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Bacteriology Of Urinary Tract Infection and antibiotic susceptibility pattern among diabetic patients

Al-Shimaa Saber Abd-elmegeed [1, *], Deya Ismaeel Alamrousy [2], Khaled Mostafa Qasem [3], and Heba Saad Abd-Alrahman [1]

[1] *Department of Biology, College of science, Jazan University, P.O. Pox.114, Kingdom of Saudi Arabia, Jazan, 45142.*
[2] *Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Tanta University, Gharbia government, El Geish street, Tanta, Egypt.*
[3] *Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.*

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Abstract

Urinary tract infections are amongst the most common pathogenic infections with an increasing frequency and severity in patients with diabetes mellitus. Isolates from urine samples were identified and their susceptibility to anti-microbial agents were studied. During the study, 500 urine samples from diabetic patient (305 female and 195 males) were analyzed, 416 samples had significant bacteriuria, and the prevalence rate was higher in females 295(70.9%) than males 121 (29.1%). The gram-negative isolates (72.6%) showed more resistance than gram-positive isolates (27.4%). *Klebsiella sp*. showed 100% resistance to all antibiotics, while *S. epidermis* and *S. aureus* showed 100% susceptibility to Gentamicin. The development of bacterial resistance against antibiotics becomes necessary to manage diabetes to reduce the chances of UTI.

Keywords: Anti-microbial; Diabetic; Urine; Infection; Resistance.

1. Introduction

Glucose serves as the main energy provider for cells, which function as the foundational elements of life. Insulin delivers it to the body and facilitates the metabolic processes that sustain living beings. An imbalance in glucose levels indicates the presence of diabetes mellitus (DM), a diverse group of conditions marked by high blood sugar levels resulting from a total or partial lack of insulin production or effectiveness [1].

The International Diabetes Federation (IDF) estimated that in 2011, the total number of individuals affected by DM was 366 million, with projections suggesting it could reach 552 million by the year 2030. Insulin is crucial for lowering blood glucose levels, overseeing carbohydrate metabolism, and regulating fat synthesis. DM is mainly divided into three categories: Type 1 Diabetes (T1DM), Type 2 Diabetes (T2DM), and Pre-diabetes. T1DM represents around 10% of cases in patients younger than 30 years, whereas T2DM comprises roughly 90% of those diagnosed over the age of 30. Additionally, 318 million adults globally are estimated to have Pre-diabetes, which often goes unnoticed [2, 3].

Urine serves as a rich nutrient medium for many microorganisms. Elevated glucose levels in the urine create conditions suitable for Enterobacteriaceae. Complicated urinary tract infections linked with diabetes can result in renal and perirenal abscesses, gas-producing infections, fungal infections, and renal papillary necrosis. Such conditions contribute significantly to rising mortality rates. Individuals with type 2 diabetes mellitus face a heightened infection risk, especially within the urinary tract, which is the most affected area [4, 5].

* Corresponding author: Al-Shimaa Saber Abd-elmegeed

Diabetics have a tenfold higher risk of urinary tract infections compared to those without diabetes, indicating that as the diabetic population increases, the incidence of diabetic kidney disease will also rise. Infections tend to recur, manifesting either as relapses when the same organism lingers in the genitourinary tract after treatment, or as reinfection from new pathogens entering the tract. A recent investigation in the United States involving over 70,000 type 2 diabetes patients found that 8.2% had been diagnosed with a urinary tract infection within a year, with 12.9% of women and 3.9% of men affected, and higher rates observed with advancing age [6, 7].

Urinary tract infections arise when bacteria or fungi colonize the urethra and, in women, the vagina, then ascend to the bladder and kidneys. The attachment of bacteria to the uro-epithelium is the critical initial event that allows for bacterial persistence and triggers early responses from the innate immune system [8]. *Escherichia coli* is responsible for around 90% of acute urinary tract infections in diabetic individuals. Other bacteria implicated include various species of *Proteus, Klebsiella, Enterobacter, Enterococci*, along with *Staphylococcus aureus*, *Staphylococcus saprophyticus, Serratia*, and *Pseudomonas* [9, 10, 11].

