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Original Article

# Detection of Antimicrobial Susceptibility and Virulence Factors for Some Bacterial Species Caused Urinary Tract Infection among Type 1 and Type 2 Diabetes Mellitus Patients

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## Abstract

**Background:** Diabetes mellitus (DM), is a metabolic illness caused by a deficiency in insulin production, action, or both, it is characterized by hyperglycemia and disruptions of the metabolism of carbohydrates, proteins, and fats. Urinary tracts infections (UTIs) are conditions when one or more urinary tract structures become contaminated after germs get past the body's robust natural defenses. The most prevalent illness, UTIs can happen at any moment during a person's lifetime. UTIs and DM have been linked for many years. Owing to a shift in the host defense mechanism, a micro-vascular illness in the kidney, and the existence of diabetic cystopathy, the incidence of UTIs is significant in DM. Urinary colonization by bacteria is made possible by high glucose concentrations in urine flow. **Objective:** Detection of antimicrobial susceptibility and virulence factors among bacteria causing UTIs isolated from type 1 and type 2 diabetic patients. **Materials and Methods:** Between April and July 2022, 178 patients with diabetes who had signs of a UTI had their urine samples taken. Midstream urine samples from DM patients were inoculated into blood agar and MacConkey agar using a calibrated loop (0.001 mL). For 24 h, cultures were incubated at 37°C in an aerobic environment. To determine whether there had been any considerable growth, colonies were counted. Diagnostic microorganisms were tested for their susceptibility to various antibiotics, and virulence factors were found. **Results:** Significant bacteriuria was observed in 28 of 69 (40.5%) and 52 of 109 (47.7%) urine samples cultured from patients with type 1 and 2 diabetes, respectively. Sensitivity to isolates which isolated from patients with type 1 and type 2 diabetes was highest for meropenem as 90% and 100%, respectively. With regard to virulence factor, it was investigated of biofilm formation, hemolysin production, protease production, urease production, and β-lactamase production. The highest ratio of these factors production in bacteria which isolated from patients with type 1 diabetes was β-lactamase (75.8%). As for patients with type 2 diabetes, the highest ratio was for biofilm formation (79.0%). **Conclusion:** According to this study, common antimicrobials are very resistant to many different drugs. UTIs are more common than previously thought, and type of diabetes was discovered to be one of the key contributing factors. We found an association between virulence factors of pathogens and type of diabetes.

**Keyword:** Antimicrobial susceptibility, diabetes mellitus, UTI, virulence factor

## Introduction

Diabetes mellitus (DM), is a metabolic condition caused by a deficiency in insulin synthesis, action, or both,[1] and it is characterized by hyperglycemia and abnormalities of carbohydrate, protein, and fat metabolism. In 2012, diabetes contributed to 1.5 million fatalities. In 2014, it was projected that 422 million adults worldwide had diabetes. Globally, it is expected that 550 million people will have the illness by 2045.[2] The dysfunction and harm to numerous organs, such as the kidneys, heart, nerves, eyes, and blood vessels, are long-term problems of DM.[3] Owing to a decline in physical activity and an increase in obesity, the prevalence of diabetes is rising day by day in both emerging and industrialized nations.[4] In less

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developed nations such as Iraq, diabetes raises mortality risk by 7.2 times.[5]

Urinary tract infections (UTIs) and DM have a long history of mutually reinforcing relationships.[6] Due to a shift in the host defense mechanism, a microvascular illness in the kidney, and the existence of diabetic cystopathy, the incidence of UTIs is significant in DM.[7,8] Microorganisms can colonize the bladder when there is a high glucose concentration in the urine flow.[9]

UTIs are conditions where one or more structures in the urinary tracts become infected after microorganisms overcome its strong natural defenses.[10] UTIs are the most common of all infections and can occur at any time in the life of the individual.[11] UTIs are classified according to the site of infection as lower and upper UTIs.[12] Gram-negative bacteria from the Enterobacteriaceae family, including *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Morganella morganii*, and *Pseudomonas aeruginosa*, are the most common causes of UTIs.[13] However, coagulase-negative Staphylococci and species of gram-positive Staphylococci, including *Staphylococcus aureus* and *Enterococcus faecalis*, can also cause these infections.[14] These bacteria have a variety of virulence factors, including hemolysin, biofilm, and other kinds, which have been linked to pathogenicity and the development of UTIs.[15] Hemolysin is a protein that is cytotoxic to multiple different types of human cells and can cause osmotic lysis of erythrocytes.[16] When bacteria form a biofilm on living tissue or a medical device like a catheter, they are shielded from the immune system of the host tissue, which increases their resistance to antibiotics and increases their capacity to proliferate and invade host tissue, which successfully starts an infection.[17]

Lack of data regarding the variation of antimicrobial susceptibility patterns and virulence factors among bacteria causing UTIs isolated from type 1 and type 2 diabetic patients prompted us to carry out this study.

