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Prevalence and Antibiogram Pattern of Uropathogens Among Diabetic and Non-Diabetic Patients in Erbil-Kurdistan region of Iraq

Ahmed O. Pirdawid[1] **Zubaidah H. Shukur**[2] **Sozan M. Sharo**[1]

[1]*Department of Biology/ College of Science/ University of Duhok/ Kurdistan Region/ Iraq*
[2]*Department of Medical Biochemical Analysis/ Cihan University-Erbil/ Kurdistan Region/ Iraq*

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corresponding author:
Sozan M. Sharo
sozan.sharo@uod.ac

ABSTRACT

The increased rate of Urinary Tract Infections (UTIs) in immunocompromised patients such as those with diabetes mellitus has become a major international health issue in developing countries. Therefore, this study was conducted to determine the differences in the prevalence of etiological agents between diabetic and non-diabetic individuals, to study the influence of diabetes mellitus on the antibiogram patterns, and to demonstrate extended spectrum β-lactamases (ESBLs) producing bacteria. This prospective cross-sectional study was conducted among 60 non-duplicated diagnosed diabetic and 60 non-diabetic patients. Midstream urine sample were collected and cultured for the diagnosis of bacteriuria. All Gram-negative uropathogens were phenotypically studied for β-lactamase production. Among all 120 patients, the incidence rate of bacteriuria in diabetic and non-diabetic female participants (76.7%, 80%) was significantly higher than in males (23.3%, 20%), respectively. The most frequent isolated uropathogens in patients with and without diabetes mellitus were *Escherichia coli* (53.3%, 46.7%) followed by *Staphylococcus aureus* (23.3%, 16.7%), respectively. Antibiogram patterns revealed that most bacterial isolates were highly susceptible to Imipenem, Meropenem, Ertapenem, Nitrofurantoin, and Vancomycin. Moreover, multi-drug resistance was observed among 70% of UTI patients. The overall prevalence of ESBL in the non-diabetic group was significantly higher than diabetic (68.8%,35.3%). *E. coli* was the predominant *b*-lactamase producer in diabetic (37.5%) and non-diabetic (64.3%) individuals. ESBL producers showed a higher resistance level against 20 used antibiotics than non-ESBL producers. This study has shown a significant relationship between the proportion of bacterial isolates and urinary tract infections in diabetic and non-diabetic patients.

Keywords: UTI, Bacteriuria, Diabetes mellitus, multidrug resistance.

INTRODUCTION

Urinary tract infection is common and is usually caused by bacteria. Studies showed that the most common causative pathogen of bacteriuria in patients with and without diabetes mellitus was *E. coli*, followed by coagulase-negative *Staphylococci*, *Enterococcus* species, *Candida albicans*, and non-albicans *Candida spp.* (Bollestad *et al*., 2018).

Urinary tract infections may be symptomatic or asymptomatic. Symptoms that occur frequently include burning micturition, urgency, dysuria, lower abdominal cramping, mental irritability, back or flank pain, chill, nausea, fever, vomiting, fatigue, and weakness (Woldemariam *et al*., 2019). Diabetic patients are more prone to urinary tract infections than non-diabetic individuals and most patients with diabetes mellitus are relatively asymptomatic, so to prevent the progression of renal complications and severe renal failure, it is important to screen diabetic patients for UTIs and diagnose them promptly (Jia *et al*., 2024).

The treatment of UTIs is contingent on an accurate diagnosis of the causative microorganism. A limitation of therapeutic options is caused by the heavy use of Oxyimino-Cephalosporins, Quinolones, Cotrimoxazole, Aminoglycosides, Sulfamethoxazole, and Metronidazole, which increases the risk for acquisition of ESBL-producing organisms (Likus *et al*., 2024).

Extended-spectrum β-lactamases are plasmid-mediated enzymes produced by Gram-negative bacteria that belong to the family *Enterobacteriaceae,* which hydrolyze and cause resistance to the third-generation Cephalosporins and Monobactams but are still susceptible to Cephamycin's and Carbapenems. The inhibition of these enzymes occurs by b-lactamase inhibitors like clavulanic acid, sulbactam, and tazobactam (Ghafourian *et al*., 2015).

So far, there have been no previous studies on the prevalence of ESBL in patients with UTIs in diabetic and non-diabetic individuals conducted in Iraq. Hence, the study was designed to identify ESBL-producing isolates in diabetic and non-diabetic patients with bacteriuria and to investigate if differences exist in the microbiological characteristics of UTI between diabetic and non-diabetic individuals, to study the influence of diabetes mellitus on the spectrum of uropathogens and antimicrobial resistance patterns in patients with urinary tract infections.