The high frequency of antibiotic prescriptions, including broad-spectrum options, for urinary tract infections in these patients may also contribute to the emergence of antibiotic-resistant urinary pathogens. Fluoroquinolones and β-lactams are frequently chosen as empirical antimicrobial treatments for patients experiencing urinary tract infections [12].

The occurrence of fluoroquinolone-resistant *E. coli* associated with urinary tract infections in the United States was around 25%, while fluoroquinolone-resistant Gram-negative urinary isolates exceeded 40% in the Asia-Pacific region. Nevertheless, since ciprofloxacin and levofloxacin are mainly eliminated through urine, they can achieve significantly high concentrations in urine, allowing these medications to potentially remain effective against urinary tract infections caused by fluoroquinolone-resistant bacteria [13, 14].

Broad-spectrum antibiotics are commonly used for treating UTIs, with empirical treatment often initiated without conducting culture and sensitivity tests. This improper and indiscriminate use of antibiotics has led to a rise in global bacterial resistance to antibiotics, resulting in the emergence of multi-resistant bacterial pathogen strains [15]. Carrillo-Larco et al. (2022) found that individuals with type 2 diabetes mellitus (T2DM) were twice as likely to suffer from resistant infections in the urinary tract or respiratory system. The goal of the current research was to evaluate the prevalence of UTI pathogens in diabetic individuals and their patterns of antimicrobial susceptibility [8, 16, 17].

2. Materials and Methods

2.1. Specimen collection

This study involved diabetic individuals aged 16 and above who visited Tanta hospital between May 1, 2025, and September 31, 2025. A total of 500 midstream urine samples were obtained from patients diagnosed with diabetes mellitus using sterile disposable containers (20 mL) fitted with secure lids to prevent any leakage. The sample bottles recorded the patient's name, age, gender, and collection time. Diabetes mellitus was traditionally defined by fasting blood glucose levels of ≥ 125 mg/dL, blood glucose levels of ≥ 200 mg/dL alongside symptoms like frequent urination, increased thirst, or excessive hunger, blood glucose of ≥ 200 mg/dL two hours post an oral glucose tolerance test, or glycated hemoglobin (HbA1c) levels of ≥ 6.5%; all cases required medical intervention.

2.2. Microscopic examination

All samples were subjected to microscopic analysis to identify pus, red blood cells (RBCs), and bacterial cells. Approximately 50 microliters of each urine sample were placed on a clean glass slide, then carefully covered with a slide cover for microscopic examination using a light microscope.

2.3. Bacteriological analyses

Within a timeframe of 2 hours, a loop of well-mixed urine samples was inoculated onto nutrient agar, MacConkey agar, and blood agar for the purpose of culturing and isolating pathogenic microorganisms. All plates were incubated aerobically at 37 ºC for 24 hours. Bacterial identification was performed according to established protocols.

2.4. Antibiotic susceptibility test (Disk diffusion method)

Antibiotic susceptibility tests were conducted in vitro using the Kirby-Bauer disk diffusion method [18]. Bacterial suspensions were created by emulsifying 3-4 pure colonies in normal saline, then adjusted to meet the 0.5 McFarland standard. A sterile cotton swab was dipped in the bacterial suspension and smeared onto Mueller-Hinton agar (Oxoid, Ltd, UK). Standard antibiotic discs were placed aseptically onto the agar; the plates were then incubated at 37 °C for 16-18 hours. The diameter of the inhibition zones was measured using calipers (in mm). The inhibition zone results were categorized as susceptible, intermediate, or resistant in accordance with CLSI 2022 guidelines. A total of nine antibiotic discs were utilized for this research (refer to Table 1). Standard reference strains of *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were employed as controls for culture and sensitivity evaluations.

3. Results and Discussion

A sum of 500 urine samples was gathered from individuals with diabetes mellitus, comprising 195 males and 305 females. The total occurrence of urinary tract infections was noted at 416 (83.2%), with a higher rate observed in females at 295 (70.9%) compared to males at 121 (29.1%) (Fig. 1). Research conducted by Simkhada (2013) indicated that among the 100 patients studied, 53% were females and 47% were males. Within the female group, those aged 41-50 exhibited a higher incidence of UTIs at 42%, followed by the 31-40 age group at 37% (Fig. 2). It is also noted that women living with diabetes face a two to three times greater risk of developing urinary tract infections compared to those without diabetes [19].

