OPEN ACCESS

GRS Journal of Multidisciplinary Research and Studies
*Abbreviate Tittle- GRS J Mul Res Stud*
ISSN *(Online) - 3049-0561*
https://grspublisher.com/journal-details/GRSJMRS

Vol-2, Iss-5 (May- 2025)

# Antibiotic resistance among some bacterial isolated from patients with urinary tract infection in south of Baghdad

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**Received: 28.04.2025** **Accepted: 17.05.2025** **Published: 25.05.2025**

## Abstract:

**Background:** Antibiotic resistance (ABR) is one of the main series global public health threats in the world. Its emergency among bacterial pathogens for local understand for the epidemiological situation. We needed information about that by gather both for clinical treatment decision-making purposes in addition to for the revision of present care guidelines.

**Objective:** Our study was aimed to identify the local bacterial Uropathogens in south of Baghdad that appear antimicrobial resistance situation for major antibiotic in group of patients have history with Urinary tract infections (UTIs)

**Methodology:** From February 2022 to October 2023, 51 patients which referred from hospital in south of Baghdad, Iraq with history of UTI. Five species of bacteria was isolated from samples which defined after culturing by microscopic, biochemical tests.

**Results:** the study shown high significant differences at (P≤0.01) in bacterial infection in female compared with male, communal bacteria that reason UTI *Escherichia coli (E.coli)* appear high rates in female at 50%, while in male about 30.7%, as well as, *staphylococcus aureus* showed high rates after *E.coli* then other bacteria like *Enterococcus sp*., *Klebsiella sp*. *E.coli* showed high resistance against many antibiotic like Amikacin (AK), Amoxicillin-clavulanate (AUG), Azithromycin (AM), Cefotaxime (CTX), ceftriaxone (CRO) and other Beta-lactamase antibiotics, Gentamycin (CN), Levofloxacin (LEV), Tetracycline (TE) and Doxycycline (DXT). These isolates showed sensitive against Imipenem. Other results appeared most of bacteria like *Klebsiella, Staphylococcus aureus* and *Pseudomonas sp*. have resistance against Azithromycin and Gentamycin.

**Conclusion**: Many studies performed to a dangerous about use antibiotic without any warnings that make most of UTIs pathogenic bacteria resistance especially *E. coli* for wide range of antibiotics that make us thinking thousands of times before using antibiotics without consult a doctor.

**Keywords:** *UTIs, Antibiotic resistance, E. coli, other pathogenic bacteria*.

## Introduction

Antibiotic resistance (ABR) is defined as a microorganism's resistance to the effects of drugs, meaning that it can resist exposure to antibiotics that would normally be able to kill it or stop its growth e.g., antibiotics, the germs are not killed and their growth is not stopped. The microorganisms to be tested and their prior antibiotic exposures determine the resistance to a particular antibiotic. (1).

Although antibiotic resistance develops naturally, human and animal abuse of antibiotics has sped up the process. *Escherichia coli, staphylococcus aureus*and other bacteria like *Enterococcus sp., Klebsiella sp., Proteus mirabilis, Acintobacter sp. and Enterobacter sp.* resistance against many antibiotics like Amikacin (AK), Amoxicillin-clavulanate (AUG), Azithromycin (AM), Cefotaxime (CTX), ceftriaxone (CRO) and other Beta-lactamase antibiotics, Gentamycin (CN), Levoflaxacin (LEV), Tetracycline (TE) and Doxycycline (DXT) (2).

Urinary tract infections (UTIs) are a common bacterial disease that affects the urinary tract's various sections and can affect both males and females (3). Both the sexes are susceptible to the infection, but because of differences in their structure and reproductive systems, women are more susceptible than men. Analysis of UTIs is based on the confluence of the signs, a positive urine culture, then an analysis. Cystitis, or lower UTI, and pyelonephritis, or upper UTI, are distinguished clinically. (4).