## Materials and Methods

### Study setting

In Mosul, Iraq, Al-Wafa Specialization Center for Diabetes and Endocrinology, the study was carried out between April and July of 2022.

### Exclusion criteria

The study did not include pregnant women with DM and diabetic patients who had undergone catheterization within the previous 2 weeks.

### Culture media

#### *The commercially prepared culture*

MacConkey agar, Mannitol Salt Agar, Nutrient Agar, Mueller-Hinton Agar, and brain heart infusion broth were used, as the media were prepared and pH values were adjusted and sterilized as per manufacturer's instructions.

#### *Culture media prepared in the laboratory*

*Blood agar medium* It was prepared by adding sterilized sheep blood with a concentration of 5% V/V to the prepared blood agar base to the medium according manufacturer's instructions.[18]

*Skimmed milk agar* In order to make it, 42 g of nutritional agar was dissolved in a liter of distilled water and 50 g of powdered nonfat dry milk was dissolved in 500 mL of distilled water. Sterilize the first solution in autoclave at a temperature of 121°C for 20 min. As for nonfat dry milk solution, sterilize it also with the same autoclave temperature for 15 min, then the solutions were left to cool to a temperature of 50°C, then the milk solution was added to the nutrient agar medium, mixed well, then poured into petri dishes and left to solidify, then kept in the refrigerator until use.[19]

#### *Urea agar medium*

It was prepared as follows:

1) Dissolve (2.1 g) of urea base agar in (95 mL) of distilled water, then sterilized by autoclave, and cooled the medium after the completion of sterilization.
2) Add 5 mL of urea solution prepared at a concentration of 40% and sterilized by filtering, and distributed the medium on sterile test tubes.
3) It is sealed and kept in a slanted form until use.[20]

### Specimen collection

After directing the enrolled DM patients to clean their genitalia with soap and water, 178 freshly void midstream urine samples (10–20 mL) were obtained using a prelabeled (date, time, and identification number) leak-proof, wide mouth, sterile, screw-capped plastic container.[21]

### Specimen transportation

The gathered samples were delivered right away to the Ibn Sina Teaching Hospital's Division of Microbiology utilizing a cool box, where they were processed in 30 min. Urine samples were maintained chilled at 4°C until processing when a delay of greater than 30 min was predicted.[21]

### Culturing and identification procedure

Midstream urine samples from DM patients were inoculated into blood agar and MacConkey agar using a calibrated loop (0.001 mL). For 24 h, cultures were incubated at 37°C in an aerobic environment.[22] To determine whether there had been any considerable growth, colonies were counted. Significant bacteriuria was

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defined as colony counts that produced bacterial growth of 105 Colony Forming Unit (CFU)/mL of urine.[10] Gram staining and colony traits allowed us to further distinguish between all positive cultures. Using the VITEC2 compact system, additional identification was completed.

## Virulence factors

The virulence factors are many and exhibit extraordinarily complicated interactions that affect pathogenicity when combined.[23] Biofilm formation, hemolysin generation, protease production, urease production, and lactamase production are some common virulence factors. These markers are expressed in a variety of illnesses and promote bacterial attachment to uroepithelial cells, which help bacteria fend off the bactericidal effects of serum and antibiotics and cause tissue damage.[15]

## Biofilm formation

Using the tube method, brain heart infusion broth (10 mL) was injected with a loopful of microbe from overnight culture plates and cultured for 24 h at 37°C to measure the quality of biofilm formation. After being decanted, the tubes underwent a phosphate-buffered saline wash (pH 7.3) and drying. Crystal violet was used to dye the dried tubes (0.1%). Tubes were cleansed with deionized water after extra stain was removed. After drying inverted, the tubes were checked for the development of biofilms. Positive biofilm formation was defined as the presence of a discernible coating on the tube's bottom and wall. Ring growth at the liquid–liquid interface was not a sign of the development of a biofilm. The experiments were carried out three times in triplicate.[24]

## Hemolysin production

The capacity of the bacteria to cause hemolysis on blood agar was assessed in order to find isolates that produce hemolysin. A blood agar plate was injected with bacteria, and it was left to grow overnight at 37°C. The partial or complete zone of hemolysis around the colonies served as a marker for the presence of hemolysin.[25]

## Urease production

This is the ability of bacteria to produce the enzyme urease, which breaks down urea and produces ammonia. The test was carried out by inoculating the medium of urea agars with a young colony of bacteria, then the medium was incubated at a temperature of 37°C for a period of 24 h. The positive result was indicated after the end of the incubation period by changing the color of the medium from yellow to pink.[26]

## Protease production

This is the ability of the studied bacterial isolates to produce protease enzyme, which is one of the most important factors of virulence. This was done by inoculating the previously prepared skimmed milk agar with pure bacterial isolates in a straight line in the culture medium, and then the dishes were incubated at (37°C) for a period of (24 h), and when a transparent area is formed around the planting line, it is evidence of the ability of this bacteria to produce protease enzyme and casein hydrolysis is positive.[27]

## β-Lactamase production

β-Lactamase production test was performed using the filter paper method described by Otu *et al*.[28] for isolates displaying resistance to one or more beta-lactam drugs.

