*Bulletin of Biological and Allied Sciences Research*
***ISSN:* 2521-0092**
www.bbasr.org
DOI: https://doi.org/10.54112/bbasr.v2023i1.56
*Bull. Biol. All. Sci. Res., Volume, **8**: 56*

*Original Research Article*

# MOLECULAR ANALYSIS OF AMINOGLYCOSIDES AND B-LACTAMS RESISTANT GENES AMONG URINARY TRACT INFECTIONS

**REHMAN A[1], BASHIR K[1], FAROOQ S[1], KHAN A[1], HASSAN N[1], SHERAZ M[1], NAWAZ K[2], KHAN J[3], KHAN MI[4], ULLAH A*[1]**

[1]*Department of Health and Biological Sciences, Abasyn University, Peshawar, 25000, Khyber Pakhtunkhwa Pakistan*
[2]*Department of Zoology, University of Peshawar, Peshawar 25000, Pakistan*
[3]*Department of Microbiology, Immunology, Infectious diseases, Transplantation and Related Diseases University of Rome Tor Vergata, Italy*
[4]*Department of statistics, Virtual University Peshawar, Peshawar 25000, Pakistan*
**Correspondence author email address: ibrarwazir.qau@gmail.com, aminbiotech7@gmail.com*

*(Received, 11th February 2023, Revised 21th December 2023, Published 22nd December 2023)*

***Abstract*** *Urinary tract infection (UTI) is a multifactorial disease with a range of pathogenic microbes as causative agents including parasites, fungi and bacteria. However, the main causative pathogen in UTIs is bacteria. The UTI is one of the common reasons for nosocomial infection in the community. The current study aimed to determine the prevalence of UTIs followed by investigating the most effective antibiotics and the amplification of β-lactamase (bla-TEM) gene in isolated bacteria. The Present study was conducted in the tertiary care hospitals of various areas in Peshawar, Pakistan Mid-stream urine samples were collected from UTI patients. Inoculation of entire samples on MacConkey and Blood agar followed by urease, catalase test for gram-positive and citrate, and indole test for gram-negative bacteria was done. Culture sensitivity analysis for various antibiotics was done according to CLSI guidelines. DNA extraction and molecular characterization of bla-TEM resistance genes was also performed using specific primers by Polymerase Chain Reaction (PCR) technique. 117/531 (22.03%) samples were positive for UTI. Among these, most of the samples belong to female population 64.9%. In the age-wise distribution, the middle to older age group has the highest prevalence of UTI. 117 isolates separated with E. coli (62.4%) followed by Klebsiella spp (11.1%) and 7.7% of Pseudomonas as the most prevalent urinary tract infectious microbes. Antibiotic susceptibility showed gentamicin and meropenem as the most resistive antibiotics. Meropenem and ciprofloxacin were the most sensitive antibiotics. Molecular characterization showed 69/117 (58.9%) and 61/117 (52.1%) sample amplification for β-lactamase and aminoglycoside-resistant genes, respectively. It is concluded that UTI is a serious multi-pathogenic problem prevailing in District Peshawar. Most of the uropathogens show resistance towards different antibiotics based on the resistance genes detected in them.*

*[Citation: Rehman, A., Bashir, K., Farooq, S., Khan, A., Hassan, N., Sheraz, M., Nawaz, K., Khan, J., Khan, M.I., Ullah, A. (2023). Molecular analysis of aminoglycosides and β-lactams resistant genes among urinary tract infections. Bull. Biol. All. Sci. Res. **8**: 56. doi: https://doi.org/10.54112/bbasr.v2023i1.56]*
**Keywords*:*** UTI; Pathogen; *Klebsiella spp.; Pseudomonas spp*.; bla-TEM; PCR

## Introduction

Urine tract infections (UTIs) are multifactorial diseases as they have a range of pathogenic microbes as their causative agents, including parasites, fungi, and bacteria. However, the main causative pathogen of UTIs is bacteria. The UTI is one of the common reasons for nosocomial infection in the community (Soltani et al., 2014). These bacteria are found in urinary tract tissue extending from the renal cortex to the meatus and organs involved in urine storage and release from the body, such as the kidney, bladder, urethra and ureters (Akobi et al., 2014). Urinary tract is divided into two: the upper urinary tract, including the kidney, also known as pyelonephritis, and the lower urinary tract, including the ureters, bladder and urethra, collectively called cystitis (Nicolle, 2016). In the male population, the prevalence of UTIs is very low because the urethra has less contamination, followed by a strong bactericidal effect due to prostatic secretions and an excellent immunological defense in children of age 3 or below, UTI is also very common because they have no developed specific immunity. Urine collection followed by result interpretation is not very easy at this age, making it difficult to diagnose and treat bacteria (Ullah et al., 2018).

*Klebsiella, Staphylococci, Proteus, Enterobacter, Enterococci* species and *Pseudomonas* are the most

common UTI bacteria found in the human gut, intestine, soil and water. The most common gram-positive bacteria are CONS (coagulase –Negative *Staphylococcus saprophyticus*), accounting for 5-15%, followed by *Klebsiella, Proteus mirabilis,* and *Enterococcus* account for <5% (Woldemariam et al., 2019). The two major routes of UTI bacteria entry into the urinary tract are hematogenous and ascending routes. In cases of UTI in children, bacteria enter through the ascending route. However hematogenous spread is common in the first 12 weeks of infant life (Robinson et al., 2014).