MATERIALS AND METHODES

Study design

A total of 120 urine samples were obtained from a heterogeneous cohort of diabetic and non-diabetic patients with clinically diagnosed urinary tract infections who attended to Layla Qasim Hospital and Media Lab in Erbil from the period between November 2022 to October 2023 diabetic participants were diagnosed based on their blood sugar levels, glycated hemoglobin (HbA1c), random blood sugar (RBS), and fasting plasma glucose (FPG) test results.

The study population comprised both males and females, aged between 10-85 years, who were attending both inpatients and outpatients at the selected hospital. Patients who have previously been treated with antibiotics for UTIs and pregnant women were excluded from the study. The protocol of the study was ethically approved by the ethics committee of the public health directorate, Erbil/ Iraq.

Sample collection

Socio-demographic data (age, gender, signs and symptoms of UTIs, and duration of diabetes), along with clinical characteristics such as previous history of UTIs and glucose level were collected from each study participant through a structured questionnaire.

The clean-catch midstream urine specimen was collected from participants according to the method explained by Gezmu *et al.,* in 2016. About 10 ml of urine sample collected in a sterile, dry, and screw-capped container was labeled with a unique sample number, date, and time of collection. The collected samples were transported within 2 hours to the microbiological laboratory following appropriate safety precautions and Standard Operating Procedures (SOPs) as described in 2006 by Cheesbrough.

Urine microscopy

A direct microscopic examination test is used for all samples before culturing to confirm the presence of urine infections. A wet preparation can detect and measure the amount of blood, leukocytes, epithelial cells, casts, and other cells in centrifuged midstream urine for 4 ± 5 min. at 1500 rounds per minute by using a 40x objective lens. A high level of white blood cells is usually a sign of inflammation associated with a bacterial infection.

Cultivation and identification of uropathogenic bacteria

The culture of the urine specimen was performed within 2 hours of sampling. The samples were inoculated onto various isolation media such as Blood, Chocolate, MacConkey, and Mannitol Salt Agar (Lab M, UK). Subsequently, the inoculated plates were incubated in both aerobic and anaerobic conditions at 37°C for 24 to 48 hrs. A culture was considered positive for bacteriuria if a single isolated uropathogen was recovered at a concentration of more than 10^5 cfu/ml of urine.

Isolated pathogens were identified by using conventional microbiological methods such as colony morphology, Gram staining, and biochemical assays, while species identification was confirmed by using Vitek2® automated system (BioMerieux® -USA).

Antimicrobial susceptibility test

The antibiotic susceptibility profile was determined by the Kirby–Bauer disk diffusion method on Mueller-Hinton agar (Himedia/ India) by using thirty-three commercially available antibiotic discs (Bioanalyse/Turkey); 13 for Gram-positive and 20 for Gram-negative bacteria. The results were detected after 24 hours of incubation at 37 ºC through measuring the zone of inhibition according to the National Committee for Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2024).

Multi-drug resistant (MDR), Extensive drug resistant (XDR), and Pan drug resistant (PDR) isolates were recognized according to the criteria described by the Centre for Disease Control & Prevention (CDC), and European Centre for Disease Control (ECDC). (Nerway and Al-Delaimi, 2021).

Detection of Extended-Spectrum Beta-Lactamase (ESBL)

Screening for ESBL-producing Gram-negative uropathogens

All Gram-negative isolates were screened for ESBL production according to the CLSI (2024) guidelines. ESBL-uropathogens were identified first by disc diffusion method using Ceftazidime 30µg, Ceftriaxone 30µg, and Aztreonam 30µg; if the bacterial strains showed a zone diameter of ≤22 mm, ≤27 mm, and ≤25 mm of the used antibiotic, respectively considered as suspected ESBL-producing isolates.

Confirmatory test for the detection of ESBL-producing bacteria

Double Disc Synergy Test

Double-disk synergy test (DDST) was performed for all suspected ESBL-producing Gram-negative isolates according to Singh and Singh (2014), through evaluations of the synergy between the third-generation Cephalosporin (Ceftriaxone 30 µg, and Ceftazidime 30 µg) and Amoxicillin clavulanic acid. Extension of the edge of the inhibition zone of third-generation cephalosporin discs on the side exposed to the Amoxicillin clavulanic acid; is considered positive for ESBL production.