Table 1 Antibiotic used and its conc

Antibiotic used	**Abbreviation**	**Conc.**
Streptomycin	S	10 ug
Amikacin	AK	30 µg
Fusidic acid	FA	10 µg
Amoxycillin	AML	10 µg
piperacillined	PRL	100 µg
Gentamicin	CN	10 µg
vancomycin	VA	30 µg
Rifamycin	Rf	30 µg
Clarithromycin	CLR	15 µg

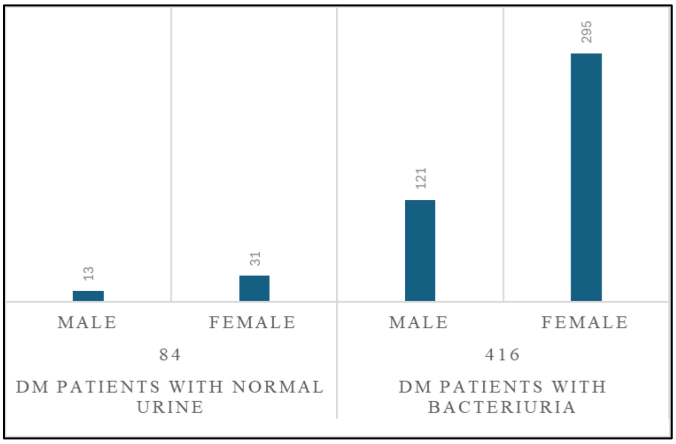

Figure 1 Sex wise distribution of DM patients with normal and bacteriuria

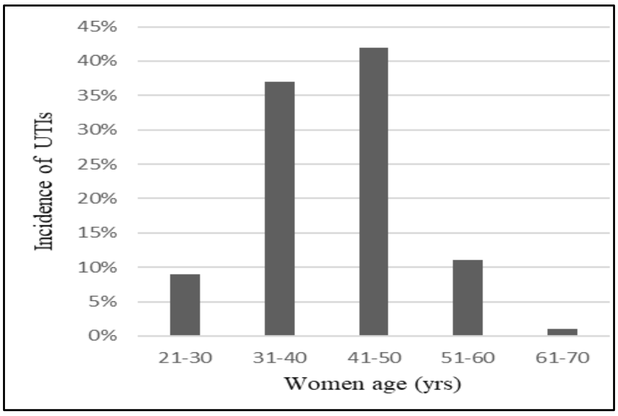

Figure 2 Incidence of UTIs in women patients related to age groups

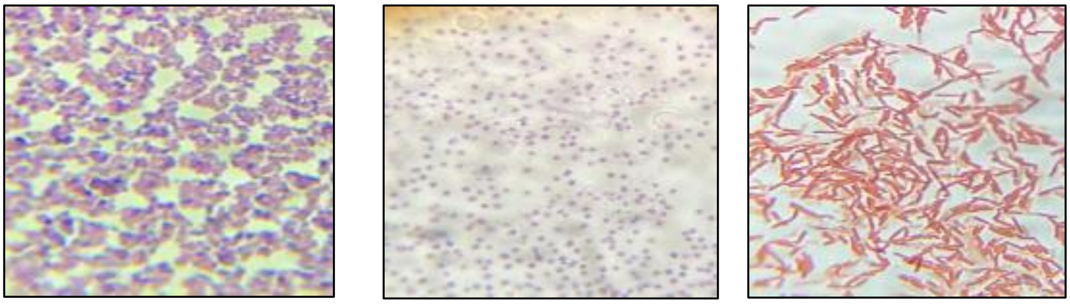

Figure 3 Gram stain differentiation of Some isolated bacteria

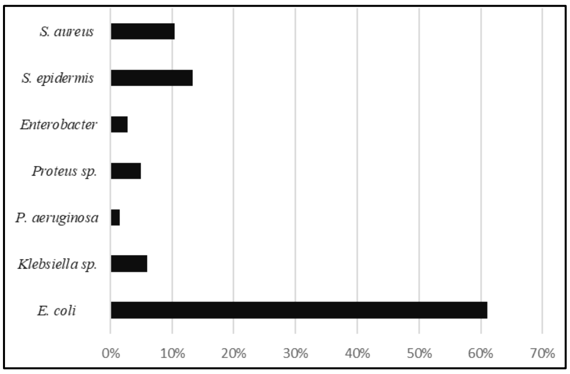

Figure 4 Uropathogens isolated from DM patients tested for UTIs.