The empirical therapy of suspected UTIs is ultimately started before identifying and diagnosing uropathogens like bacteria and parasites. The best option for complete UTI treatment depends upon the clinical status and age of the patients linked with the predominant uropathogens, and choosing a suitable antibiotic against the isolate is found to be sensitive (Chu and Lowder, 2018). The antibiotic treatment should be started simultaneously after obtaining a proper urine culture. Some antibiotics resistant to E. coli include amoxicillin, trimethoprim, sulfamethoxazole, nitrofurantoin and cephalosporin (Bryce et al., 2016).

## Material and methods

### Study Demographics

The study was carried out in the laboratory of microbiology at different hospitals of district Peshawar including Khyber Teaching Hospital (KTH), Hayatabad Medical Complex (HMC), and Lady Reading Hospital (LRH), in collaboration with Antimicrobial Resistance Lab, Veterinary Sciences Department, Pir Mehr Ali Shah ARID Agriculture (PMAS-ARID) University, Rawalpindi and Abasyn University Peshawar, Khyber Pakhtunkhwa, Pakistan.

### Sample Collection and Processing

A total of 531 patients suffering from UTI, who visited hospital medical and surgical OPD and ICU admitted with UTI infection complaints were included in the study. The urine of the infected individuals was collected in wide mouthed urine container and transferred to the laboratory for further processing for the identification of aerobic microorganisms as used by (Shenoy et al., 2017).

### Urine Sample Collection

According to the mid-stream clean catch tool, urine samples were collected in a sterile tube from (4-5 ml), transported to the laboratory, and stored in a cold box for further processing (Baron et al., 2013).

### Physical and Routine Examination of Urine

The physical characteristics of the urine samples, such as colour, flow, pH, and specific gravity, were recorded. 5 ml of the urine samples were poured into tubes and were centrifuged at 5000 rpm for 5 minutes. The supernatant was discarded, and the remaining was stored. A sample drop was then examined under the microscope through glass slide preparation using high and low-power objective lenses (40x and 100x).

### Urine Culture and Sensitivity Analysis

Each urine sample was inoculated in Blood Agar, MacConkey Agar and Cysteine Lactose Electrolyte Deficient Agar (CLED).

### Urine Sample Culture on CLED

Urine samples were streaked on CLED by a sterilized loop and incubated at 37°C for 24 hours. Plates with less or no growth was incubated for the the next 48 hours. A colony count of 10 cfu/ml in the urine sample was considered positive. Colony morphology, selective media growth, gram staining, and lactose tests were done according to standard microbiology methods. For the drug sensitivity, Muller-Hinton agar was used.

### Bacterial Isolates Identification

Gram staining was performed to identify isolated bacterial species, followed by observing colony morphology and biochemical test confirmation.

### Biochemical Tests for Confirmation Bacteria

For Confirmation of Gram-negative Bacteria, Indole and Citrate tests were performed. Similarly, for confirmation of gram-positive bacteria urine, catalase and DNAse tests were performed.

### Culture Sensitivity Analysis

The urine samples were collected at the microbiology laboratory and were plated on MacConkey and Blood agar plates. These plates were incubated at 37°C for 24 hours. The culture-positive samples were then identified by collecting pure colonies and re-cultured them on different growth media. Morphological characteristics were determined, and different biochemical tests were performed to identify isolates. Bergey's bacteriology manual was used as a reference in identifying species (Ahmad et al., 2013). Antibiotics used for antimicrobial susceptibility testing included for piperacillin/Tazobactam (TZP) 30µg, clotrimazole (CO) 25 µg, ciprofloxacin (CIP) 5 µg, Imipenem (IPM) 10 µg, Amikacin (AK) 30 µg, Amoxicillin clavulanic acid (AMC) 20 µg, Cefoperazone + Sulbactam (SCF) 30 µg, Ampicillin (AMP) 10 µg, Cefepime (FEP) 30 µg, Cefoxitin (FOX) 30 µg, Ceftriaxone (CRO) 30 µg, Linezolid (LZD) 30 µg and Meropenem (MEM) 10 µg.

### DNA Extraction

From 24 hours of bacterial cultures, the phenol-chloroform method extracted total nucleic acid. The 20mg of each sample was thawed, and suspended in 5 ml PBS (pH 7.2). Samples were then centrifuged at 4°C at 10000 rpm for 15 minutes. The resultant pellet was discarded, and the supernatant was transferred to a new sterile micro-centrifuge tube. Centrifugation of supernatant was done again at 10000 rpm for 10 minutes. The resultant pellet was washed with 1.5 ml acetone solution 3 times. The supernatant was discarded, and the purified pellets was used for DNA extraction through TE boil extraction method, according to Li *et al*., (2003). A 200 µl TE buffer was added to the pellet and briefly vortexed. Resultant mixture was incubated for 1 minute at 100°C followed by centrifugation for 5 minutes at 10000 rpm.

Resultant pellet was dissolved in 100µl TE buffer and stored at -20°C until the molecular characterization.