Boiling was used to dissolve 0.2 g of soluble starch that was added to 100 mL of distilled water. 1 g of penicillin was added after chilling. Strips of Whatman filter paper (1 × 5 cm) were cut and soaked in the mixture. They then underwent a two-hour air-drying process. The colonies were rubbed into the paper strip in a circular motion to cover an area measuring about 5 mm in diameter, and then the strips were infected with a loop-full of the freshly isolated test organism. A petri dish was placed on top of the inoculated filter paper, and it was then incubated at 37°C for 30 min. The filter paper was covered with an iodine solution flood, and any extra solution was drained. When the injection site became colorless after 10 min whereas the other non-beta-lactamase producers kept their purple color, the test for beta-lactamase was deemed positive.

## Antimicrobial susceptibility testing

In accordance with CLSI 2022 recommendations,[29] antimicrobial susceptibility test was performed on Mueller-Hinton agar using the Kirby-Bauer disk diffusion method. A loopful of bacteria (three to five pure colonies) was emulsified in 5 mL of sterile normal saline and gently stirred to create a homogeneous suspension. The suspension's turbidity was then corrected to meet the McFarland 0.5 requirement for density. To standardize the inoculum size, a sterile cotton swab was dipped into the suspension and any excess was removed by gently rotating the swab against the surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of Mueller-Hinton agar. The inoculation plates were dried for 3–5 min at room temperature. Then, by using a disk dispenser, selected antimicrobial disks were positioned on the plate and incubated for 16–18 h at 35–37°C. Table 1 lists the antimicrobial agents that were evaluated at the various concentrations. These antibiotic medicines were chosen in accordance with CLSI 2014 list of prescription medications for the treatment of UTIs.[30] Diameters of the zone of inhibition around the disks were measured using a digital caliper. The result was interpreted as sensitive, intermediate, and resistant-based CLSI 2022 guidelines.

## Ethical approval

The study was carried out in conformity with the ethical standards set forth in the Helsinki Declaration. Before a

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**Table 1: The antimicrobial disks used in the study**

| Antimicrobial disks | Abbreviation | For $Gr^+$ | For $Gr^-$ | Inhibition zone (mm)* | | | Company |
|---|---|---|---|---|---|---|---|
| | | | | R | I | S | |
| Amikacin (30 µg) | AK | ✓ | ✓ | ≤14 | 15–16 | ≥17 | Bioanalyse |
| Aztreonam (10 g) | ATM | ✓ | ✓ | ≤17 | 18–20 | ≥21 | |
| Ceftazidime (30 µg) | CAZ | ✓ | ✓ | ≤17 | 18–20 | ≥21 | |
| Ciprofloxacin (5 µg) | CIP | ✓ | ✓ | ≤21 | 22–25 | ≥26 | |
| Gentamycin (10 µg) | CN | ✓ | ✓ | ≤12 | 13–14 | ≥15 | |
| Cefotaxime (30 µg) | CTX | ✓ | ✓ | ≤22 | 23–25 | ≥26 | |
| Nitrofurantoin (300 µg) | F | ✓ | ✓ | ≤14 | 15–16 | ≥17 | |
| Levofloxacin (5 µg) | LEV | ✖ | ✓ | ≤16 | 17–20 | ≥21 | |
| Meropenem (10 µg) | MEM | ✓ | ✓ | ≤19 | 20–22 | ≥23 | |
| Norfloxacin (10 µg) | NOR | ✖ | ✓ | 12≥ | 13–16 | ≥17 | |
| Co-trimoxazole (1.25–23.75 µg) | SXT | ✓ | ✓ | ≤10 | 11–15 | ≥16 | |
| Tetracycline (30 µg) | TE | ✓ | ✓ | ≤11 | 12–14 | ≥15 | |

R: Resistant, I: Intermediate, S: Sensitive
*According to CLSI[29]

