the inoculated plates. The susceptibility pattern of tested antibiotics was read after 24 hours of incubation at 37°C. The inhibition zone of bacteria was measured after the completion of the incubation period and was compared with Clinical and Laboratory Standards Institute (CLSI, 2017) guidelines. The isolated bacteria were subjected to susceptibility test for amikacin (30µg), amoxicillin/clavulanate (10µg), aztreonam (30µg), ceftriaxone (30µg), ciprofloxacin (10µg), clarithromycin (5µg), erythromycin (15µg), imipenem/cilastatin (10µg), meropenem (10µg), nitrofurantoin (100µg), ofloxacin (10µg), co-trimazazole (10µg), amoxicillin (10µg), ampicillin (10µg), cefoperazone (75µg), cefotaxime (30µg), cefotaxime (30µg), doxycycline (30µg), gentamicin (30µg), levoflaxacin (1µg), minocycline (30µg), norfloxacin (10µg), pipemidic acid (20µg) and cefoperazone+Sulbactum (30µg).

**III. Results**

A total of 150 urine samples were collected from patients showing symptoms of urinary tract infections. Among the total samples, 62% (n=93) were from female patients and 38% (n=57) were collected from male patients. Samples were classified according to age groups to determine which age group is more affected by UTIs. Most of the cases were from the age group 3 which was 20.66% and the number of patients was 3, other age groups were as follows group 4 having 19.33% (n=29), group 1 having 18.66% (n=28), group 5 having 17.33 (n=26), group 2 having 12.66% (n=19), group 6 had 7.33% (n=11) however a smaller number of patients were found in the age group 7 having 4% (n= 6).

In this study, 6 different bacteria were isolated from collected urine samples. The frequency of bacteria causing urinary tract infections was calculated. From the results of biochemical tests, it was observed that around 77.30% of isolated pathogens were identified as *E. coli*. The percentages of other bacteria identified in the collected samples were *Klebsiella* 16%, *Pseudomonas* 4%, *Proteus* 1.33%, *E. fecalis* 0.66%, and *Citrobacter* 0.66%, respectively. It was
observed that among the collected samples, the highest number of infections were due to *E. coli* whereas, the least number of infections were observed for *E. fecalis* and *Citrobacter* species (Figure 1).

![Percentage of isolated organisms](image)

**Figure 1:** Percentage of isolated bacteria from urine samples.

When the antimicrobial susceptibility pattern was checked on Muller- Hinton agar, it was found that *E. coli* showed the highest resistance to Clarithromycin (100%), Ampicillin (98%), Amoxicillin (97.18%), Co-Trimaxazole (95.65%), Amikacin (93.57%), Cefuroxime (91.89%), Pipemidic Acid (91%) and showed least resistant against Fosfomycin (0%), Imipenem (6.42%), Cefoperazone/Sulbactam (8.10%), Nitrofuration (9.09%), Meropenam (10.61%).

When different antibiotics were tested against *Klebsiella*, it was observed that *Klebsiella* showed resistance against antibiotics drugs such as Amoxicillin (100%), Amoxicilin (83.3%), Ampicillin (100%), Aztronam (100%), Co-Trimaxazole (100%), Cefuroxime (95.4%), Cefixime (95%), Cefotaxime (91.3%), Ceftazidime (85.7%), Naladixic acid (76.12%), Norfloxacin (71.4%) and was found least resistant against Chloramphenical (5.88%), Colistan sulphate (6.25%), Cefoperazone/Sulbactam (36.8%), Meropenam (34.7%) and Imipenem (21.7%).
However, *Klebsiella* sp. showed 100% sensitivity to Minocyclin, Nitrofurantoin, Fosfomycin, and Polymyxin.

Among generally used antibiotics for *Pseudomonas*, it was observed that *Pseudomonas* showed 100% resistivity for Amoxicillin, Ofloxacin, Cefixime, Cefuroxime, and other antibiotics, followed by Amoxicillin (83.3%), Cefotaxime (80%), Pipemidic acid (80%), Norfloxacin (66.6%), Ceftazidime (60%), Naladixic acid (60%), Co-Trimoxazole (60%), Meropenem (60%), Amikacin (40%), Imipenem (40), Ciprofloxacin (33.3%), Colistin sulfate (33.3%), Polymyxin (33.3%), and Chloramphenicol (25%).

The *Proteus* sp. showed 100% resistivity against Cefotaxime, Polymyxin, Chloramphenicol whereas, 50% resistivity was against the Amikacin, Amoxicillin, Ciprofloxacin, and Naladixic acid. *Proteus* showed 100% sensitivity for Meropenem, Norfloxacin, Pipemidic acid, Co-Trimoxazole, Sulbactam, Cefixime, Cefuroxime, and Ceftazidime.

*Citrobacter* showed 100% resistivity to Amikacin, Amoxicillin, Cefixime, Ciprofloxacin, Cefotaxime, Imipenem, Norfloxacin, Cefixime, Naladixic acid, Cefuroxime, and Ceftazidime whereas, complete sensitivity was observed when Co-Trimoxazole, Colistin sulfate, Polymyxin antibiotics were tested.