VITEK2 ESBL testing

This is an automated identification method for the conformation of ESBL isolates, each suspected Gram-negative ESBL isolate was tested using the VITEK 2 compact system with the ESBL test panel containing Cefepime (1 g⁄ml), Cefotaxime (0.5 g⁄ml), or Ceftazidime (0.5 g⁄ml), either alone or in combination with (10, 4 and 4 g⁄ml) of clavulanic acid, respectively. An isolate is considered ESBL positive if a proportional reduction in the growth is observed in wells containing Cephalosporin with clavulanic acid compared to those without clavulanic acid. (Rahman *et al.*, 2014).

ESBL-Chromogenic media

ESBL-CHROMagar (Conda pronadisa, Spain) was used to detect ESBL-producing bacteria. The media contain a mixture of chromogenic compounds that allows the identification of ESBL-producing microorganisms based on colony colors (Salihu *et al.,* 2020) and antibiotics in the

form of supplements (Cat. 6042) which inhibit the growth of all non-ESBL-producing bacteria. ESBL Chromogenic agar has been prepared according to the manufacturer's instructions on their container.

Statistical data analysis

The results obtained from this study were carefully documented. A statistical study was analyzed by using the Statistical Package for the Social Sciences (SPSS) version 23, and Microsoft Excel (2016). The correlation between variables becomes significant and highly significant if the probability value is <0.05 or <0.01, respectively.

RESULTS AND DISCUSSION

The current study was carried out to find out the frequency of etiological agents and antibiogram patterns among diabetic and non-diabetic patients in Erbil City. During this study, 120 urine samples were collected from 60 diabetic (14 male and 46 female) and 60 non-diabetic (12 male, 48 female) patients, who had glycosylated hemoglobin (HbA1c) > 6.5, and clinical signs and symptoms of UTIs such as urgency, dysuria, urinary frequency, loin pain, and nausea.

As shown in (Table 1), the attribution of participants with UTIs was higher and statistically significant among female patients with diabetic and non-diabetic than among men. Our results were consistent with earlier studies conducted in Iraq by Al-Tulaibawi (2019); Jameel and Artoshi (2019); Kamal and Atiyea, (2022). Females are more prone to have UTIs than males due to anatomical and physiological factors such as short urethra in females making the distance between the anal canal and urogenital meatus smaller, and easily contaminating the urinary channel with fecal flora, while in males the prostatic fluid have antibacterial properties which inhibit the bacterial growth in the urinary tract by preventing biofilm formation and reducing the inflammatory response. (Awaness *et al*., 2000).

The participants were aged between 10 and 85 years, including both diabetic and non-diabetic individuals. The mean ages of the diabetic and non-diabetic patients were 53±13.4 years and 37.7±16.6 years, respectively. Statistically, no significant correlation was found between respondents age and bacteriuria in patients with and without diabetes mellitus. As shown in(Table 1), the occurrence of UTI in diabetic individuals was more frequent in the age group (50-70 years), this is in agreement with a study done by (Kande *et al*. 2021; Oumer 2022) increasing the age of diabetic patients makes them vulnerable to UTIs because of high levels of glucose in the urine, which make the urinary bladder a breeding ground for uropathogenic bacteria and cause immunological disorders, lowering the human defense system, and this has been associated with increasing the rate of bacteriuria in patients with diabetes mellitus (Priyadarshini *et al*., 2022).

Table 1: Sociodemographic data of diabetic and non-diabetic individuals.

Variables		Diabetic patients with UTIs (60)	Non-diabetic patients with UTIs (60)	P-value
Gender	Male	14(23.3%)	12 (20%)	P<0.01
	Female	46(76.7%)	48 (80%)	
Age	(10-30)	4 (6.7%)	16(26.7%)	P>0.05
	(30-50)	12 (20%)	28 (46.7%)	
	(50-70)	42 (70%)	14(23.3%)	
	(> 70)	2(3.3%)	2 (3.3%)	

Etiology of bacteriuria

Bacteriological findings of our study reveal that Gram-negative bacteria were the predominant 66(55%) uropathogens, of which 34(51.5%) were from diabetic and 32(48.5%) non-diabetic participants, while Gram-positive bacteria were responsible for UTI infection in 54(45%) individuals. Meanwhile, there were significant associations between UTIs among patients with and without diabetes mellitus and the proportions of bacterial isolates.

As shown in (Table 2) the most common causative agents among all patients with bacteriuria,

identified by using conventional microbiological methods and Vitek2 ® automated system, were *E. coli* 50%, followed by *S. aureus* 20%, *Streptococcus agalactiae* 13.3%, and *Enterococcus faecalis* 11.7%, while *Klebsiella pneumoniae* and *Citrobacter. diversus* were found only in 3.3% -1.7% respectively. Akinnibosun and Iriakpe in (2016) and Shah *et al*. in (2019) showed that *E. coli* is the predominant pathogen isolated from both non-diabetic and diabetic patients, which is in agreement with the current situation. This could be because they are a part of commensals in the intestines, infections are mostly caused by fecal contamination due to improper sanitation, and another reason is the structure of *E. coli*, which promotes strong adherence between virulent type1- fimbriated of pathogen and uroepithelial cells of individuals with diabetes. (Geerlings *et al*., 2002).