**Table 2: Numbers and species of bacterial isolates of urinary tract infections were isolated from patients with type 1 diabetes and type 2 diabetes**

| Causative agents of UTIs in T1DM $n = 30$ | | Causative agents of UTIs in T2DM $n = 55$ | |
|---|---|---|---|
| $Gr^-$ bacteria 21(70%) | $Gr^+$ bacteria 9(30%) | $Gr^-$ bacteria 40 (72.7%) | $Gr^+$ bacteria 15 (27.3%) |
| *Escherichia coli* 10 (47.7%) | *Staphylococcus aureus* 5 (55.6%) | *Escherichia coli* 15 (37.5%) | *Staphylococcus aureus* 7 (46.7%) |
| *Klebsella pneumonia* 4 (19%) | *Enterococcus faecalis* 3 (33.3%) | *Klebsella pneumonia* 7 (17.5%) | *Enterococcus faecalis* 4 (26.7%) |
| *Proteus mirabilis* 3 (14.3%) | *Staphyllococcus lentus* 1 (11.1%) | *Proteus mirabilis* 7 (17.5%) | *Kocuria kristinae* 3 (20%) |
| *Morganella morganii* 4 (19%) | | *Morganella morganii* 3 (7.5%) | *Staphyllococcus hemolyticus* 1 (6.6%) |
| | | *Pseudomonas aeruginosa* 3 (7.5%) | |
| | | *Serratia marcescens* 3 (7.5%) | |
| | | *Vibrio fulviales* 2 (5%) | |

sample was taken, it was done with the patient's verbal and analytical consent. A local ethics committee examined and approved the study protocol, subject information, and permission form in accordance with document number 4 (which includes the number and the date in April 10, 2022) to receive this approval.

## Results

### Culture results

Twenty-eight of the sixty nine (40.5%) urine samples cultured from type 1 diabetic patients showed significant bacteriuria.

A total of 30 bacterial species were found; of these 21 (70%) were gram-negative bacteria, with *E. coli* being the most common isolate (47.7%), and 9 (30%) were gram-positive bacteria, with *S. aureus* being the most common isolate (at 55.5%).

Significant bacteriuria was found in 52 of 109 (47.7%) urine samples from patients with type 2 diabetes.

A total of 55 isolates were found, of these 40 (72.7%) were Gram-negative bacteria, with *E. coli* accounting for the majority (37.5%), and 15 (27.3%) were gram-positive, with *S. aureus* accounting for the majority (46.7%). Numbers and species of bacterial isolates of UTIs were isolated from patients with type 1 and type 2 diabetes are shown in Table 2.

### Bacterial susceptibility pattern

The antimicrobial susceptibility test was performed on bacterial species that cause UTIs isolated from patients with type 1 and type 2 diabetes. As for those that were isolated from patients with one of the two types of diabetes and did not isolate from those with the other type, they were excluded. Gram-negative isolates which

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were isolated from patients with type 1 ($n$ = 21) and type 2 ($n$ = 32) diabetes, respectively, were tested against 12 types of antibiotics whereas gram-positive isolates which were isolated from patients with type 1 ($n$ = 8) and type 2 ($n$ = 11) diabetes, respectively, were tested against 10 types of antibiotics [Table 1]. Sensitivity to Gram-negative isolates, which were isolated from patients with type 1 diabetes, was highest for meropenem, with a rate of 19/21 (90%), norfloxacin and gentamicin 14/21 (67%) for each of them, whereas the lowest level of sensitivity was observed for ceftazidime, with a rate of (0%), cefotaxime and tetracycline (14%) for each of them [Table 3].

*Proteus mirabilis* was highly resistant to ceftazidime, cefotaxime, nitrofurantoin, co-trimoxazole, and tetracycline (100%) for each of them. *Escherichia coli*, the most frequently isolated bacteria, was highly resistant to ceftazidime, cefotaxime and tetracycline (100%) for each of them and aztreonam (80%). *Morganella morganii* was highly resistant to ceftazidime, ciprofloxacin, and nitrofurantoin (100%) for each of them all isolates were highly resistant to ceftazidime (100%).

Sensitivity to Gram-negative isolates, which were isolated from patients with type 2 diabetes, was highest for meropenem, with a rate of 32/32 (100%), whereas the lowest level of sensitivity was observed for ceftazidime, cefotaxime with a rate of (0%) for each of them, ciprofloxacin (3%), co-trimoxazole (6%), and tetracycline (7%) [Table 4]. *Escherichia coli*, the most frequently isolated bacteria, was highly resistant to aztreonam, ceftazidime, ciprofloxacin, cefotaxime, co-trimoxazole, and tetracycline (100%) for each of them and amikacin, nitrofurantoin, levofloxacin, and norfloxacin (80%) for each of them. *Proteus mirabilis* was highly resistant to ceftazidime, cefotaxime, nitrofurantoin, co-trimoxazole,