**Molecular Characterization**

Extracted DNA of the Gentamicin and Ampicillin resistant UTI positive isolates were amplified through Polymerase Chain Reaction (PCR) for 2 genes encoding Aminoglycoside and β-lactamase antibiotics, namely, aac (6')-Ib-cr and blaTEM, respectively, as described in table 1. A total of 20$\mu$l PCR mixture was prepared using 4$\mu$l DNA template, 8 $\mu$l master mix, 2$\mu$l of each forward and reverse primers and 4$\mu$l sterile PCR water with the PCR condition as initial denaturation for 5 minutes at 95°C, followed by 35 cycles with denaturation for 45 seconds at 95°C, annealing for 30 seconds at 52°C and 53.5°C (Aminoglycoside and β-lactamase genes respectively) and 45 seconds initial extension at 72°C followed by a final extension at 72°C for 8 minutes. Amplified PCR products were visualized under UV transilluminator.

**Table 1: Primers sequences and amplicon size**

| S. No. | Gene name | Primer sequence | Size | Reference |
|---|---|---|---|---|
| **1** | Aminoglycosides (aac (6')-Ib-cr) | F: 5' TTGCGATGCTCTATGAGTGGCTA3'<br>R: 5' CTCGAATGCCTGGCGTGTTT 3' | 482bp | Eftekhar *et al*., 2015 |
| **2** | β-lactamase (blaTEM) | F: 5' TACGATACGGGAGGGCTTAC3'<br>R: 5' TTCCTGTTTTTGCTCACCCA3' | 716bp | Alabi *et al*., 2017 |

**Results**

A total of 531 samples with UTI symptoms were included in the study. Out of which, 117 (22.03%) showed growth in the culture medium, having E. coli (62.4%) followed by Klebsiella spp. (11.1%) and 7.7% of Pseudomonas. Details of the isolates are shown in table 2.

**Table 2: Frequency of Isolated bacteria in male and female population**

| Bacteria | Positive (%) | Male | Female |
|---|---|---|---|
| ***E. coli*** | 73 (62.4) | 26 (22.2%) | 47 (40.2%) |
| ***Klebsiella spp.*** | 13 (11.1) | 5 (4.3%) | 8 (6.8%) |
| ***Pseudomonas*** | 9 (7.7) | 3 (2.6%) | 6 (5.1%) |
| ***Enterococci*** | 8 (6.8) | 4 (3.4%) | 4 (3.4%) |
| ***Enterobacter*** | 7 (6) | 2 (1.7%) | 5 (4.3%) |
| ***S. aureus*** | 4 (3.4) | 1 (0.8%) | 3 (2.6%) |
| ***S. proteus*** | 3 (2.6) | 0 | 3 (2.6%) |

**Gender Wise Distribution**

Positive samples were divided gender-wise and age wise. Gender-wise distributions show that more samples belong to the female population (64.9%), as shown in table 3 .

**Table 3: Gender wise distribution**

| Gender | Total | Positive |
|---|---|---|
| **Male** | 195 | 41 (35.1%) |
| **Female** | 336 | 76 (64.9%) |

**Age Wise Distribution**

The samples were taken from different age groups divided into 4 groups. The results showed that age group 3 (36-50 years) has the highest number of infected individuals, 53 (45.3%), followed by age group 2 (21-35 years), 34 (29.1%) as shown in Table 4.

**Table 4: Age wise distribution**

| Age groups (years) | Positive |
|---|---|
| **5-20** | 9 (7.7%) |
| **21-35** | 34 (29.1%) |
| **36-50** | 53 (45.3%) |
| **>50 years** | 21 (17.9%) |

**Antibiotic Susceptibility**

All the positive samples were tested for antibiotic susceptibility using various antibiotics. Antibiotic susceptibility shows that *E. coli* was resistant to Gentamicin (83.5%), followed by Ciprofloxacin (79.5%). However, it shows the highest sensitivity against Cefoxitin (71.2%) followed by Cefuroxime (63.1%). *Klebsiella spp*. was the second most prevalent microbe that was sensitive to Amikacin (76.9%) and Ampicillin (69.2%) while showing resistance to Ciprofloxacin (76.9%), Levofloxacin (76.9%) and Amoxicillin (69.2%). The 3rd most prevalent microbe was *Pseudomonas*, showing resistivity against Meropenem and linezolid (77.8%), followed by Cefepime (66.7%), while it showed sensitivity by Ceftriaxone (77.8%) and Ciprofloxacin (66.7%). *Enterococci* was susceptible to ciprofloxacin (87.5%), and Meropenem (75%). This microbe resisted Gentamicin (87.5%) and Amikacin (75%). Meropenem and Amikacin (71.4%) and Ampicillin (57.2%) showed resistance in *Enterobacter*, while Norfloxacin (71.4%) and Ciprofloxacin (57.2%) were sensitive. Piperacillin and Ampicillin (75%) were sensitive, while Gentamicin and Meropenem (75%) were resistant. *S. proteus* showed equal resistance (66.7%) by Imipenem, Linezolid, Levofloxacin and was sensitive to Meropenem and Ciprofloxacin (66.7%). Detail of the antibiotic susceptibility is shown in table 5 below.