Table 2: Distribution of uropathogens among diabetic and non-diabetic patients.

Bacterial isolates	Total no. of isolates	Diabetic No. (%)	Non-diabetic No. (%)	P-value
E. coli	60	32 (53.3%)	28 (46.7%)	P<0.01
S. aureus	24	14(23.3%)	10(16.7%)	
S. agalactia	16	8(13.3%)	8(13.3%)	
E. faecalis	14	4 (6.7%)	10(16.7%)	
K. pneumoniae	4	0	4(6.7%)	
C. diversus	2	2 (3.3%)	0	
Total	120	60	60	

Antimicrobial susceptibility pattern

The antibiotic-resistant profile of uropathogens isolated from patients with and without diabetes mellitus showed broad variation in their susceptibility to the tested antibiotics. The antibiogram revealed that both Gram-negative and Gram-positive bacteria showed high (>80%) to intermediate (80% to 60%) resistance levels to most of the antibacterial agents being tested. The most effective antibiotics against etiological agents isolated in diabetic and non-diabetic patients with UTIs in this study were Imipenem, Meropenem, Ertapenem, Vancomycin, and Nitrofurantoin. This partially disagrees with that of Okwume *et al.* 2021, who observed Carbapenem to be the most effective against all uropathogens, but Nitrofurantoin has a less to moderate effect on pathogens isolated from diabetic and nondiabetic group patients with UTIs. The high efficacy of these antibiotics is due to the fact that these drugs have relatively higher costs and/or limited availability compared to other antibiotics. Consequently, they could serve as potential therapeutic options for the empirical treatment of UTIs in the study population.

The reported results in (Table 3) showed that the Gram-negative bacterial pathogens isolated from non-diabetic patients were significantly $P<0.05$ more resistant to Cephalosporins, Aztreonam, and Trimethoprim/Sulphamethoxazole than diabetic patients. Regarding the Gram-positive uropathogens, the best overall sensitivity 100% was to Tetracycline. As shown in (Table 4), there is a significant difference ($P<0.05$) in the resistance pattern with Gentamycin, Ciprofloxacin, Levofloxacin, and Chloramphenicol between diabetic and non-diabetic patients with UTIs.

The results reported in Iraq by Hadi *et al.* (2014) and Saeed *et al.* (2015) revealed the high resistance levels to Tetracycline, Ampicillin, and Ceftriaxone. In neighboring countries (Arslan *et al.* 2014) in Turkey and (Alsohaili *et al.* 2015) from Jordan reported the same result. The high resistance level may be attributed to the easy availability and frequent misuse in the empirical treatment of UTIs. Alternatively, the increase in resistance could also be linked to the inappropriate use of antimicrobials in empirical therapies and the lack of effective infection control measures, which may lead to the spread of MDR organisms in the community.

Table 3: Antibiotic sensitivity pattern of Gram-negative bacteria isolated from diabetic and non-diabetic patients with UTIs.

Antimicrobial agents (Disk potency)		Diabetic		Non-diabetic		p-value
		E. coli %	*C. diversus* %	*E. coli* %	*K. pneumoniae* %	
Gentamycin (10 µg)	R*	12.5	0	14.3	100	P>0.05
	S**	87.5	100	85.7	0	
Amikacin (10 µg)	R	25	0	28.6	0	P>0.05
	S	75	100	71.4	100	
Imipenem (10 µg)	R	0	0	0	0	P>0.05
	S	100	100	100	100	
Meropenem (10 µg)	R	0	0	0	0	P>0.05
	S	100	100	100	100	
Ertapenem (10 µg)	R	0	0	0	0	P>0.05
	S	100	100	100	100	
Ceftriaxone (30 µg)	R	37.5	0	64.3	100	P<0.05
	S	62.5	100	35.7	0	
Cefotaxime (30 µg)	R	37.5	0	64.3	100	P<0.05
	S	62.5	100	35.7	0	
Cefdinir (30 µg)	R	37.5	0	64.3	100	P<0.05
	S	62.5	100	35.7	0	
Cefixime (30 µg)	R	43.75	0	64.3	100	P<0.05
	S	56.25	100	35.7	0	
Aztreonam (30 µg)	R	37.5	0	64.3	100	P<0.05
	S	62.5	100	35.7	0	
Ciprofloxacin (5 µg)	R	56.25	0	35.7	100	P>0.05
	S	43.75	100	64.3	0	
Levofloxacin (5 µg)	R	56.25	0	35.7	100	P>0.05
	S	43.75	100	64.3	0	
Norfloxacin (10 µg)	R	56.25	0	42.9	100	P>0.05
	S	43.25	100	57.1	0	
Amoxicillin-clavulanic acid (30 µg)	R	75	0	85.7	100	P>0.05
	S	25	100	14.3	0	
Amoxicillin (30 µg)	R	93.75	100	71.4	100	P>0.05
	S	6.25	0	28.6	0	
Piperacillin-Tazobactam (110 µg)	R	0	0	14.3	50	P>0.05
	S	100	100	85.7	50	
Ampicillin-cloxacillin (30 µg)	R	68.75	100	85.7	100	P>0.05
	S	31.25	0	14.3	0	
Trimethoprim/Sulfamethoxazole (25 µg)	R	25	0	57.1	50	P<0.05
	S	75	100	42.9	50	
Nitrofurantoin (100 µg)	R	43.75	0	14.3	50	P>0.05
	S	56.25	100	85.7	50	
Ampicillin (25 µg)	R	100	100	85.7	100	P>0.05
	S	0	0	14.3	0	