UTI is one of the most prevalent infectious disorders, mostly affecting women, caused by Presence of bacteria in urine with symptoms, and occasionally indicators, of inflammation, such as recurrent micturation, fever, pyuria, dysuria, and nucturia. Antimicrobial agents are amongst the most commonly agreed medications for UTIS, On the other hand, the main causes of high-level resistance are the widespread use of intrusive equipment, various pathologies, crowding, and the large consumption of frequently misprescribed antibiotics (5).

Depending Depending on the severity of the disease, urinary tract infections can be divided into complicated and uncomplicated contagions. Whereas uncomplicated are common For healthy, nonpregnant women, complicated urinary tract infections have a specific host (with elevated risks to develop problems). Predisposing factors for complicated UTIs include male gender, pregnancy, Immunosuppression (eg, diabetes mellitus), urinary

tract anatomy or function abnormalities (eg, kidney stones, indwelling catheters or other drainage devices, renal failure, renal transplantation, neurogenic bladder, etc.) (6).

UTIs are commonly affected by gram-negative aerobic bacilli initiate in the gastrointestinal tract identified as Enterobacteriaceae most commonly *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa Enterococcus faecalis*, and *Staphylococcus aureus* (5,7).

The aim of study was to identify the local bacterial Uropathogens in south of Baghdad that appear antimicrobial resistance situation for major antibiotic in group of patients have history with Urinary tract infections (UTIs).

## Materials and Methods

### Sample collection and study population

Fifty-one of clinical specimens included in the study that were collected from Iraqi patients, who were referred to hospitals in south of Baghdad city in a period of time about five months between October 2022 and February 2023, 41 of samples gave positive result while 10 of them was considered as control. Patients age ranged between (18- 60) with clinical criteria including: pain in the lower abdomen and during urination, repeated urination, in microscope examination noted full filled with pus cells and 10-20 Red blood cells (RBC).

### Isolation and Identification

Clinical sample was isolated from urinary tract infection (UTI) patients, then cultured on MacConkey agar. After incubation for 24 hours at 37C°under aerobic condition. Cultures were examined after incubation to determine whether they had expanded noticeably. (8). The bacteria were identified as lactose fermenting and lactose non-fermenting. After that the subcultures have been transferred to plates made of nutrient aga r and left to grow for an additional twenty-four hours. Initial Cultures of bacteria were identified depend on colony colors and shape. blood agar and EMB agar were also used for some cultures. The agar medium was prepared from dehydrated powder in compliance with the manufacturer's guidelines.

### Microscopic and morphological features

Gram stain was applied to the bacterial isolates in order to distingui sh between gram-positive and gram-negative isolates. Gram staining technique was followed; subsequently and the slides were examined under microscope with observation of morphological character (shape: cocci or bacilli) of bacteria (9).

### Biochemical tests

The urinary tract infection isolates have been identified by using some biochemical tests including: Indol test, lysine iron agar, Kliglar iron agar, motility test, Simmon citrate, catalase and oxidase (10).

### Susceptibility to antimicrobials test

The KirbyBauer test, which uses disk diffusion to test for antimicro bial susceptibility, was used to find the sensitivity of the isolates according to the CLSI guidelines. Urinary tract infection isolates were inoculated at a turbidity of 0.5 McFarland standards antibiotic disks on the surface of the agar after being added to the Mueller-Hinton agar culture media. Following a 24-hour incubation period at 35°C. Results are assigned as sensitive (S), intermediate (I), or resistant R) based to the millimeter-long zone of bacterial inhibition surrounding the disk (Moulavi et al., 2019.

Among the disks used in the antimicrobial susceptibility test were : Gentamicin, Doxycycline, Nitrofurantoin, Ciprofloxacin, Trimethoprim, Chloramphenicol, Cefotaxim, Azithromycin, Levofloxacin, Nalidixic, Tetracycline, Impenem, Amikacin, Amoxicillin, Ceftazidime, Meropenem, Aztreunam, Cefatriaxene (11).