**Table 5. Antibiotic susceptibility/resistance in collected isolates**

| Antibiotics | | *E. coli* | *Klebsiella spp.* | *Pseudomonas* | *Enterococcus* | *Enterobacter* | *Staphylococcus aureus* | *S. proteus* |
|---|---|---|---|---|---|---|---|---|
| **Piperacillin /Tazobactam** | S | 24 (32.9%) | 4 (30.8%) | 5 (55.6%) | 3 (37.5%) | 4 (57.2%) | 3 (75%) | 2 (66.7%) |
| | R | 49 (67.1%) | 9 (69.2%) | 4 (44.4%) | 5 (62.5%) | 3 (42.8%) | 1 (25%) | 1 (33.3%) |
| **Gentamycin** | S | 12 (16.5%) | 3 (23.1%) | 4 (44.4%) | 1 (12.5%) | 4 (57.2%) | 1 (25%) | 2 (66.7%) |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | R | 61 (83.5%) | 10 (76.9%) | 5 (55.6%) | 7 (87.5%) | 3 (42.8%) | 3 (75%) | 1 (33.3%) |
| **Meropenem** | S | 17 (23.3%) | 7 (53.8%) | 2 (22.2%) | 6 (75%) | 2 (28.6%) | 1 (25%) | 2 (66.7%) |
| | R | 56 (76.7%) | 6 (46.2%) | 7 (77.8%) | 2 (25%) | 5 (71.4%) | 3 (75%) | 1 (33.3%) |
| **Trimethoprim-sulfamethoxazole** | S | 41 (56.2%) | 5 (38.5%) | 5 (55.6%) | 3 (37.5%) | 5 (71.4%) | 1 (25%) | 2 (66.7%) |
| | R | 32 (43.8%) | 8 (61.5%) | 4 (44.4%) | 5 (62.5%) | 2 (28.6%) | 3 (75%) | 1 (33.3%) |
| **Ciprofloxacin** | S | 15 (20.5%) | 3 (23.1%) | 6 (66.7%) | 1 (12.5%) | 4 (57.2%) | 1 (25%) | 2 (66.7%) |
| | R | 58 (79.5%) | 10 (76.9%) | 3 (33.3%) | 7 (87.5%) | 3 (42.8%) | 3 (75%) | 1 (33.3%) |
| **Vancomycin** | S | 35 (47.9%) | 4 (30.8%) | 3 (33.3%) | 3 (37.5%) | 4 (57.2%) | 2 (50%) | 1 (33.3%) |
| | R | 38 (52.1%) | 9 (69.2%) | 6 (66.7%) | 5 (62.5%) | 3 (42.8%) | 2 (50%) | 2 (66.7%) |
| **Imipenem** | S | 31 (42.5%) | 6 (46.2%) | 4 (44.4%) | 6 (75%) | 4 (57.2%) | 2 (50%) | 1 (33.3%) |
| | R | 42 (57.5%) | 7 (53.8%) | 5 (55.6%) | 2 (25%) | 3 (42.8%) | 2 (50%) | 2 (66.7%) |
| **Norfloxacin** | S | 36 (49.3%) | 5 (38.5%) | 6 (66.7%) | 5 (62.5%) | 5 (71.4%) | 1 (25%) | 1 (33.3%) |
| | R | 37 (50.7%) | 8 (61.5%) | 3 (33.3%) | 3 (37.5%) | 2 (28.6%) | 3 (75%) | 2 (66.7%) |
| **Amikacin** | S | 41 (56.2%) | 10 (76.9%) | 3 (33.3%) | 2 (25%) | 2 (28.6%) | 2 (50%) | 1 (33.3%) |
| | R | 32 (43.8%) | 3 (23.1%) | 6 (66.7%) | 6 (75%) | 5 (71.4%) | 2 (50%) | 2 (66.7%) |
| **Linezolid** | S | 28 (38.4%) | 7 (53.8%) | 2 (22.2%) | 5 (62.5%) | 5 (71.4%) | 2 (50%) | 1 (33.3%) |
| | R | 45 (61.6%) | 6 (46.2%) | 7 (77.8%) | 3 (37.5%) | 2 (28.6%) | 2 (50%) | 2 (66.7%) |
| **Amoxicillin clavulanic acid** | S | 30 (41.1%) | 4 (30.8%) | 3 (33.3%) | 2 (25%) | 3 (42.8%) | 2 (50%) | 1 (33.3%) |
| | R | 43 (58.9%) | 9 (69.2%) | 6 (66.7%) | 6 (75%) | 4 (57.2%) | 2 (50%) | 2 (66.7%) |
| **Cefoperazone+ Sulbactam** | S | 46 (63.1%) | 4 (30.8%) | 6 (66.7%) | 4 (50%) | 4 (57.2%) | 2 (50%) | 1(33.3%) |
| | R | 27 (36.9%) | 9 (69.2%) | 3 (33.3%) | 4 (50%) | 3 (42.8%) | 2 (50%) | 2 (66.7%) |
| **Ampicillin** | S | 39 (53.4%) | 10 (76.9%) | 2 (22.2%) | 2 (25%) | 3 (42.8%) | 3 (75%) | 2 (66.7%) |
| | R | 34 (46.6%) | 3 (23.1%) | 7 (77.8%) | 6 (75%) | 4 (57.2%) | 1 (25%) | 1 (33.3%) |
| **Cefepime** | S | 32 (43.9%) | 9 (69.2%) | 3 (33.3%) | 2 (25%) | 2 (28.6%) | 2 (50%) | 1 (33.3%) |
| | R | 41 (56.1%) | 4 (30.8%) | 6 (66.7%) | 6 (75%) | 5 (71.4%) | 2 (50%) | 2 (66.7%) |
| **Cefoxitin** | S | 52 (71.2%) | 6 (46.2%) | 2 (22.2%) | 4 (50%) | 4 (57.2%) | 1 (25%) | 2 (66.7%) |
| | R | 21 (28.8%) | 7 (53.8%) | 7 (77.8%) | 4 (50%) | 3 (42.8%) | 3 (75%) | 1 (33.3%) |
| **Ceftriaxone** | S | 35 (47.9%) | 5 (38.5%) | 7 (77.8%) | 3 (37.5%) | 3 (42.8%) | 3 (75%) | 1 (33.3%) |
| | R | 38 (52.1%) | 8 (61.5%) | 2 (22.2%) | 5 (62.5%) | 4 (57.2%) | 1 (25%) | 2 (66.7%) |
| **Cefuroxime** | S | 46 (63%) | 4 (30.8%) | 2 (22.2%) | 5 (62.5%) | 2 (28.6%) | 2 (50%) | 1 (33.3%) |
| | R | 27 (37%) | 9 (69.2%) | 7 (77.8%) | 3 (37.5%) | 5 (71.4%) | 2 (50%) | 2 (66.7%) |
| **Levofloxacin** | S | 23 (31.5%) | 3 (23.1%) | 3 (33.3%) | 5 (62.5%) | 3 (42.8%) | 2 (50%) | 1 (33.3%) |
| | R | 50 (68.5%) | 10 (76.9%) | 6 (66.7%) | 3 (37.5%) | 4 (57.2%) | 2 (50%) | 2 (66.7%) |

