Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i6.6394

# ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF ISOLATED MICROBES CAUSING URINARY TRACT INFECTIONS (UTIS) AMONG PATIENTS AT HEALTH FACILITIES IN GUJRANWALA DIVISION, PAKISTAN

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**Abstract:**

**Background:** An estimated 150 million UTIs occur annually across the globe, making it one of the most prevalent diseases seen in medical practice and a significant public health concern in terms of morbidity and financial cost. It is also main cause of illness and mortality in the underdeveloped countries like Pakistan. Prevalence of UTIs has been reported in both gender men (30%) and most common among the female (70%) with a peak incidence in the old age (>45). The most common pathogen of UTIs, whether acquired in the community or in a hospital, is *Escherichia coli*. Usually, antibiotics are drug of choice for patients with UTIs Possible development of MDR due to the excessive use of broad-spectrum agents is main reason behind less effectiveness of antibiotics.

**Aim:** Study is aimed to compare MDR and sensitivity patterns of antimicrobials against uropathogens

**Objectives:** To assess the prevalence of UTIs and causative microbial agents among suspected UTI patients.

**Method:** A cross-sectional descriptive study conducted. Inclusion criteria was based on CLSI and CAP standards; This is hospital & clinical LAB based study, conducted in Saddique Family Hospital, Chughtai lab & Islamabad diagnostic center to analyse the existing data from urine

samples to check the microbial resistance and antibiotics from January 2024 to March 2024. For microbial identification high-tech mass spectrometry (MALDI-ToF) on the latest, state of the Art, VITEK-2 MS was performed. Kirby-Bauer Disk Diffusion was used for the antibiotic susceptibility identification. Micro broth dilution was used for testing MIC susceptibility. All the data was collected from microbiology department and analysed by GRAPHPAD 10.2.3.

**Results:** 56 (90%) out of 62 urine sample found positive for urinary pathogens. *Escherichia coli* (37.09%), *Klebsiella spp.* (14.5%), *Enterobacter spp.* (11.29%), *Canidada spp.* (8.06%), *Staphylococcus aureus* (6.45%), *streptococcus agallactiae* (6.45%), *Proteus mirabillis* (3.22%), *Morganella morganii* (1.61%) *and Pseudomonas aeruginosa* (1.61%) *were isol*ated from urine culture samples.

**Conclusion:** Most frequent causative microbes in UTIs are *E.coli* followed by *Klebsiella spp.*, *Enterobacter spp.* Continuous monitoring is highly recommended to cope with MDR and selection of microbial therapy according to susceptibility pattern of microbes.

**Keywords:** *Urinary tract infection (UTIs), MDR,* Escherichia coli, Klebsiella, *uropathogens, broad-spectrum antibiotics*

## Introduction:

Urinary tract infection (UTI) can be defined as "A condition in which bacteria invade and grow in the urinary tract (kidney, bladder, uterus and urethra)." Stated by WHO, UTI is common in all age groups but it more prevalent in females as compared to males (1).

UTIs are among the most common bacterial diseases in the world. The kidneys, ureters, bladder, and urethra make up the urinary system, which filters blood by eliminating waste materials and extra water. One important mechanism in the elimination of metabolic waste products from the bloodstream is the urinary system. Among the extra-intestinal bacterial illnesses that occur most frequently are (UTIs) (2).These might arise from the entry of disease-causing bacteria into the urinary tract through the skin or rectum. The bacteria then proceed up the urethra and to the bladder, where it causes an infection known as cystitis (Reynard et al., 2019).UTIs are divided into two categories: lower (limited to the bladder) and upper (pyelonephritis), as well as simple or complex. A simple UTI is one that affects a healthy host without any anatomical or functional problems. All other types of UTIs are thought to be complex (Foxman, 2010).UTIs classified as complicated like urinary obstruction, neurological disease-related urine retention, immunosuppression, renal failure, renal transplantation, pregnancy, and the presence of foreign objects like calculi, indwelling catheters, or other drainage devices, linked to conditions that impair the urinary system or the immune system of the host

The prevalence of UTI has been reported in all age groups, from newborns to the elderly, and is frequently encountered in medical practice (Foxman, 2002).Females are more prone to UTIs (Minardi *et al.,* 2011). Un-treated UTIs result in different complications such as recurrent infections, pyelonephritis with sepsis (Kalal & Nagaraj, 2016). Recurrent UTIs (rUTIs) affect 20–30% of women with acute cystitis (3). It is hypothesized that bladder remodelling due to overreaction to the original infection may result in repeated UTIs (4). Factors like bacterial colonization and decreased therapeutic efficacy impact UTIs (5). *E. coli and Pseudomonas spp, Proteus spp, klebsiella spp*. are the major cause of UTI (6). Currently many antibiotics which have been commonly used without the urine culture test in UTIs resulted in antibiotics resistance. (Waller et al., 2018). Clinicians must have complete knowledge of the susceptibility pattern of the uropathogens to prevent the antimicrobial resistance (Ahmed et al., 2019) during UTIs treatment, which in turn improve Patient's suffering from ailment (Nerurkar *et al.,* 2012). Current study was designed to check patients with MDR towards different antibiotics and susceptibility pattern of microbes in UTI. Urine culture reports of patients were collected, observed and data was recoded analysed to assess above.