*Resistance ** Sensitive

Table 4: Antibiotic sensitivity pattern of Gram-positive bacteria isolated from diabetic and nondiabetic patients with UTIs.

Antimicrobial agents (Disk *potency)*		Diabetic			Non-diabetic			p-value
		S. aureus %	*S. agalactia* %	*E. faecalis* %	*S. aureus* %	*S. agalactia* %	*E. faecalis* %	
Gentamycin (10 µg)	R*	42.9	75	100	0	25	20	P<0.05
	S**	57.1	25	0	100	75	80	
Ciprofloxacin (5 µg)	R	14.3	50	0	100	25	80	P<0.05
	S	85.7	50	100	0	75	20	
Levofloxacin (5 µg)	R	14.3	50	0	80	25	60	P<0.05
	S	85.7	50	100	20	75	40	
Trimethoprim/Sulfamethoxazole (25 µg)	R	0	25	0	40	50	0	P>0.05
	S	100	75	100	60	50	100	
Tetracycline (10 µg)	R	100	100	100	100	100	100	P>0.05
	S	0	0	0	0	0	0	
Chloramphenicol (30 µg)	R	0	25	0	60	25	60	P<0.05
	S	100	75	100	40	75	40	
Vancomycin (30 µg)	R	0	0	0	0	0	0	P>0.05
	S	100	100	100	100	100	100	
Clindamycin (10 µg)	R	28.6	50	100	60	25	100	P>0.05
	S	71.4	50	0	40	75	0	
Penicillin G (10 µg)	R	71.4	100	100	100	100	100	P>0.05
	S	28.6	0	0	0	0	0	
Ampicillin (25 µg)	R	42.9	100	100	100	100	60	P>0.05
	S	57.1	0	0	0	0	40	
Azithromycin (15 µg)	R	71.4	25	50	100	100	100	P>0.05
	S	28.6	75	50	0	0	0	
Erythromycin (10 µg)	R	71.4	25	50	100	100	100	P>0.05
	S	28.6	75	50	0	0	0	
Nitrofurantoin (100 µg)	R	0	0	0	0	0	0	P>0.05
	S	100	100	100	100	100	100	

*Resistance ** Sensitive

Incidence of multidrug resistant pattern in all bacterial isolates studied

(Table 5) shows the frequency of MDR and XDR uropathogenic bacteria for selected antibiotic classes in diabetic and non-diabetic patients. The overall MDR (the isolates that are resistant to at least one agent in ≥3 antimicrobial classes) was observed in 70% of diabetic and nondiabetic patients with UTIs; while 13.3% and 16.7% of the isolates remain susceptible to only one or two classes of antibiotics in both patients with and without diabetes mellitus, respectively. In our study, there was no PDR (the isolate that is resistant to all tested antibiotic agents). However, this incidence rate is higher than the findings of other studies conducted by Hamdan *et al*. (2015); Alemu *et al*. (2020).

According to our findings, nearly half of the *E. coli* isolates were found to be MDR strains, which are in line with previous studies in the same field conducted by Nayaju *et al*. (2020). The high

frequency of MDR might be due to a consequence of inappropriate use of antibiotics for a long time, over-the-counter availability of antibiotics, and a lack of laboratory diagnostic tests. Moreover, the Gram-negative bacterial efflux system contributes to the emergence of MDR strains. Additionally, the formation of biofilm by uropathogens inside the urinary bladder, and a plasmid carrying drug-resistant genes, leads to developing ESBL strain. (Joshi *et al*., 2011).