**Table 3: Antimicrobial sensitivity to Gram-negative isolates which were isolated from patients with type 1 diabetes**

| Antimicrobial agent | Pathogens | | | | |
|---|---|---|---|---|---|
| | *E. coli* $n$ = 10 | *K. pneumoniae* $n$ = 4 | *P. mirabilis* $n$ = 3 | *M. morganii* $n$ = 4 | Total $n$ = 21 |
| | S (%) | S (%) | S (%) | S (%) | S (%) |
| AK | 4 (40) | 3 (75) | 1 (33) | 2 (50) | 10 (48) |
| ATM | 2 (20) | 3 (75) | 2 (67) | 2 (50) | 9 (43) |
| CAZ | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| CIP | 4 (40) | 3 (75) | 1 (33) | 0 (0) | 8 (38) |
| CN | 6 (60) | 4 (100) | 2 (67) | 2 (50) | 14 (67) |
| CTX | 0 (0) | 1 (25) | 0 (0) | 2 (50) | 3 (14) |
| F | 6 (60) | 1 (25) | 0 (0) | 0 (0) | 7 (33) |
| LEV | 4 (40) | 3 (75) | 3 (100 | 2 (50) | 12 (57) |
| MEM | 8 (80) | 4 (100) | 3 (100 | 4 (100) | 19 (90) |
| NOR | 4 (40) | 3 (75) | 3 (100 | 4 (100) | 14 (67) |
| SXT | 4 (40) | 1 (25) | 0 (0) | 2 (50) | 7 (33) |
| TE | 0 (0) | 1 (25) | 0 (0) | 2 (50) | 3 (14) |

**Table 4: Antimicrobial sensitivity to Gram-negative isolates which isolated from patients with type 2 diabetes**

| Antimicrobial agent | Pathogens | | | | |
|---|---|---|---|---|---|
| | *E. coli* $n$ = 15 | *K. pneumoniae* $n$ = 7 | *P. mirabilis* $n$ = 7 | *M.morganii* $n$ = 3 | Total $n$ = 32 |
| | S (%) | S (%) | S (%) | S (%) | S (%) |
| AK | 3 (20) | 1 (14) | 5 (71) | 1 (33) | 10 (21) |
| ATM | 0 (0) | 1 (14) | 7 (100 | 3 (100) | 11 (34) |
| CAZ | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| CIP | 0 (0) | 1 (14) | 0 (0) | 0 (0) | 1 (3) |
| CN | 9 (60) | 3 (43) | 5 (71) | 0 (0) | 17 (53) |
| CTX | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| F | 3 (20) | 2 (29) | 0 (0) | 0 (0) | 5 (16) |
| LEV | 3 (20) | 5 (71) | 2 (29) | 2 (67) | 12 (37) |
| MEM | 15 (100 | 7 (100) | 7 (100) | 3 (100) | 32 (100) |
| NOR | 3 (20) | 4 (57) | 7 (100) | 3 (100) | 17 (53) |
| SXT | 0 (0) | 2 (29) | 0 (0) | 0 (0) | 2 (6) |
| TE | 0 (0) | 3 (43) | 0 (0) | 0 (0) | 3 (7) |

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and tetracycline (100%) for each of them. *Morganella morganii* was highly resistant to ceftazidime and nitrofurantoin (100%) for each of them. All isolates were highly resistant to ceftazidime and cefotaxime (100%) for each of them.

Overall, gram-positive isolates which isolated from patients with type 1 diabetes ($n$ = 8) showed 100% resistance to aztreonam, ceftazidime and cefotaxime, whereas the lowest level of resistance was observed for meropenem 25%.

*Enterococcus faecalis* showed 100% resistance to amikacin, aztreonam, ceftazidime, gentamycin, cefotaxime, and tetracycline whereas they showed 100% sensitivity to nitrofurantoin.

*Staphylococcus aureus* showed 100% sensitivity to gentamycin and meropenem [Table 5].

As for gram-positive which isolated from patients with type 2 diabetes ($n$ = 11) showed 100% resistance to ceftazidime and cefotaxime, and 82% to aztreonam, whereas the lowest resistance was for ciprofloxacin 18%.

*Enterococcus faecalis* showed 100% resistance to amikacin, aztreonam, ceftazidime, gentamycin, cefotaxime, meropenem, and tetracycline.

*Staphylococcus aureus* showed 100% sensitivity to ciprofloxacin, gentamycin, and meropenem [Table 6].