**Statement of Problem:** Kahlmeter (7) found no correlation between antibiotic resistance and the use of the same or similar drugs. While according to (8) when antibiotics are used inappropriately, it can result in insufficient therapy and exacerbate drug resistance. The improper use of antibiotics in low-income nations may be attributable to a combination of factors, including a lack of education about the drugs and treatment guidelines (9) or the fact that antibiotics are easily available over-the-counter and may have been prescribed by unskilled GPs (10).

**Objectives of the Study**

**Broad Objective**

To determine (UTIs) caused by *E coli* in the Gujranwala Division, Pakistan, and their susceptibility to various antibiotics

**Specific Objectives**

- Purpose of this study is to identify the microbes causing (UTIs) in urine samples collected from male & female patients visiting (OPDs) at various hospitals in Gujranwala Division.
- To find out which common bacteria and other microorganisms are resistant to antibiotics by analysing urine samples from trial participants
- To evaluate the efficacy of various antibiotics against various bacterial strains found in OPDs

**Methodology:**

**Study design:**

Based on Clinical&Laboratory standards institute (CLSI) guidelines and College of American Pathologists (CAP) standards (Humphries et al., 2021). A cross sectional descriptive study including systemic sampling. To execute, Clinical data, Urine culture samples were tested at City Lab, Chughtai lab Wazirabad & Islamabad diagnostic centre of patients suspected of UTIs to assess microbial resistance and antibiotics sensitivity to specific urinary pathogens from January to March 2024. Subject selection for study was based on patients who contacted hospital OPDs for UTI complaints and written and verbal consent was taken from them about data collection from their urine culture report. General Physicians from DHQ Gujranwala, THQ Wazirabad and Saddique Family Hospital referred patients to above labs for urine culture reports. Urine sample collection was done after prior approval from 62 patients who want to participate in study. Patients who were able to urinate were treated using a clean-catch midstream technique. In the meantime, sterile bladder samples were taken during intermittent catheterization or, in the case of patients unable to void their bladders voluntarily, from indwelling catheters. Cultured and isolated microbes were identified at the authorized clinical labs of City Lab, Chughtai lab Wazirabad & Islamabad diagnostic centre from all specimens were. Urine sample data for culture was collected from Lab reports and analysed on GRAPHPAD version 10.2.3. Data was segregated on the basis of age, gender, microbial sensitivity and antibiotics resistance.

**Culture and identification:** Identification of microbes was done via collection of microbial growth from urine sample in maintained sterile conditions. MacConkey agar and blood agar are used when the urine samples were plated on Cystine Lactose Electrolyte-Deficient (CLED) medium, using calibrated wire loops (0.001 ml) and then incubated aerobically at 37 °C for 24 h. High-tech mass spectrometry (MALDI-ToF) on the latest, state of the Art, VITEK-2 MS was performed. Samples with more than $10^5$ CFU/mL colony count were considered positive.

**Antibiotic susceptibility testing:** Kirby-Bauer Disk Diffusion was used to determine the Antibiotic susceptibility. E-test/Micro broth dilution/Colistin Broth Disk Elution was used for testing the minimum inhibitory concentration ug/ml (MIC) susceptibility.

**Result:** Demographic distribution of patients (*n*=62) alongwth age&gender was correlated with different isolated microbes. 56 (90%) cultures among total were found to be positive for uropathogens. Gender & age wise data of UTI patients tabulated in Table:1. Isolated microbes included *Escherichia coli* (41.07%), *Klebsiella spp*. (16.07%), *Enterobacter spp.* (12.50%),

*Canidada spp*. (8.93%), *Staphylococcus aureus& Streptococcus agallactiae* each was (7.14%), *Proteus mirabillis* (3.57%), *Morganella morganii* (1.79%) and *Pseudomonas aeruginosa* (1.79%).

**Table: 1 Prevalence of UTI among participant patients**

| Age | Male | Female | Total |
|---|---|---|---|
| | *n* | | |
| 19-29 | 5 | 12 | 17 |
| 30-39 | 6 | 13 | 18 |
| 40-49 | 4 | 2 | 6 |
| >49 | 5 | 16 | 21 |
| **Mean&SD** | 6.3±3.2 | 11±6.1 | 16±6.6 |

Among total 56 culture samples of patients with positive UTIs, *E.Coli* found to be most prevalent among male & females (1.3±0.58) and (6.3±0.21) respectively. Other microbes which were detected in culture samples of both genders and in all selected age groups were compared with one-way ANOVA with a significant (*p=0.0214*) (Table:2).