**Molecular Characterization**

A total of 117 samples were run on the thermocycler to confirm the presence of β-lactamase (blaTEM). Amplification shows that a total of 69/117 (58.9%) and 61/117 (52.1%) samples show band on UV transillumination showing the presence of resistance gene (β-lactamase and Aminoglycoside genes, respectively, in all observed bacterial strains as shown in figures 1 and 2.

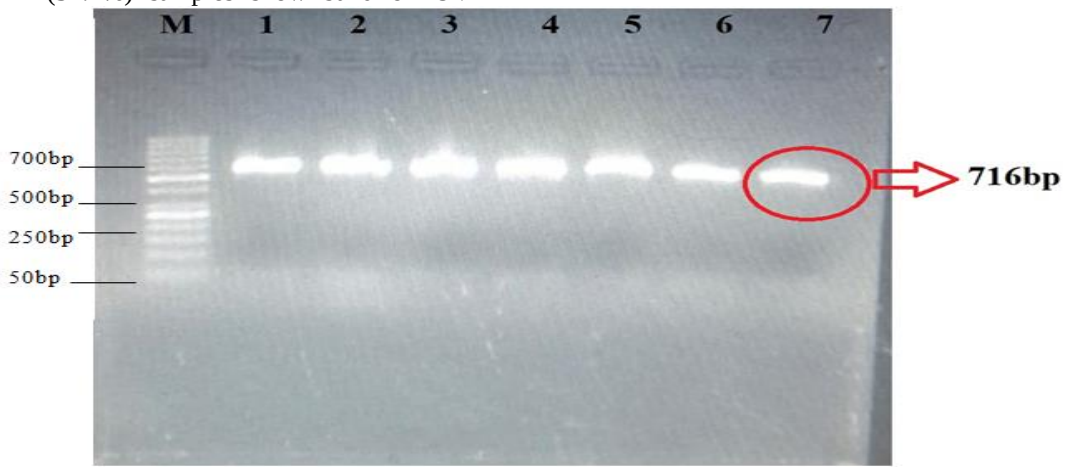


**Figure 1. PCR amplified product of β-lactamase (blaTEM) gene**

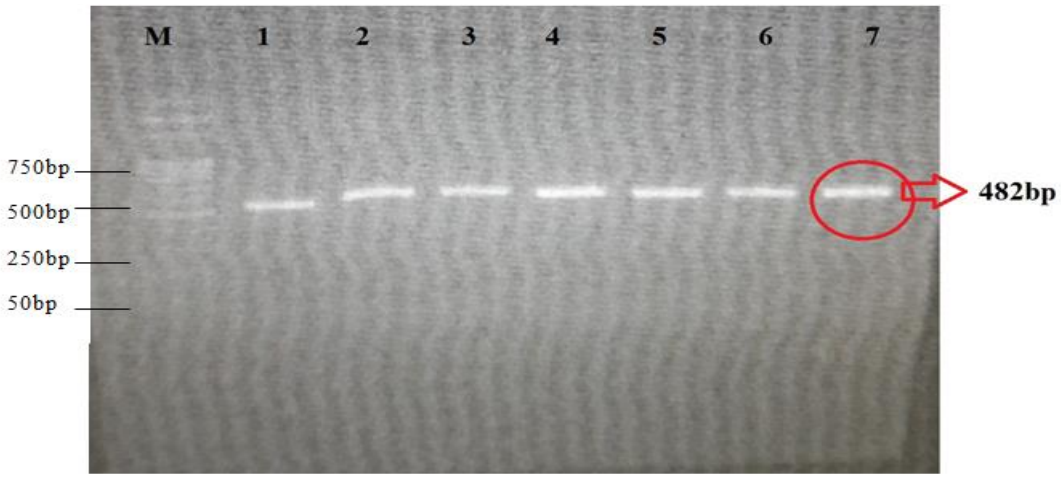


**Figure 2. PCR amplified product of Aminoglycosidase gene**

**Discussion**

Various pathogenic bacteria invade urinary tract tissues and start multiplication inside the Urinary tract system, causing UTI. The UTI prevalence is reported in pediatric healthcare and pregnant women more frequently than in the general population. Patients with a good prognosis have the highest complications and morbidity due to UTI (Ranjan et al., 2017). The UTI varies from asymptomatic to severe infections. In most cases, if population is more infected as they have short urethra than males (Geerlings, 2016). Most of the pathogenic bacteria are non-pathogenic as they are in normal microflora of the human body. But when these bacteria move to the urinary tract, they become pathogenic due to environmental changes and multiply, followed by infection. Some of the bacteria reside in an alkaline environment, and the rest is found in alkaline urine.

The current study found a total prevalence of 22.03% which is in agreement with a study from Pakistan carried out by (Ullah et al., 2018). A lower prevalence rate of 11.6% (Ullah et al., 2018) and a higher prevalence rate of 53.82%, and 49%, respectively were reported by (Prakash and Saxena, 2013; Preeja et al., 2021). The difference in the findings of various reports is due to the infection's differentiation from country to country, state to state, small hospital to large hospital and even hospital to community (Preeja et al., 2021). Zakka *et al*., and Preeja *et al*., found higher UTI prevalence in females compared to males (Zakka et al., 2018)(Preeja et al., 2021). The above findings are similar the current study finding showing that females are more infected than males her prevalence of UTI in females might be due to physiological and anatomical differences in males and female's Urinary systems, which drier in the case of the male urethra, which leads to the prevention of bacterial optimal growth as compared males. Other factors like more distance between the urethra and anus apertures and prostate secretions containing antimicrobial activity among males are responsible for variations in the prevalence of uropathogenic infection among different genders (Ullah et al., 2018).