## Screening of virulence factors

With regard to product of virulence factors, the highest ratio of these factors product from gram-negative isolates which isolated from patients with type 1 diabetes was β-lactamase (86%) followed by formation of biofilm (76%), urease (52%) whereas the lowest was for hemolysin and protease (38%) for each of them, as for patients with type 2 diabetes. The highest ratio was biofilm formation (81.0%) followed by production of β-lactamase (75%),

**Table 5: Antimicrobial susceptibility test to gram-positive isolates which isolated from type 1 diabetic patients**

| Antimicrobial agent | Pathogens | | | | | |
|---|---|---|---|---|---|---|
| | *S. aureus* ($n$ = 5) | | *E. faecalies* ($n$ = 3) | | Total ($n$ = 8) | |
| | R (%) | S (%) | R (%) | S (%) | R (%) | S (%) |
| AK | 2 (40) | 3 (60) | 3 (100) | 0 (0) | 5 (62.5) | 3 (37.5) |
| ATM | 5 (100) | 0 (0) | 3 (100) | 0 (0) | 8 (100) | 0 (0) |
| CAZ | 5 (100) | 0 (0) | 3 (100) | 0 (0) | 8 (100) | 0 (0) |
| CIP | 2 (40) | 3 (60) | 1 (33) | 2 (67) | 3 (37.5) | 5 (62.5) |
| CN | 0 (0) | 7 (100) | 3 (100) | 0 (0) | 3 (37.5) | 5 (62.5) |
| CTX | 5 (100) | 0 (0) | 3 (100) | 0 (0) | 8 (100) | 0 (0) |
| F | 3 (60) | 2 (40) | 0 (0) | 3 (100) | 3 (37.5) | 5 (62.5) |
| MEM | 0 (0) | 7 (100) | 2 (67) | 1 (33) | 2 (25) | 3 (75) |
| SXT | 2 (40) | 3 (60) | 2 (67) | 1 (33) | 4 (50) | 4 (50) |
| TE | 2 (40) | 3 (60) | 3 (100) | 0 (0) | 5 (62.5) | 3 (37.5) |

**Table 6: Antimicrobial susceptibility test to gram-positive isolates which isolated from patients with type 2 diabetes**

| Antimicrobial agent | Pathogens | | | | | |
|---|---|---|---|---|---|---|
| | *S. aureus* ($n$ = 7) | | *E. faecalies* ($n$ = 4) | | Total ($n$ = 11) | |
| | R (%) | S (%) | R (%) | S (%) | R (%) | S (%) |
| AK | 3 (43) | 4 (57) | 4 (100) | 0 (0) | 7 (64) | 4 (36) |
| ATM | 5 (71) | 2 (29) | 4 (100) | 0 (0) | 9 (82) | 2 (18) |
| CAZ | 7 (100) | 0 (0) | 4 (100) | 0 (0) | 11 (100) | 0 (0) |
| CIP | 0 (0) | 7 (100) | 2 (50) | 2 (50) | 2 (18) | 9 (82) |
| CN | 0 (0) | 7 (100) | 4 (100) | 0 (0) | 4 (36) | 7 (64) |
| CTX | 7 (100) | 0 (0) | 4 (100) | 0 (0) | 11 (100) | 0 (0) |
| F | 3 (43) | 4 (57) | 0 (0) | 4 (100) | 4 (36) | 7 (64) |
| MEM | 0 (0) | 7 (100) | 4 (100) | 0 (0) | 4 (36) | 7 (64) |
| SXT | 2 (29) | 5 (71) | 2 (50) | 2 (50) | 4 (36) | 7 (64) |
| TE | 2 (29) | 5 (71) | 4 (100) | 0 (0) | 6 (54.5) | 5 (45.5) |

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**Table 7: Rate of virulence factors product from gram-negative isolates which isolated from patients with type 1 and type 2 diabetes**