Table 5: Frequency of MDR and XDR bacteria isolated from diabetic and non-diabetic patients with UTIs.

Isolated pathogens	Diabetic		Non-diabetic	
	MDR*	XDR**	MDR*	XDR**
E. coli	56.25%	18.75%	50%	21.4%
S. aureus	100%	0	100%	0
S. agalactia	75%	25%	100%	0
E. faecalis	100%	0	100%	0
K. pneumoniae	-	-	0	100%
C. diversus	0	0	-	-
Total	70%	13.3%	70%	16.7%

* Multi-drug resistance ** Extensive-drug resistance

Prevalence of ESBL producers

The empirical use of 3rd and 4th generation Cephalosporin (β-lactam antibiotics) is a significant contribution to ESBL isolates. ESBLs are a group of plasmid-mediated Gram-negative bacteria. Concurring with our result, most of the isolates belonging to the *Enterobacteriaceae* family exhibited high resistance levels against Oxyimino-cephalosporin and Monobactams. This is most likely because the uropathogens isolates can produce the beta-lactamase enzyme, which targets the β-lactam ring in the antibiotic structures and dampens their activity. Beta-lactamase enzymes are inhibited by β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam. (Farag *et al*., 2024)

To characterize ESBL production by these uropathogens, several phenotypic confirmatory methods were used. Among 66 *Enterobacterial* isolates, 57.6% and 51.5% were ESBL producers by the antibiotic screening test and the confirmatory test, respectively. As illustrated in (Table 6), 41.2% and 75% of Gram-negative bacterial isolates of diabetic and nondiabetic patients were suspected of being ESBL positive, respectively. The results were confirmed by DDST as shown in Fig. (1), ESBL-Chrom agar, and by using automation (vitek 2). In 2024, Aneesha *et al*. revealed that DDST has a higher sensitivity than the screening method for the detection of ESBL among UTI patients with and without diabetes mellitus.

Table 6: Rate of ESBL and non-ESBL Ur pathogenic organisms in diabetic and non-diabetic patients with UTIs using phenotypic methods.

Phenotypic detection methods		Patients with UTIs		Diabetic patients with UTIs		Non-diabetic patients with UTIs	
		ESBL	Non-ESBL	ESBL	Non-ESBL	ESBL	Non-ESBL
ESBL screening test	Disc diffusion	57.6%	42.4%	41.2%	58.8%	75%	25%
ESBL confirmatory tests	DDST	36.4%	63.6%	23.5%	76.5%	50%	50%
	Vitek	45.5%	54.5%	29.4%	70.6%	62.5%	37.5%
	CHROMagar	51.5%	48.5%	35.3%	64.7%	68.8%	31.3%

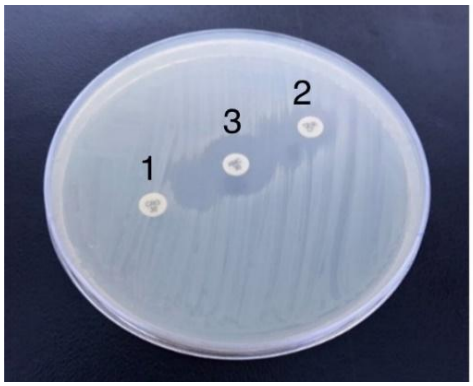

Fig. 1: Positive DDST result for ESBLs detection showing synergy between third-generation Cephalosporin (1-Ceftriaxone, 2-Ceftazidime) and 3-Amoxicillin clavulanic acid disk.

The incidence of ESBL-producing bacteria in our study was significantly higher among the non-diabetic patients with bacteriuria (68.8%) than the patients with DM (35.3%). Among all ESBL-positive isolates, (37.5%) and (64.3%) were *E. coli* in diabetic and non-diabetic individuals, respectively, while *K. pneumoniae* was isolated only from non-diabetic patients, as shown in Table (7). These are dissimilar to the findings of Saber *et al.* (2010) and Alemu *et al.* (2020), which isolated *K. pneumoniae* only from patients with diabetes mellitus.

Table 7: Frequency of ESBLs and non-ESBLs producing Gram-negative uropathogens.