Age wise distribution shows that age group 3 (36-50 years) is more infected (45.3%) than the other ages. Similar observations are recorded by Shaifali *et al*., Akram *et al*., and Ullah *et al*., in their studies (Akram et al., 2007; Shaifali et al., 2012; Ullah et al., 2018). This difference might be due to the competent immune system and high treatment-seeking behavior in young age (Shaifali et al., 2012).

The current finding showed that gram-negative bacteria (n=105, 89.7%) were frequently involved in UTI as compare to gram positive bacteria (n=12, 10.3%) accordingly observed in the studies of (Derese et al., 2016; Hisano et al., 2015; Melaku et al., 2012; Sibi et al., 2014) respectively. The highest number of gram-negative bacterial isolates is due to their uniqueness in the cell walls which the leads to strong attachment, invasion and multiplication within the epithelial cells of urinary tract system (Lavigne et al., 2011). The current study showed that, *E. coli* (62.4%) and *Klebsiella* (11.1%) isolates were dominating uropathogens likewise reported someone else (Kumar et al., 2017; Lavigne et al., 2011; Marschall et al., 2013; Smaill and Vazquez, 2019). The presence of various virulence factors in E. coli involved in the invasion and colonization of epithelial cells of the urinary tract system, favors the habitat for E. coli inside the urinary tract system (Lavigne et al., 2011).

Difference in antibiotic sensitivity and increasing drug resistance to antimicrobials has already been reported (Kumar et al., 2017). Multidrug resistant (MDR) strains were high in this study, as entire isolates resisted at least two antibiotics. The antibiotic resistance patterns reported in this study are highest for ampicillin (73.32%) followed by gentamicin (70.34%). This finding aligns with the high resistance to gentamicin and ampicillin (Derese et al., 2016; Kumar et al., 2017). Majority of the gram positive isolates including enterococci and S. aureus showed a higher rate of resistance to gentamicin and ciprofloxacin, which are similar to the observation of Kumar *et al*., (Kumar et al., 2017) and opposite to a study of Ahmed *et al*., (Ahmed et al., 2019). This study's high prevalence of MDR isolates might be due

to higher empirical antibiotic treatment. Other factors like wide spread of antibiotic resistance genes among bacterial isolates due to non-availability or inadequate of antibiotic resistance surveillance programs, contravene of standard antibiotic therapy guidelines, miss-use of antibiotics and uncontrolled self-medication are responsible for the evolution of MDR isolates (Gebrekirstos et al., 2017).