| Virulence factor | | Pathogens | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *E. coli* | | *K. pneumonia* | | *P. mirabilis* | | *M. morganii* | | Total | |
| | | T1D | T2D | T1D | T2D | T1D | T2D | T1D | T2D | T1D | T2D |
| | | *n* = 10 | *n* = 15 | *n* = 4 | *n* = 7 | *n* = 3 | *n* = 7 | *n* = 4 | *n* = 3 | *n* = 21 | *n* = 32 |
| Hemolysin | α | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | β | 2 (20) | 0 (0) | 1 (25) | 2 (28.5) | 3 (100) | 7 (100) | 2 (50) | 1 (33.3) | 8 (38) | 10 (31) |
| | γ | 8 (80) | 15 (100) | 3 (75) | 5 (71.5) | 0 (0) | 0 (0) | 2 (50) | 2 (66.7) | 13 (62) | 22 (69) |
| Biofilm | + | 8 (80) | 12 (80) | 3 (75) | 6 (86) | 3 (100) | 7 (100) | 2 (50) | 1 (33.3) | 16 (76) | 26 (81) |
| | – | 2 (20) | 3 (20) | 1 (25) | 1 (14) | 0 (0) | 0 (0) | 2 (50) | 2 (66.7) | 5 (24) | 6 (19) |
| Urease | + | 0 (0) | 0 (0) | 4 (100) | 6 (86) | 3 (100) | 7 (100) | 4 (100 | 3 (100) | 11 (52) | 16 (50) |
| | – | 10 (100) | 15 (100) | 0 (0) | 1 (14) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 10 (48) | 16 (50) |
| Protease | + | 3 (30) | 1 (7) | 2 (50) | 5 (71.5) | 3 (100) | 7 (100) | 0 (0) | 0 (0) | 8 (38) | 13 (41) |
| | – | 7 (70) | 14 (93) | 2 (50) | 2 (28.5) | 0 (0) | 0 (0) | 4 (100 | 3 (100) | 13 (62) | 19 (59) |
| β- lactamase | + | 9 (90) | 11 (73) | 3 (75) | 7 (100) | 3 (100) | 5 (71.5) | 2 (50) | 1 (33.3) | 18 (86) | 24 (75) |
| | – | 1 (10) | 4 (27) | 1 (25) | 0 (0) | 0 (0) | 2 (28.5) | 2 (50) | 2 (66.7) | 3 (14) | 8 (52) |

**Table 8: Rate of virulence factors product from gram-positive isolates which were isolated from patients with type 1 and type 2 diabetes**

| Virulence factor | | Pathogens | | | | | |
|---|---|---|---|---|---|---|---|
| | | *S. aureus* | | *E. faecalies* | | Total | |
| | | T1D | T2D | T1D | T2D | T1D | T2D |
| | | *n* = 5 | *n* = 7 | *n* = 3 | *n* = 4 | *n* = 8 | *n* = 11 |
| Hemolysin | α | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| | β | 5 (100) | 7 (100) | 3 (100) | 4 (100) | 8 (100) | 11 (100) |
| | γ | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) |
| Biofilm | + | 2 (40) | 4 (57) | 3 (100) | 4 (100) | 5 (62.5) | 8 (72.7) |
| | – | 3 (60) | 3 (43) | 0 (0) | 0 (0) | 3 (37.5) | 3 (27.3) |
| Urease | + | 4 (80) | 4 (57) | 0 (0) | 0 (0) | 4 (50) | 4 (36.3) |
| | – | 1 (20) | 3 (43) | 3 (100) | 4 (100) | 4 (50) | 7 (63.7) |
| Protease | + | 2 (40) | 1 (14) | 0 (0) | 0 (0) | 2 (25) | 1 (9) |
| | – | 3 (60) | 6 (86) | 3 (100) | 4 (100) | 6 (75) | 10 (91) |
| β-lactamase | + | 5 (100) | 7 (100) | 0 (0) | 0 (0) | 5 (62.5) | 7 (63.6) |
| | – | 0 (0) | 0 (0) | 3 (100) | 4 (100) | 3 (37.5) | 4 (36.7) |

urease (50%), protease (41%) whereas the lowest was for hemolysin (31%).

Production of virulence factors of each gram-negative isolate which was isolated from patients with type 1 and 2 diabetes is shown in Table 7.

As for gram-positive isolates which were isolated from patients with type 1 diabetes, the highest rate of production of these factors was β-hemolysin (100%), whereas the lowest was for protease (25%), as for patients with type 2 diabetes, the highest ratio was hemolysin production (100%) also, whereas the lowest was also for protease (9%). Production of virulence factors of each gram-positive isolate which was isolated from patients with type 1 and 2 diabetes is shown in Table 8.

## Discussion

In this study, we find significant bacteriuria in 28 of 69 (40.5%) urine samples cultured from type 1 diabetic patients, and we showed significant bacteriuria in 52 of 109 (47.7%) urine samples from type 2 diabetic patients. Similar outcomes were found in Saudi Arabia,[31] Iraq,[32] Egypt,[33] and India.[34] Thirty bacterial species from type 1 diabetic UTIs were identified, of which 70% were gramnegative; the majority of which were *E. coli* (47.7%), and 30% were gram-positive the majority, of which were *S. aureus* (55.6%). As for type 2 diabetic UTIs 55 isolates were discovered, of which 40 (72.7%) were gram-negative bacteria, the majority of which were *E. coli* (37.5%), and 15 (27.3%) were gram-positive bacteria, the majority of which were *S. aureus* (46.6%). Because it can connect to receptors on urinary tract epithelial cells and spread