## Statistical Analysis

The Statistical Analysis System- SAS (2018) program was used to detect the influence of differential factors in study parameters. The chi-square test was used to significantly compare between percentage (0.05 and 0.01 probability) in this study (12).

## Results

Case control study that compromised fifty-one of patient which referred to hospital in south of Iraq. 41 of samples have been shown different bacterial infections by many types of bacteria and 10 samples as control, as shown in Table (1). Bacterial infection in male showed lower percentage compared with bacterial infection in female. There are many types of bacteria caused Urinary tract infection in both male and female, E. coli appeared high percentage about 30.7% in male and 50% in female compared with other types of bacteria that showed different percentage. Our results shown high significant for bacterial infection in male and female.

***Table (1): Distribution of infection bacteria by gender.***

| Type of Bacteria | male | Percentage% | Female | Percentage% |
|---|---|---|---|---|
| ***E.coli*** | 4 | 30.7 % | 14 | 50 % |
| ***Klebsiella spp*** | 2 | 15.3 % | 4 | 14.2 % |
| ***Staphylococcus aureus*** | 5 | 38.4 % | 5 | 17.8 % |
| ***Pseudomonas aeruginosa*** | 1 | 7.6 % | 2 | 7.14% |
| ***Enterococcus ssp*** | 1 | 7.6 % | 3 | 10.7 % |
| **Total** | 13 | 100% | 28 | 100% |
| **Chi-square** | -- | 3.874 * | -- | 8.027 ** |
| **P-value** | -- | 0.0396 | -- | 0.0052 |
| *** (P≤0.05), ** (P≤0.01).** | | | | |

## Bacterial identification for Uropathogens

Urinary tract infection can be produced by a wide range of bacterial species such as *E. coli*, *Klebsiella spp*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus ssp*. Which grow in aerobic or facultative anaerobic conditions. Microbial examination of bacterial species in this study appeared varied in

microscopic examination and showed various characteristics like, colony sizes, shapes and colors on different culture media such as MacConkey agar, blood agar, Mannitol salt agar. Biochemical tests were done for five species of bacteria that isolated from patients with Urinary tract infection, as shown in table (2).

***Table (2): Diagnostic tests results for bacteria isolated for urinary tract infection (UTI)***

| Diagnosis tests | *Escherichia coli* | *Klebsiella spp.* | *Staphylococcus aureus* | *Pseudomonas aeruginosa* | *Enterococcus sp* |
|---|---|---|---|---|---|
| **Growing on MacConkey agar** | **Pink color dry colony** | **Pink color mucoid colony** | - | **Colorless** | - |
| **Growing on blood agar** | - | - | - | - | **Grayish-white** |
| **Growing on Mannitol salt agar** | - | - | **Yellow colony** | - | - |
| **Gram stain** | **Negative (-)** | **Negative (-)** | **Positive (+)** | **Negative (-)** | **Positive (+)** |
| **Oxidase** | - | - | + | + | - |
| **Citrate** | - | + | + | + | - |
| **Urease** | - | + | + | - | - |
| **Indole** | + | - | - | - | - |
| **Catalase** | + | + | + | + | - |
| **Methyl red** | + | - | + | - | + |
| **Kliglar iron agar (KIA)** | **A/A with gas** | **A/A** | **A/A with gas** | **K/K** | **K/K with gas** |
| **Lactose fermentation** | + | + | + | - | + |
| **Coagulase** | - | - | + | - | - |

Five species of bacteria were isolated from UTI were: *E. coli, Klebsiella sp., Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus sp.* These bacteria were cultured on different media and appeared different characteristics as shown in table (2) and Figure (1).