**Table:2. Number of Bacteria detected in culture isolates of both male&females**

| **Age (yrs.)** | ***(E. Coli)*** | | ***(S areus)*** | | ***(Klebsiella spp)*** | | **F** | ***P-value*** |
|---|---|---|---|---|---|---|---|---|
| | Male | Female | Male | Female | Male | Female | | |
| **19-29** | 1 | 4 | 2 | 1 | 1 | 2 | | |
| **30-39** | 1 | 8 | | | 2 | 2 | 6.4 | 0.0214 |
| **> 49** | 2 | 7 | | | | | | |
| **Mean± SD** | **1.3±0.58** | **6.3±2.1** | **2** | **1** | **1.5±0.71** | **2** | | |

Presence of causative microbes in urine culture reports of only females (30.36%) and only males (8.92%) mentioned in Table:3(a, b) respectively.

**Table:3a. Bacteria detected in culture isolates of females**

| | **Females** | | | | |
|---|---|---|---|---|---|
| **Bacteria found in isolates** | **19-29** | **30-39** | **40-49** | **>49** | **Total** |
| *Staphylococcus auerus* | | | | 1 | 1 |
| *Canidada spp.* | | 1 | 4 | | 5 |
| *Morganella morganii* | | 1 | | | 1 |
| *Enterobactor Spp.* | 3 | 2 | | | 5 |
| *Pseudomonas aeruginosa* | | | | 1 | 1 |
| *Proteus mirabilis* | | | 1 | 1 | 2 |
| *Streptococcus agalactiae* | | | | 2 | 2 |
| *n=17* | | | | | |

**Table:3b. Bacteria detected in culture isolates of males**

| **Bacteria found in isolates** | **Males** | | | |
|---|---|---|---|---|
| | 19-29 | 30-39 | 40-49 | >49 |
| *Staphylococcus auerus* | | | | |
| *Canidada spp.* | | | | |
| *Morganella morganii* | | | | |
| *Enterobactor Spp.* | | | 1 | 1 |
| *Pseudomonas aeruginosa* | | | | |
| *klebsiella sp.* | | | | 1 |
| *Proteus mirabilis* | | | | |
| *Streptococcus agalactiae* | 1 | | 1 | |
| *n*=5 | | | | |

Different culture tests were performed to check resistance and sensitivity of antibiotics and their relative class. Among all bacteria detected in urine culture reports, *E.coli* was mostly found resistant (8±6.1) in different class of antibiotics including Ampicillin, Oxacillin/methicillin, Amoxicillin/Calvulanic acid and cefradine while it was found sensitive (5.2±7.6) in Oxacillin/methicillin, Doxycycline, Minocycline, Tazobactam, Amphotericin B, Imipenem, Meropenem, Ceftizoxime, Chloramphenicol. Other than this, *E.coli* was found both resistant and sensitive to similar antibiotic in different urine culture reports of different patients. *Enterobacter Spp.klebsiella sp. S Areus* was also found resistant, sensitive and both to different antibiotics tested for urine culture sample of different patients. One-way ANOVA and one sample and Wilcoxon test were used to check significant differences between resistant, sensitive or both status of bacteria detected in urine culture test of patient (Table 4 a&b).

**Table:4 (a) Comparison of either resistant & sensitive microbes or both detected in urine culture samples of patients**

| **Bacteria detected in urine culture samples of patients** | **Resistant** | **Sensitive** | **Both** | **F** | ***P-Value*** | |
|---|---|---|---|---|---|---|
| ***E.coli*** | 8±6.1 | 5.2±7.6 | 12±6.6 | 4.1 | 0.0237 | Significant |
| ***Enterobacter Spp.*** | 2±1.4 | 1.5±0.81 | 2.8±1.2 | 3.8 | 0.0336 | |
| ***klebsiella sp.*** | 1.5±0.58 | 1.2±0.45 | 3.6±1.7 | 7 | 0.0048 | |
| ***S Areus*** | 4 | 3.5±0.71 | 3.8±0.5 | 0.5 | 0.6339 | Not significant |

**Table:4 (b) Comparison of either resistant & sensitive microbes detected in urine culture samples of patients**

| One sample and Wilcoxon test | | | | |
|---|---|---|---|---|
| ***P value* (two tailed)** | **Resistance** | **Sensitive** | **EXACT OR ESTIMATE** | |
| **Morganella morganii** | 0.0005 | 0.0039 | Exact | Exact |
| | *** | ** | | |
| **P aeruginosa** | 0.0625 | 0.125 | Exact | Exact |
| | ns | Ns | | |
| **P-mirabillis** | 0.0039 | 0.0156 | Exact | Exact |
| | ** | * | | |
| S alagltacea | 0.0039 | 0.25 | Exact | Exact |
| | ** | Ns | | |

MDR of different isolated uropathogens to monotherapy or combination antibiotic therapy explained in Table:6.