Gram negative isolates cause majority of UTI. Aminoglycosides, the broad-spectrum antibiotics, show high potency, and that's why they are traditionally used for the treatment of serious gram-negative infections ((Ahmed et al., 2021). Furthermore, 58.9% of the ESBL phenotypes show blaTEM gene in the current study. Similarly, the presence of the blaTEM gene among UTI isolates was reported worldwide (Manoharan et al., 2011; Pishtiwan and Khadija, 2019). The easy and high spread of these resistance genes among Enterobacteriaceae (especially in *K. pneumoniae* and *E. coli*) might be due to its presence and transfer through mobile genetic elements. Further research on antibiotic resistance genes will guide us about the exact situation of antibiotic resistance genes and will help us in the prevention and dissemination of antibiotic resistance genes by choosing the appropriate antibiotics for the treatment of UTI (Al-Zahrani et al., 2019; Eftekhar and Seyedpour, 2015).

**Conclusion**

The study concludes that UTI is a serious multi-pathogenic problem prevailing in District Peshawar, with most of the resistant bacterial species involved. It is also concluded that most of the uropathogens are resistant to many different antibiotics in use, and the resistance genes were detected in most of them. The problem needs to be explored further, and proper preventive measures and treatment strategies should be developed to cope with increasing UTIs involving such resistant bacterial species.

**References**

Ahmad, B., Nigar, S., Shah, S. S. A., Bashir, S., Ali, J., Yousaf, S., and Bangash, J. A. (2013). Isolation and identification of cellulose degrading bacteria from municipal waste and their screening for potential antimicrobial activity. *World Appl. Sci. J* **27**, 1420-1426.

Ahmed, Suhail, S., Shariq, A., Alsalloom, A. A., Babikir, I. H., and Alhomoud, B. N. (2019). Uropathogens and their antimicrobial resistance patterns: Relationship with urinary tract infections. *International Journal of Health Sciences* **13**, 48.

Ahmed, O. B., Asghar, A. H., Bahwerth, F. S., Assaggaf, H. M., and Bamaga, M. A. (2021). The prevalence of aminoglycoside-resistant genes in Gram-negative bacteria in tertiary hospitals. *Applied Nanoscience*, 1-7.

Akobi, O. A., Inyinbor, H. E., Akobi, E. C., Emumwen, E. G., Ogedengbe, S. O., Uzoigwe, E. O., Agbayani, R. O., Emumwen, E. F., and Okorie, I. E. (2014). Incidence of urinary tract infection among pregnant women attending antenatal clinic at Federal Medical Centre, Bida, Niger-State, North Central Nigeria. *Am J Infect Dis Microbiol* **2**, 34-38.

Akram, M., Shahid, M., and Khan, A. U. (2007). Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India. *Annals of clinical microbiology and antimicrobials* **6**, 1-7.

Al-Zahrani, J., Al Dossari, K., Gabr, A. H., Ahmed, A.-f., Al Shahrani, S. A., and Al-Ghamdi, S. (2019). Antimicrobial resistance patterns of Uropathogens isolated from adult women with acute uncomplicated cystitis. *BMC microbiology* **19**, 1-5.

Baron, E. J., Miller, J. M., Weinstein, M. P., Richter, S. S., Gilligan, P. H., Thomson Jr, R. B., Bourbeau, P., Carroll, K. C., Kehl, S. C., and Dunne, W. M. (2013). A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2013 recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM) a. *Clinical infectious diseases* **57**, e22-e121.

Bryce, A., Hay, A. D., Lane, I. F., Thornton, H. V., Wootton, M., and Costelloe, C. (2016). Global prevalence of antibiotic resistance in paediatric urinary tract infections caused by Escherichia coli and association with routine use of antibiotics in primary care: systematic review and meta-analysis. *bmj* **352**.

Chu, C. M., and Lowder, J. L. (2018). Diagnosis and treatment of urinary tract infections across age groups. *American journal of obstetrics and gynecology* **219**, 40-51.

Derese, B., Kedir, H., Teklemariam, Z., Weldegebreal, F., and Balakrishnan, S. (2016). Bacterial profile of urinary tract infection and antimicrobial susceptibility pattern among pregnant women attending at Antenatal Clinic in Dil Chora Referral Hospital, Dire Dawa, Eastern Ethiopia. *Therapeutics and clinical risk management* **12**, 251.

Eftekhar, F., and Seyedpour, S. M. (2015). Prevalence of qnr and aac (6')-Ib-cr genes in clinical isolates of Klebsiella Pneumoniae from Imam Hussein Hospital in Tehran. *Iranian journal of medical sciences* **40**, 515.

Gebrekirstos, N. H., Workneh, B. D., Gebregiorgis, Y. S., Misgina, K. H., Weldehaweria, N. B., Weldu, M. G., and Belay, H. S. (2017). Non-prescribed antimicrobial use and associated factors among customers in drug retail outlet in Central Zone of Tigray, northern Ethiopia: a cross-sectional study. *Antimicrobial Resistance & Infection Control* **6**, 1-10.

Geerlings, S. E. (2016). Clinical presentations and epidemiology of urinary tract infections. *Microbiology spectrum* **4**, 4-5.

Hisano, M., Bruschini, H., Nicodemo, A. C., Gomes, C. M., Lucon, M., and Srougi, M. (2015). The bacterial spectrum and antimicrobial susceptibility in female recurrent urinary tract infection: how different they are from sporadic single episodes? *Urology* **86**, 492-497.

Kumar, N., Nahid, F., and Zahra, R. (2017). Association of virulence factors, phylogenetic groups and antimicrobial resistance markers in Escherichia coli from Badin city, Pakistan. *Journal of Chemotherapy* **29**, 8-13.