***Figure (1): Pathogenic bacteria colonies cultured on (A) Staphylococcus aureus on mannitol salt agar (B)&(C) E. coli & Klebsiella sp cultured on MacConkey agar respectively (D) Enterococcus sp cultured on blood agar (E ) Pseudomonas aeruginosa cultured on MacConkey agar.***

**(A)** **(B)** **(C)**

**(D)**

**(E)**

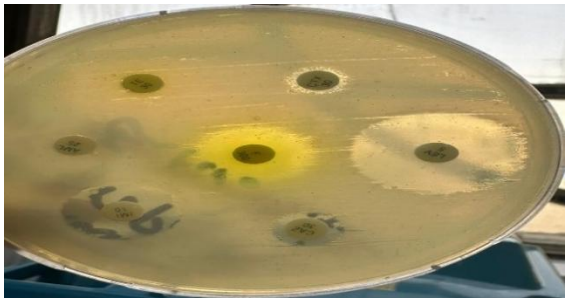
## Biochemical tests

Pathogenic bacteria isolates appeared negative results for some biochemical tests and positive results for others as shown in table (2). *E. coli* has been shown negative results for Oxidase, citrate, Urase and coagulase when showed positive results for Indole, Catalase, Methyl red and lactose fermentation. Whereas *Klebsiella sp*. Has been given negative results for Oxidase, Indole, Methyl red and coagulase while appeared positive results Citrate, Urase, Catalase and lactose fermentation. In addition to *Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterococcus* which showed different results for various biochemical tests.

## Susceptibility of antimicrobial for 5 pathogenic bacteria species

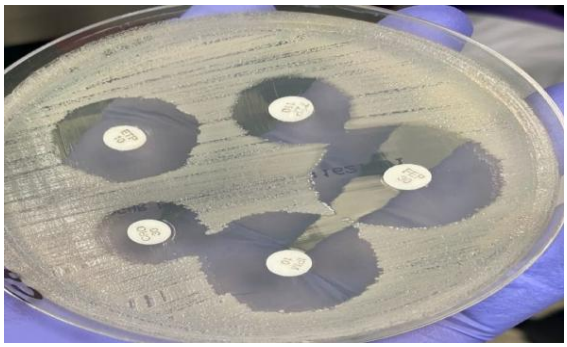
Antibiotic sensitivity was detected for *E. coli, Klebsiella sp, Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterococcus sp*. By using Disk diffusion method against different types of antibiotics as showed in figure (2).

***Figure (2): The sensitivity of antibiotics for five pathogenic bacteria against group of antibiotics: (A) Pseudomonas aeruginosa (B) Staphylococcus aureus (C) Klebsiella sp. (D) E. coli (E ) Enterococcus sp.***