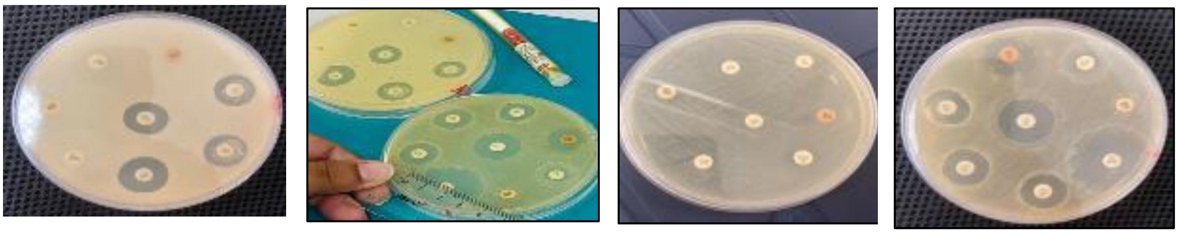

Figure 5 Antibacterial susceptibility test and inhibition zones

Table 2 Resistance rate of bacterial UTI among DM patients against antibiotics

Isolated Uropathogens	Resistance rate against antibiotics; n (%)								
	S	**AK**	**FA**	**AML**	**PRL**	**CN**	**VA**	**RF**	**CLR**
E. coli (n=254)	211(83%)	224(88%)	241(95%)	230(91%)	247(97%)	217(85%)	220(87%)	250(98%)	247(97%)
Klebsiella sp. (n=25)	25(100%)	25(100%)	25(100%)	25(100%)	25(100%)	25(100%)	25(100%)	25(100%)	25(100%)
P. aeruginosa (n=6)	5(83%)	5(83%)	6(100%)	3(50%)	6(100%)	4(67%)	3(50%)	6(100%)	6(100%)
Proteus sp. (n=20)	16(80%)	14(87%)	20(100%)	17(85%)	20(100%)	19(95%)	20(100%)	20(100%)	20(100%)
Enterobacter (n=13)	9(69%)	7(54%)	8(62%)	6(46%)	12(92%)	5(38%)	10(77%)	13(100%)	13(100%)
S. epidermis (n=55)	32(58%)	29(53%)	30(55%)	40(73%)	39(71%)	0	12(22%)	39(71%)	30(55%)
S. aureus (n=43)	34(79%)	37(86%)	40(93%)	29(67%)	18(42%)	0	27(63%)	34(79%)	37(86%)
Total (n=416)	332(80%)	341(82%)	370(89%)	350(84%)	367(88%)	270(65%)	273(67%)	347(83%)	378(91%)

S Streptomycin, AK Amikacin, FA Fusidic acid, AML Amoxycillin, PRL Piperacillined, CN Gentamicin, VA Vancomycin, Rf Rifamycin, CLR Clarithromycin