### IV. Discussion

UTI is a general medical problem in healthcare-associated settings and is responsible for large morbidity and mortality (Ali *et al.*, 2017). The 2nd widespread infections are urinary tract infection in society and clinical practice. All over the world, fifty million people are diagnosed with urinary tract infections per year (Polse *et al.*, 2016). A large number of enteropathogenic organisms have been identified that are responsible for urinary tract infections (Dereje *et al.*, 2017). Generally, UTIs are bacterial infections that damage the kidneys, bladder, and urethra, and mainly it is caused by bacteria belonging to different genera. In some cases, the lower urinary tract infection can damage the kidneys and cause acute pyelonephritis which subsequently results in bacteremia and sepsis (Abduzaimovic *et al.*, 2016).

In the present study, it was observed that females (62%) were more affected by urinary tract infections as compared to males (38%). Similar results have been reported by previous
studies and it has been found that females have the highest occurrence of urinary tract infections, which is frequently attributed due to the smaller urethra that facilitates organisms to colonize easily. On the other hand, the presence of antibacterial agents in the prostate fluid may contribute to the low frequency of urinary tract infections in men (Dereje et al., 2017; Shakya et al., 2017). According to gender, female cases (64.5%) of urinary tract infections have been reported to be more prevalent as compared to males (Chowdhury and Parial, 2015).

When the prevalence of infection among different age groups was studied, it was observed that the majority of the patients having urinary tract infection belonged to the age group of 21-30 and 61-70 years. Furthermore, the previous studies showed that females mostly suffer from UTIs especially at reproductive age while males were mainly affected at older ages (Asaduzzaman et al., 2018). In another study, it has been reported that the middle-aged population was mostly affected by urinary tract infections (Alam et al., 2017).

The present study revealed that *E.coli* was the predominant causative agent for UTIs as it caused infection in 73.30% of individuals. Similarly in other studies, it has been identified; that *E. coli* (83.8%) was the most common organism responsible for this disease (Abduzaimovic et al., 2016). However, 53.7% of cases of *E. coli* infections have been reported in patients suffering from urinary tract infections (Dereje et al., 2017). A recent study showed that the most commonly isolated bacteria causing urinary tract infection was *E. coli* (Chowdhury and Parial, 2015).

In the present study, the most frequently found bacterial isolates after *E. coli* were *Klebsiella* (16%), *Pseudomonas* (4%), *Proteus* (1.33%), and *E. fæcalis* (0.66%) and *Citrobacter* (0.66%), respectively. However, Asaduzzaman et al., have reported that the second most widespread organism after *E. coli* was *Klebsiella* followed by *Staphylococcus aureus, Pseudomonas, and Enterococcus* spp., *Proteus*, and *Enterobacter* (Asaduzzaman et al., 2018).

Antibacterial resistance is the main health issue in public health and patient care and it is a major problem especially in developing countries (Shakya et al., 2017). Patterns of antimicrobial resistance in micro-organisms fluctuate equally in time and space (Ali et al., 2016). Population and health professionals require knowledge regarding the importance for the accurate
use of antibiotics, and it is necessary to get into account the result of antibiotics susceptibility tests (Alam et al., 2017).

In the present study *E. coli* was found to be more resistant to clarithromycin, ampicillin, amoxicillin, co-trimaxazole, amikacin, and least resistant to imipenem, cefoperazone/sulbactam, nitrofuration, and meropenam. Similarly, Shakya et al. has also reported that the isolated *E. coli* strain from urinary tract infections when subjected to antibiotic susceptibility testing, showed resistance to amoxicillin and was found sensitive to imipenem (Sabir et al., 2014).

In the present study, the second most commonly isolated bacteria were *Klebsiella* which was highly resistant to amoxicillin, ampicillin, aztronam, co-trimaxazole, and least resistant to chloramphenical, colistan sulfate, cefoperazone/sulbactam, meropenam, and imipenem/cilastatin. Likewise, from previous studies, it was found that *Klebsiella* showed a high resistivity pattern against amoxicillin (Shakya et al., 2017).

Among the isolated bacteria, the 3rd most prevalent bacteria were *Pseudomonas*, which showed 100% susceptibility for amoxicillin, ofloxacin, cefixime, cefuroxime were showed 100% resistance to imipenem and other tested antibiotics. In contrast to this study, Yasmeen et al. reported that ciprofloxacin effectively inhibited the growth of fifty percent isolated *Pseudomonas* strains (Yasmeen et al., 2015). On the other hand, *Proteus* sp. showed 100% resistivity against cefotaxime, polymaxin, and chloramphenical whereas, the least resistance was observed for cefotaxime, meropenam, norfloxacin, pipemidic acid, co-trimaxazole, cefixime, and cefuroxime. However, Kahlmeter reported that *Proteus* was found least resistant to ampicillin (Goldstein and Group, 2000).

From the present study, it has been concluded that *E. coli* was the most commonly isolated bacteria and the most common cause of urinary tract infections among the studied population. Mostly, the urinary tract infection was reported in females and the infectious bacterial isolates showed multi-drug resistance against the tested antibiotics that are commonly used to treat urinary tract infections.
References