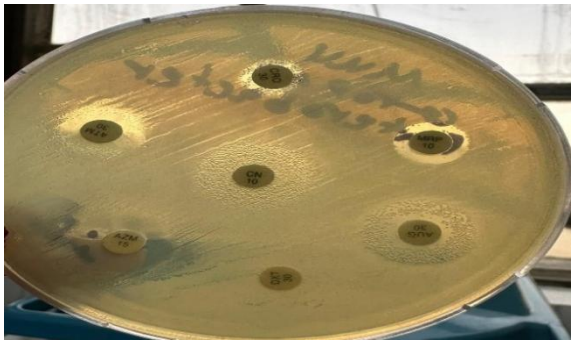


**(A)**

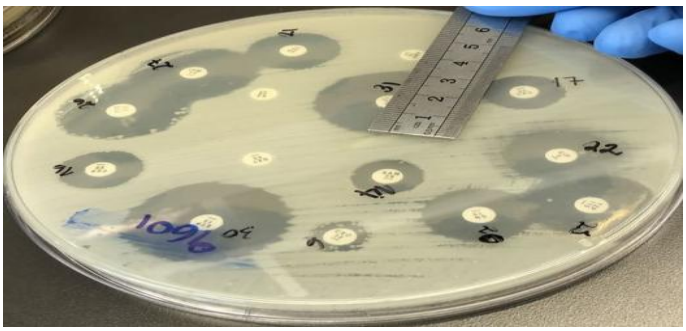
**(B)**



**(C)**



**(D)**



**(E)**

As shown in table (3) *Pseudomonas aeruginosa* isolate showed resistance against Tetracycline (TE), Aztreonam (AM) and Cefotaxime (CTX), while showed sensitivity against Levofloxacin (LEV) with big inhibition zone and Imipenem (IMI). *Staphylococcus aureus* has been shown resistance for Cefpodoxime (CFM) and Trimethoprime/sulfamethoxazole (SXT), whereas gave sensitive inhibition zone for Amikacin (AK), Cefoxitin (FOX), Cefepime (FEP), Chloramphenicol (C ) and gave high inhibition zone for Ciprofloxacin(CIP). Isolates of *Klebsiella sp* appeared sensitive against Imipenem (IM), Cefepime (FEP) and resistance for many antibiotics such as Amikacin (AK) and Cefotaxime (CTX). For *E. coli* isolates showed resistance for most of antibiotic that we used in this study except Aztreonam (AM) and Imipenem (IM) which appeared some sensitivity against it. Finally isolates of *Enterococcus sp* which gave sensitive inhibition zone against many antibiotics like

Amikacin (AK), Azithromycin (AZM) and Ciprofloxacin (CIP) and resistance against Cefpodoxime (CFM) and Trimethoprime/sulfamethoxazole (SXT).

***Table (3): Antibiotic resistance for pathogenic bacteria against many of antibiotics***

| Antibiotic | *E. coli* | *Klebsiella sp.* | *Staphylococcus aureus* | *Pseudomonas aeruginosa* | *Enterococcus sp.* |
|---|---|---|---|---|---|
| **Amoxicillin-clavulanate (AUG)** | R | R | S | R | R |
| **Amikacin (AK)** | R | R | S | R | S |
| **Azithromycin (AZM)** | R | R | R | R | S |
| **Aztreonam (AM)** | S | S | R | R | S |
| **Cefepime (FEP)** | R | S | S | R | R |
| **Cefotaxime (CTX)** | R | S | S | R | S |
| **Cefoxitin (FOX)** | R | R | S | R | S |
| **Cefpodoxime (CFM)** | R | R | R | R | R |
| **Ceftriaxone (CRO)** | R | S | S | R | R |
| **Chloramphenicol ©** | R | R | S | R | S |
| **Ciprofloxacin (CIP)** | R | S | S | R | S |
| **Gentamycin (CN)** | R | S | R | R | S |
| **Imipenem (IM)** | S | S | S | S | R |
| **Levofloxacin (LEV)** | R | R | S | S | S |
| **Tetracycline (TE)** | R | S | S | R | S |
| **Doxycycline (DXT)** | R | R | R | R | R |
| **Trimethoprime/sulfamethoxazole (SXT)** | R | R | R | R | R |

## Discussion

Urinary tract infection (UTI) is The most prevalent nosocomial and community acquired infectious diseases, which caused by a group of bacteria, 150 million of people suffering from UTI every year in the worldwide (13).

Our research objective to determine the main pathogenic agent which causes the UTI in group of patients living in south of Baghdad, in this study we found the bacterial infection in female more than male. The major pathogenic bacteria that cause UTI was E. coli (30.7 %) in male and about (50%) in female, while our results appeared Klebsiella spp. about (15.3%) in male and (14.3%) in female these findings were agreed with other study which *E. coli* was determine in the urine cultures of 64.1%, Klebsiella *spp.* of 17.1% of the cases (14). Additional study was reported the public pathogenic bacteria was *E. coli* with the percentage of 66.8%. Parallel to our study, it has been showed that the primary pathogen responsible for UTI was *E. coli* in study reported from different countries (15). In this research the Uropathogens were isolated from female compared with male patients, other study by Deshpande et al which shown the fact that UTI is more common in women than in men is well known, and our data support this generalization (16).