Lavigne, J.-P., Boutet-Dubois, A., Laouini, D., Combescure, C., Bouziges, N., Marès, P., and Sotto, A. (2011). Virulence potential of Escherichia coli strains causing asymptomatic bacteriuria during pregnancy. *Journal of clinical microbiology* **49**, 3950-3953.

Manoharan, A., Premalatha, K., Chatterjee, S., Mathai, D., and Group, S. S. (2011). Correlation of TEM, SHV and CTX-M extended-spectrum beta lactamases among Enterobacteriaceae with their in vitro antimicrobial susceptibility. *Indian journal of medical microbiology* **29**, 161-164.

Marschall, J., Piccirillo, M. L., Foxman, B., Zhang, L., Warren, D. K., and Henderson, J. P. (2013). Patient characteristics but not virulence factors discriminate between asymptomatic and symptomatic E. coli bacteriuria in the hospital. *BMC infectious diseases* **13**, 1-7.

Melaku, S., Kibret, M., Abera, B., and Gebre-Sellassie, S. (2012). Antibiogram of nosocomial urinary tract infections in Felege Hiwot referral hospital, Ethiopia. *African Health Sciences* **12**, 134-139.

Nicolle, L. E. (2016). Urinary tract infections in the older adult. *Clinics in geriatric medicine* **32**, 523-538.

Pishtiwan, A. H., and Khadija, K. M. (2019). Prevalence of blaTEM, blaSHV, and blaCTX-M genes among ESBL-producing Klebsiella pneumoniae and Escherichia coli isolated from thalassemia patients in Erbil, Iraq. *Mediterranean journal of hematology and infectious diseases* **11**.

Prakash, D., and Saxena, R. S. (2013). Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in urban community of meerut city, India. *International scholarly research notices* **2013**.

Preeja, P. P., Kumar, S. H., and Shetty, V. (2021). Prevalence and characterization of methicillin-resistant Staphylococcus aureus from community-and hospital-associated infections: a tertiary Care Center Study. *Antibiotics* **10**, 197.

Ranjan, A., Sridhar, S. T. K., Matta, N., Chokkakula, S., and Ansari, R. K. (2017). Prevalence of UTI among pregnant women and its complications in newborns. *Indian Journal of Pharmacy Practice* **10**, 45-49.

Robinson, J. L., Finlay, J. C., Lang, M. E., Bortolussi, R., Canadian Paediatric, S., Community Paediatrics, C., Infectious, D., and Immunization, C. (2014). Urinary tract infection in infants and children: Diagnosis and management. *Paediatrics & child health* **19**, 315-319.

Shaifali, I., Gupta, U., Mahmood, S. E., and Ahmed, J. (2012). Antibiotic susceptibility patterns of urinary pathogens in female outpatients. *North American journal of medical sciences* **4**, 163.

Shenoy, P. A., Vishwanath, S., Gawda, A., Shetty, S., Anegundi, R., Varma, M., Mukhopadhyay, C., and Chawla, K. (2017). Anaerobic bacteria in clinical specimens–frequent, but a neglected lot: a five year experience at a tertiary care hospital. *Journal of Clinical and Diagnostic Research: JCDR* **11**, DC44.

Sibi, G., Kumari, P., and Kabungulundabungi, N. (2014). Antibiotic sensitivity pattern from pregnant women with urinary tract infection in Bangalore, India. *Asian Pacific Journal of Tropical Medicine* **7**, S116-S120.

Smaill, F. M., and Vazquez, J. C. (2019). Antibiotics for asymptomatic bacteriuria in pregnancy. *Cochrane database of systematic reviews*.

Soltani, R., Ehsanpoor, M., Khorvash, F., and Shokri, D. (2014). Antimicrobial susceptibility pattern of extended-spectrum β-lactamase-producing bacteria causing nosocomial urinary tract infections in an Iranian referral teaching hospital. *Journal of research in pharmacy practice* **3**, 6.

Ullah, A., Shah, S. R. H., Almugadam, B. S., and Sadiqui, S. (2018). Prevalence of symptomatic urinary tract infections and antimicrobial susceptibility patterns of isolated uropathogens in kohat region of Pakistan. *MOJ Biol Med* **3**, 85-89.

Woldemariam, H. K., Geleta, D. A., Tulu, K. D., Aber, N. A., Legese, M. H., Fenta, G. M., and Ali, I. (2019). Common uropathogens and their antibiotic susceptibility pattern among diabetic patients. *BMC infectious diseases* **19**, 1-10.

Zakka, S., Oluwatosin, O. C., Cosmas, E. U., John, S., Rebecca, E. F., and Isa, B. E. (2018). Prevalence of urinary tract infection in HIV patients on antiretroviral drugs in Jos Metropolis, Nigeria. *World* **3**, 57-60.

**Declarations**

**Acknowledgments**

Not applicable

**Funding**

Not applicable

**Author's contributions**

AR Conducted research and wrote the manuscript. KB, SF, AK, NH, MS, KN, JK, MIK and AU edit the manuscript in original. All authors have read and approved the final manuscript.

**Ethics approval and consent to participate**

Not applicable

**Consent for Publication**

Not applicable

**Competing interests**

The authors declare that they have no competing interests.