	Patients with UTIs		Diabetic patients with UTIs		Non-diabetic patients with UTIs	
	ESBL	Non-ESBL	ESBL	Non-ESBL	ESBL	Non-ESBL
E. coli	50%	50%	37.5%	62.5%	64.3%	35.7%
K. pneumoniae	100%	0	-	-	100%	0
C. diversus	0	100%	0	100%	-	-

Antimicrobial susceptibility pattern among ESBL and non-ESBL isolates

(Table 8) exhibits the antibiotic-resistant pattern of 20 commonly used antimicrobial agents. The drug resistance profile revealed that ESBL-producers were more resistant to antimicrobial substances than non-ESBL isolates in diabetic and non-diabetic patients, this is in agreement with the findings of Alemu *et al.* (2020). In our study, a significant relationship ($P<0.05$) was recorded in the resistant pattern with Monobactam, Cephalosporins (Cefuroxime, Ceftriaxone, Cefixime, and Cefdinir), and Penicillin (Amoxicillin-clavulanic acid, and Ampicillin-cloxacillin) between ESBL and non-ESBL producers in both diabetic and non-diabetic individuals with bacteriuria.

Additionally, it was found that all ESBL and non-ESBL isolates in UTI patients with and without DM were susceptible 100% to Carbapenems (Imipenem, Meropenem, and Ertapenem). Nayaju *et al.* in 2020 found that ESBL-producing *E. coli* were highly resistant to the first line of antibiotics, while non-ESBL *E. coli* isolates exhibit high sensitivity to the same antibiotics. Additionally, it was found that ESBL isolates were more susceptible to a second line of antimicrobial drugs than non-ESBL isolates. An increase in the level of resistance rate is typically caused by the production of β- lactamase enzymes, which lead to resistance to Cephalosporin and Monobactam antibiotics, however resistance to Quinolone and fluoroquinolone antibiotics correlated with plasmid-mediated quinolone resistance genes, which inhibit the quinolone binding DNA gyrase and topoisomerase enzymes. (Shahbazi *et al*., 2018).

Table 8: Antibiotic resistance pattern of ESBL and non-ESBL producers isolated from diabetic and nondiabetic patients with bacteriuria.

Antimicrobial agents (Disk potency)		Diabetic		Non-diabetic		p-value
		ESBL %	Non-ESBL %	ESBL %	Non-ESBL %	
Gentamycin (10 µg)	R	33.3	0	36.4	0	P>0.05
	S	66.7	100	63.6	100	
Amikacin (10 µg)	R	16.7	27.3	36.4	0	P>0.05
	S	83.3	72.7	63.6	100	
Imipenem (10 µg)	R	0	0	0	0	P>0.05
	S	100	100	100	100	
Meropenem (10 µg)	R	0	0	0	0	P>0.05
	S	100	100	100	100	
Ertapenem (10 µg)	R	0	0	0	0	P>0.05
	S	100	100	100	100	
Ceftriaxone (30 µg)	R	100	0	100	0	P<0.05
	S	0	100	0	100	
Cefotaxime (30 µg)	R	100	0	100	0	P<0.05
	S	0	100	0	100	
Cefdinir (30 µg)	R	100	0	100	0	P<0.05
	S	0	100	0	100	
Cefixime (30 µg)	R	100	0	100	0	P<0.05
	S	0	100	0	100	
Aztreonam (30 µg)	R	100	0	100	0	P<0.05
	S	0	100	0	100	
Ciprofloxacin (5 µg)	R	66.7	45.5	63.6	0	P>0.05
	S	33.3	54.5	36.4	100	
Levofloxacin (5 µg)	R	66.7	45.5	63.6	0	P>0.05
	S	33.3	54.5	36.4	100	
Norfloxacin (10 µg)	R	66.7	45.5	72.7	0	P>0.05
	S	33.3	54.5	27.3	100	
Amoxicillin-clavulanic acid (30µg)	R	100	54.5	100	60	P<0.05
	S	0	45.5	0	40	
Amoxicillin (30 µg)	R	100	90.9	100	10	P>0.05
	S	0	9.1	0	80	
Piperacillin-Tazobactam (110 µg)	R	16.7	0	27.3	0	P>0.05
	S	83.3	100	72.7	100	
Ampicillin-cloxacillin (30 µg)	R	100	54.5	100	60	P<0.05
	S	0	45.5	0	40	
Trimethoprim/Sulfamethoxazole (25 µg)	R	50	9.1	63.6	60	P>0.05
	S	50	90.9	36.4	40	
Nitrofurantoin (100 µg)	R	50	36.4	18.2	10	P>0.05
	S	50	63.6	81.8	90	
Ampicillin (25 µg)	R	100	100	100	60	P>0.05
	S	0	0	0	40	