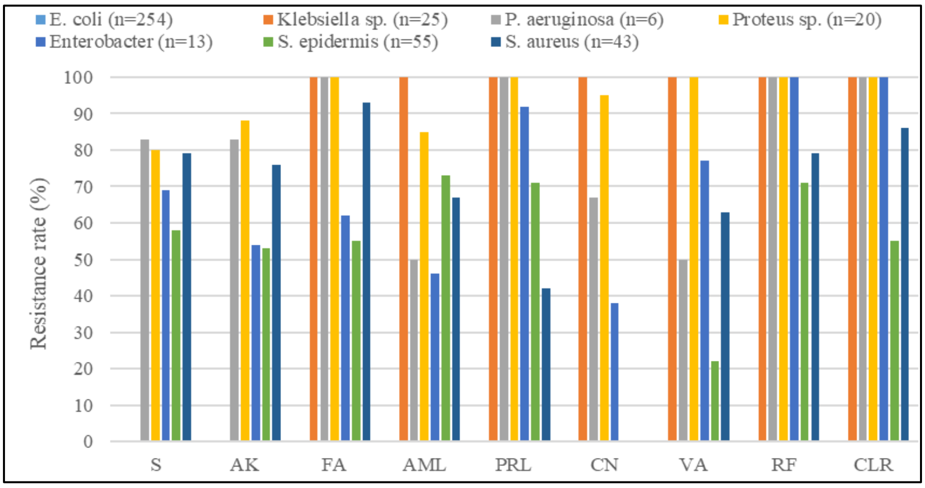

Figure 6 Resistance rate percentages of bacterial UTI among DM patients against antibiotics

A notable relationship was identified between the length of time having diabetes and the rate of bacteriuria. The likelihood of bacteriuria rose by 1.9 times every decade of having diabetes. This phenomenon is believed to be linked to autonomic neuropathy and the resulting incomplete bladder evacuation over prolonged diabetes duration [20].

3.1. Microscopic examination

Microscopic examination of the urine sample revealed that 416 (83.2%) of the specimen showed significant pyuria (≥ 10 leukocyte/hpf). All samples showed significant pus cells +++ and epithelial cells ++ and this was identical to the previous study [20, 21].

3.2. Bacteriological analyses

Among the 500 participants in the study, the total occurrence of notable positive bacteriuria was 416, representing 83.2%. Out of these cases, 72.6% involved gram-negative bacteria, whereas 27.4% involved gram-positive bacteria (Fig. 3). The pathogen most frequently isolated was *E. coli*, accounting for 61%, followed by *S. epidermidis* at 13.3% and *S.*

aureus at 11.4% (Fig. 4). Research conducted by Yismaw et al. (2012) identified gram-negative bacilli at 31.7% and gram-positive cocci at 8.5% [22].

Antimicrobial susceptibility testing (Disk diffusion method) revealed that the rate of multi-drug resistance (MDR) among gram-negative bacterial uropathogens was higher compared to gram-positive ones. Gram-negative and gram-positive uropathogens showed a high resistance rate to Clarithromycin (91.7%), Fusidic acid (89%), and Piperacillined (88%), Rifamycin (84.2%), Amoxycillin (84%), and Amikacin (82%), and Streptomycin (80%). The isolated *K. pneumonia* demonstrated complete resistance (100%) to all nine antimicrobial agents evaluated (Table 2 and Fig. 6). Furthermore, most gram-positive isolates were susceptible to many of the antibiotics tested, with a noted 100% susceptibility for gentamicin in case of *S. aureus*.

In a study by Belete et al. (2019) involving 94 cases at a primary health care facility, gram-negative pathogens represented 87.5% of the cases as the most prevalent pathogens, these pathogens exhibited the highest sensitivity to Gentamicin (100%), Nalidixic acid (100%), Augmentin (96%), and Cephalexin (96%) [23, 24]. A prior hospital-based investigation by the same researchers indicated that the detected bacteria, mainly gram-negative bacilli, were sensitive to Nalidixic acid (100%), Amikacin (100%), Cephalexin (91%), and Augmentin (94%) [25]. A separate study by Musa-Aisien et al. (2003) involving 300 participants analyzed the prevalence and antimicrobial sensitivity patterns in urinary tract infections (UTIs). The isolates were found to be moderately-to-highly sensitive to Gentamicin (80%), Augmentin (81%), Ceftriaxone (77%), and Ciproxin (77%) [26, 27].

4. Conclusion

Diabetes is a chronic disorder which offers many complications to the sufferers. Urinary tract infections are very common among the diabetics and even more prevalent in females due to anatomical differences in urogenital tract as compared to males. It also increases with growing age. This study confirms that diabetes predisposes humans to the risk of urinary tract infections. Prevalence of bacterial UTI was high. Gram negative uropathogens were the most isolated. *E. coli* was the most dominant followed by *S. epidermis*. The research further disclosed that Gram-negative isolates exhibited resistance to antibiotics, more than the resistance found in Gram-positive isolates. This underscores a major threat: the development of bacterial resistance against antibiotics. Hence, it becomes necessary to manage diabetes to reduce the chances of UTI.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declares that there is no conflict of interest.

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