Table (1) showed different percentage for infection with other pathogenic of bacteria like *Staphylococcus aureus, Pseudomonas* and *Enterococcus spp* were (38.4%, 17.8%), (7.6%, 7.14%) and (7.6%, 10.7%) in male and female respectively. *Staphylococcus aureus* was reported in another study by martin Odoki et al, as more incidence after *E. coli* isolates (17).

One of the main issues associated with common infections caused by bacteria, such as UTIs, is antibiotic resistance. In numerous impoverished and underdeveloped nations, antibiotics including azithromycin, ciprofloxacin, cephradin, nalidixic acid, amoxicillin, and cotrimoxazole are still used to treat gram-positive and gram-negative bacterial infections, such as UTIs (18)

Our findings have been shown varying levels of antimicrobial antibiotic resistance. E. coli showed resistance for most of antibiotics that used in this study except 2 from 17 antibiotics these were: Aztreonam (AM) and Imipenem (IM). In addition to another Gram-negative bacteria like *Klebsiella sp.* and *Pseudomonas aeruginosa which showed high rates of resistance to tested drugs against* Amoxicillin-clavulanate (AUG), Amikacin (AK), Azithromycin (AZM), Cefepime (FEP), Cefoxitin (FOX), Cefpodoxime (CFM), Chloramphenicol (C), Doxycycline (DXT) and Trimethoprime/sulfamethoxazole (SXT). while showed sensitive in different degree against Aztreonam (AM), Imipenem (IM), Levofloxacin (LEV) and Tetracycline (TE) as show in table (3). like to the frequency of drug resistance described by Beyene et al. when most of Gram-negative presented high variable resistance to confirmed drugs against ampicillin, Bactrim, and Augmentin which considers different classes from the same antibiotics we used (19). in another side, Gram-positive bacteria like *S. aureus* and *Enterococcus sp.* showed various result in resistant for many drugs such as Cefpodoxime (CFM), Doxycycline (DXT) and Trimethoprime/sulfamethoxazole (SXT),

Trimethoprim/sulfamethoxazole (Bactrim) is the first-line treatment indicated in Grenada for a urinary tract infection.

Ciprofloxacin and amoxicillin/clavulanate acid (Augmentin) come following. As a result, the pattern of resistance found in our study may have resulted from ciprofloxacin promotion in the past and the current recommendations for Bactrim and Augmentin for the majority of UTI infections in Grenada (20). But in our results *S. aureus* record resistance against some drugs like Azithromycin (AZM), Gentamycin (CN), whereas, *Enterococcus sp*. record resistance against Amoxicillin-clavulanate (AUG), Cefepime (FEP), Ceftriaxone (CRO) and Imipenem (IM). other studies appeared variable findings as S. aureus is ampicillin-resistant, while enterococci group D is cefuroxime-resistant.
It is not entirely clear why the resistance pattern of bacterial isolate s varies in UTI research. (21). Some studies suggested that the community behavior of the general people and the quantity of previous experience to the identical medicine may be the cause of the variance in the antimicrobial drug resistance pattern (22).

Finally, it`s important to be careful when we use the drugs or antibiotics in specially, because most of pathogenic bacteria especially Uropathogens Gram-negative bacteria have high resistance for wide range of antibiotics this make us stop and thinking many times before use these antibiotics in the future and all the world need to know the dangers about use these drugs without any medical consultation.

## Conclusion

This study was performed to describe the resistance to antibiotics subject of urine isolates in south of Baghdad. Most of isolates presented high resistance levels to many UTIs antibiotics were observed. *E. coli* appeared high rate of resistance against most of antibiotics especially ampicillin classes and cephalosporin in addition to gentamycin and Tetracycline. whereas Staphylococcus, Klebsiella, Pseudomonas and Enterococcus recorded different range of resistance against many antibiotics like Amoxicillin, Cefpodoxime (CFM), Doxycycline (DXT) and Trimethoprime/sulfamethoxazole (SXT).

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