CONCLUSIONS

In conclusion, since there have been no previous studies on the prevalence of ESBL in UTI patients with and without diabetes mellitus in Iraq, this study represents the first report on the prevalence of ESBLs in diabetic and non-diabetic individuals with bacteriuria. In view of our study findings, the UTIs were observed to be significantly higher in diabetic and non-diabetic female patients than in males. Of the diverse etiological agents, the Gram-negative bacteria were predominant over Gram-positive isolates, and *E. coli* was the most frequent pathogen in patients with and without DM followed by *S. aureus*. In vitro, the most effective antibacterial agents against Gram-positive and Gram-negative bacterial isolates were Vancomycin, Nitrofurantoin, and Carbapenem. Besides this, the majority of uropathogens were resistant to most commonly used

antibiotics. Moreover, the rate of ESBL-producing isolates was significantly higher in non-diabetic individuals than in patients with diabetes mellitus. It could be observed that non-ESBL producers were more susceptible to the antibiotics used than ESBL producers in diabetic and non-diabetic populations. Early detection and regular screening of antibiotic-resistant isolates and β-lactamase producers' bacteria are essential to prevent the development of MDR and ESBL strains and will help physicians prescribe appropriate antibiotics at the correct dosage, especially in diabetic patients to deny the development of nephritis.

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انتشار ونمط المضادات الحيوية لمسببات الأمراض البولية بين مرضى المصابين وغير المصابين بمرض السكري في منطقة أربيل-كردستان العراق

احمد عثمان بيرداوود [1] زبيدة هشام شكر [2] سوزان موسى شرو[1]

[1] قسم علوم الحياة/ كلية علوم/ جامعة دهوك/ منطقة كوردستان/ العراق

[2] قسم التحليلات الكيميائية الحياتية الطبية/ جامعة جيهان-أربيل/ منطقة كوردستان/ العراق

الملخص

أصبح ارتفاع معدل التهابات المسالك البولية لدى المرضى الذين يعانون من نقص المناعة، مثل مرض السكري، مشكلة صحية عالمية رئيسية في الدول النامية. لذلك، أُجريت هذه الدراسة لتحديد الاختلافات في انتشار العوامل المسببة بين مرضى المصابين وغير المصابين بمرض السكري، ودراسة تأثير داء السكري على أنماط المضادات الحيوية، وإثبات البكتيريا المنتجة لإنزيمات بيتا لاكتاميز واسعة الطيف .(ESBLs) أُجريت هذه الدراسة المقطعية الاستشرافية على 60 مريضًا مصاب و60 مريض غير مصاب بمرض السكري. جُمعت عينات من منتصف مجرى البول وزرعت لتشخيص جرثومة المسالك البولية. دُرست جميع مسببات الأمراض البولية سلبية الغرام ظاهريًا لتحديد انتاج إنزيم بيتا لاكتاميز. من ضمن مرضى120، كان معدل الإصابة ببكتيريا البول لدى النساء المصابات بداء السكري وغير المصابات به (76.7%−80%) أعلى بكثير منه لدى الذكور (23.3%−20%) على التوالي. وكانت الإشريكية القولونية (53.3%−46.7%) هي أكثر مسببات الأمراض البولية المعزولة شيوعًا لدى المرضى المصابين بداء السكري وغير المصابين به، تليها المكورات العنقودية الذهبية (23.3%−16.7%) على التوالي. وعند التحري عن الحساسية للمضادات الحيوية اظهرت النتائج أن معظم العزلات البكتيرية كانت مقاومة لكل من إيميبينيم، ميروبينيم، إرتابينيم، نيتروفورانتوين، وفانكومايسين. علاوة على ذلك، تم تصنيف 70% من العزلات على انها عزلات مقاومة للادوية المتعددة. كان معدل انتشار عزلات ال ESBL في المرضى غير المصابين بداء السكري أعلى بكثير منه لدى المصابين بداء السكري (68.8%−35.3%). كانت بكتيريا الإشريكية القولونية أكثر العزلات انتاجا لل ESBL لدى مرضى السكري (37.5%) وغير المصابين بالسكري (64.3%). وأظهرت البكتيريا المنتجة لإنزيم بيتا لاكتاميز مستوى مقاومة أعلى تجاه 20 مضادًا حيويًا مستخدمًا مقارنةً بالبكتيريا الغير المنتجة لهذه الإنزيم. وقد أظهرت هذه الدراسة علاقة مهمة بين نسبة العزلات البكتيرية والتهابات المسالك البولية لدى مرضى السكري وغير المصابين به.

الكلمات الدالة: التهاب المسالك البولية، جرثومة المسالك البولية، داء السكري، مقاومة الأدوية المتعددة، بيتا لاكتاميز واسع الطيف.