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infection, *E. coli* is the most common uropathogen.[35] The possession of *Staph. aureus* to the virulence factor coagulase makes them resistant to immunological means and gives them to be one of the most pathogenic species.[36] Similar results were obtained by Jameel and Artoshi[32] and Worku *et al.*[10] $Gr^-$ strains isolated from type 1 diabetic UTIs showed total resistance to antibiotics that reached 58%, whereas the rate was 73.4% in the isolated species of type 2 diabetic UTIs. The same applies to the species of $Gr^+$strains isolated from type 1 diabetic UTIs, as the total resistance was less than that of the isolated species of the type 2 diabetic UTIs, reaching 43.9% and 56.2%, respectively. Similar results were obtained by Birhan,[35] Signing *et al*.[36] and Alemu *et al.*[37] Previous history of UTIs was risk factor linked to the emergence of antimicrobial drug resistance and most of the type 2 diabetic UTIs have prior history of UTIs more than those with type 1 diabetic UTIs and this explains why antimicrobial resistance was high in type 2 diabetic UTIs compared to type 1 diabetic UTIs.[10] In fact, type 2 DM participants with a history of UTIs had a 3.5 times higher likelihood of developing resistance to antimicrobial agents than type 1 diabetic UTIs.[37] This finding explains why bacteria have a favorable environment to become resistant to antimicrobial agents in cases where UTIs have previously been contracted.[36] Bacterial isolates' susceptibility to antibiotics is not continuous and changes over time and in different environments. This necessitates the frequent testing of prevalent bacterial pathogens' antibiotic susceptibility profiles in various communities.[25]

With regard to virulence factor *S. aureus* and *E. faecalis* were blood analyzer with a rate reached (100%). Hemolysin is considered important for some isolates of bacteria to overcome host defense mechanisms; hemolysis of erythrocytes increases the availability of iron in the bacterial environment. This facilitates their survival and invasion potentials in the host tissues.[25]

Gram negative isolates which were isolated from type 1 diabetic UTIs and type 2 diabetic UTIs were products of biofilm with a rate reached 76% and 81%, respectively, whereas gram negative isolates were 62.5% and 72.7%, respectively, Bacterial growth within biofilm may facilitate plasmid exchange and enhance the spread of antimicrobial resistance. Biofilm can increase bacterial resistance to antimicrobial agents by some mechanisms, including impairment of antimicrobial agents diffusion, locally altered microenvironment that impair the activity of antimicrobial agent, reduced bacterial growth rate, and finally biofilm community facilitate transfer of resistant plasmids between bacteria and spread resistant for antibiotic.[38]

Bacterial UTIs, particularly those brought on by *K. pneumoniae*, *P. mirabilis*, are linked to a specific urease-dependent mechanism, but these urease-positive bacteria can cause infection, which can lead to the formation of infection stones that enclose and safeguard the pathogen. The minerals struvite and carbonate apatite, which are created by the binding of ammonium to magnesium ions and bicarbonate to calcium ions, respectively, precipitate to form stones.[39] In this study, we found that *K. pneumoniae*, *P. mirabilis*, *M. Morganii,* and *S. aureus* were produced of urease in certain proportions [Tables 7 and 8] in both type 1 and type 2 diabetic UTIs.

One of the key elements in bacterial invasion and colonization is the proteases system. Proteases have been shown to damage the integrity of extracellular matrix/intercellular connections, cleave particular host immune system components, and counteract the protective mechanisms of epithelia.[19,40] In this study, we found that *P. mirabilis* was 100% product of proteas in species isolated from both type 1 and type 2 diabetic UTIs.

We noticed a high rate of production of β-lactamase in the causative species of UTIs isolated from both types of isolation sources, and biofilm community facilitates the transfer of plasmids responsible for β-lactamase production between bacterial species and spread resistant for beta-lactam antibiotics.[38] β-Lactamase is expressed by the majority of resistance genes, yet some bacteria may exhibit resistance from other classes, as they pick up resistance genes from their surroundings or from other bacteria.[41] Some of β-lactamases have been imaged from the chromosomally encoded genes that occur spontaneously in some species to conjugative plasmids increasing their spreading ability among other species.[42]

## Conclusion

28 of the 69 (40.5%) urine samples cultured from type 1 diabetic patients showed significant bacteriuria. Significant bacteriuria was found in 52 of 109 (47.7%) urine samples from patients with type 2 diabetes. *Staphylococcus aureus* and *E. coli* were the most common gram-positive and gram-negative bacteria, respectively.

This study also showed a high prevalence of drug resistance to common antimicrobials.

UTIs are more common than usual, and the type of diabetes was revealed to be one of the major contributing factors.

We found relationship between virulence factors of pathogens and type of diabetes. These will help to understand the pathogenicity and appropriate management of UTI patients.

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### Conflicts of interest

There are no conflicts of interest.

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