**Table: 2 MDR of different isolated uropathogens to monotherapy or combination antibiotic therapy**

| **Antibiotics** | **MDR of microbe isolates s towards antibiotics** | | | | | |
|---|---|---|---|---|---|---|
| | ***E. coli*** | ***Morganella morganii*** | ***Enterobactor Spp.*** | ***P-aeruginosa*** | ***klebsiella spp.*** | ***P-mirabilis*** |
| **penicillins/ combination** | +++ | + | + | + | ++ | + |
| **Carbapenems** | +++ | - | + | - | ++ | - |
| **Monobactams** | +++ | + | - | - | - | - |
| **Cephalosporins** | +++ | + | + | + | ++ | + |
| **Aminoglycosides** | +++ | - | + | - | ++ | - |
| **Tetracycline** | +++ | + | - | - | - | - |
| **Fluroquinolo-nes** | +++ | + | - | + | ++ | + |
| **Fosphomycin/nitrofurantoin** | +++ | - | + | + | ++ | + |
| **Chloromphenicol** | +++ | - | - | - | - | - |

**Discussion:**

Due to the growing development of microbial resistance to conventional antibiotics,antibiotic resist ance is increasingly seen as a global public health concern(11).The rise of resistant microbes is a res ult of the widespread use of antibiotics. In current study highest cases of *E.coli* followed by *Klebseilla* species& *Enterobacter* species which is related to the study done by (12).

Antimicrobial resistance patterns of the pathogens vary widely by region, patient population and type of healthcare facility. Most of the isolates were resistant to multiple antibiotics at our setting. E. coli (41.07%) detected as most common microbe isolated in present study in accordance with previous researches (13). Enterobacter spp. (12.50%), which have been noted as a significant bacterial isolate from women with UTI in pregnancy (14). In current study different isolated microbes were detected other than above like Staphylococcus aureus & Streptococcus agallactiae each was (7.14%), Proteus mirabillis (3.57%), Morganella morganii (1.79%) and Pseudomonas aeruginosa (1.79%). Prevalence of UTI among participant patients was found to be more in females (11± 6.1) as was found in (15) . S Auerus, Morganella morganii, Enterobactor Spp, P aeruginosa, P-mirabilis and S agalactiae were found as causative bacteria among females (30.36%) (16) while Similar microbes were also detected in males (8.92%) including klebsiella sp.(17).It was observed in current study that isolated microbes were ESBL (Extended spectrum β-Lactamase) producer and were likely to be clinically resistant to all penicillins, cephalosporins, aminoglycosides, fluoroquinolones, tetracyclines and co-trimoxazole. E coli, Enterobacter Spp., klebsiella sp. known as a representative G-negative Bacilli indicator for antibiotic resistance surveillance (18) and it was found resistant to multiple antibiotic and classes of antibiotics and this is in accordance to findings of (19). Due to issue of fluoroquinolone-resistant and ESBL producers, E. coli is a growing concern in developed nations, it is significantly more severe in developing nations due to a lack of resources, controls, and surveillance (20)..The pattern of antimicrobial sensitivity of the micro-organisms causing UTI infections vary in their susceptibility to antimicrobials from patient to patient and from time to time. Susceptibility profile suggested that many bacteria are sensitive to meropenem, imipenem, nitrofurantoin and combination antibiotics (Sulbactam& cefperazone), (piperacillin &tazobactum) (21),(22).

MDR was depicted by many uropathogens like *E.coli* 96% against many antibiotics in different urine culture sample. While MDR depicted by Klebsiella spp. (89%), Enterobactor spp. 72%, P-mirabilis, P-aeruginosa and Morganella morganii also showed MDR to different classes of antibiotics.

**Conclusion:**

Microbial sensitivity fluctuates with the passage of time therefore microbes show high resistance against antibiotics. It was observed and concluded that most frequent cause of UTIs was *E.coli* followed by *Klebsiella spp*. Clinicians should prescribed antibiotics post urine culture sampling. Continuous monitoring is necessary for the positive outcomes regarding prevention of the antibiotics resistance and selection of microbial therapy according to their susceptibility pattern.

**Limitations:**

Many patients became resistant to antibiotics due to non-prescription use

**Recommendations:**

- Among the antibiotics, cost effective and safe antibiotics should be prescribed after complete analysis of resistance and sensitivity pattern of antibiotic.
- Proper screening of pathophysiological paraments of patients should be documented in nearby health facility

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