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Uropathogenic Infections Among Patients with Diabetes in Baghdad City

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Abstract

One hundred thirty four urine samples were collected from diabetic patients during the period from March –November -2014. From these samples there were 62.7% positive samples, women represented 45.3% and men represented 54.7% , some of them were a symptomatic as women represented 60.5% and men represented 58.7% and some were with clinical symptoms with or without a history of UTI. Hypertension diabetic women represented 45% and men represented 26% .Men suffering from prostate enlargement were represented 35% from the total diabetics .The *E.coli* isolates were represented 28.6% from the total cases ,*Klebsiella spp* isolates represented 20%, *S. aureus* isolates represented 16.7%,*Proteus spp* isolates represented 15.5% followed by *Streptococcus fecalis* isolates represented 13% and *Candida albicans* isolates represented 6.2%.

Both gram positive and negative isolates were tested for their susceptibility and resistance to some common antibiotics, *S.aureus* isolates were resistant to ampicillin , cefotaxime and amoxicillin (71.4%,57%) respectively , *Streptococcus fecalis* isolates were resistant to erythromycin 82% and susceptible to ampicillin 73% , *E.coil* isolates were resistant to cloramphenicol 58% and susceptible to ciprofloxacin 83% . *Klebsiella spp* isolates were susceptible to ciprofloxacin and gentamicin both (100%),*Proteus spp* isolates were susceptible to norfloxacin 92%.

Keywords: UTI, Pathogenic bacteria, diabetes mellitus

امراضية التهابات المجاري البولية بين المصابين بداء السكري في مدينة بغداد

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الخلاصة

تم جمع 143 عينة إدرار من المرضى المصابين بداء السكري خلال المدة من شهر اذار – شهر تشرين الثاني 2014 ووجد ان 62,7% عينة منها كانت موجبة ، كانت نسبة الاصابة بين النساء 45,3% والرجال 54,7% . البعض لم يكن لديه أعراض مرضية بنسبة 60,5% للنساء و 58,7% للرجال و كانت بعض الحالات مصاحبة لتاريخ مرضي مع التهابات المجاري البولية .النساء اللواتي كان لديهن ارتفاع في ضغط الدم مثلن نسبة 45% والرجال نسبة 26% كما كانت نسبة الرجال الذين يعانون من تضخم البروستات 35% . تم خلال الدراسة عزل بكتيريا للاشريشيا القولونية و نسبة 28,6% من مجموع الحالات الكلية، ثم بكتيريا الكلبسيلا بنسبة 20% و المكورات الذهبية مشكلة نسبة 16,7% ثم المتقلبات بنسبة 15,5% و المكورات المسبحية بنسبة 13% وأخيرا الكانديدا البيكانس وبنسبة 6,2%.تم إجراء فحص الحساسية لجميع العزلات البكتيرية وكانت المكورات الذهبية مقاومة للامبسلين والسيفونكساميم بنسبة 71,4% والاموكسيسلين 57% .اما المكورات المسبحية فكانت مقاومة للاريترومايسين 82% وحساسة تجاه الامبسلين 73% والاشريشيا القولونية كانت مقاومة للكورمغنيكول(58%) وحساسة للسايبيروفلوكساسين 83% . تجاه للسيفونكساميم و الجنتاميسين (74%,79%) .

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الكليسيلا كانت حساسة للسايبيروفلوكساسين و للجنتاميسين (100%) لكل منهما ، اما المتقلبات فكانت حساسة اتجاه النورفلوكساسين 92%.

Introduction:

Diabetes mellitus is one of the most challenging health problems of 21st century and is the fifth leading cause of death in developed countries [1]. Asian patients are considered to have a higher risk of developing diabetes and potentially worse prognosis. By 2025, the number of individuals with diabetes is expected to be more than double [2].

Urinary Tract Infection (UTI) has long been recognized as a significant problem in patients with diabetes mellitus ,a changed bacterial adhesion to the uroepithelium, granulocyte dysfunction and impaired antioxidant systems involved in the pathogenesis of UTI in diabetics [3,4]. Diabetes causes several abnormalities in the host system that increases the risk of urinary tract infection. These include immunologic impairments such as defective migration, phagocytic alteration of chemotaxis in polymorphonuclear leukocytes [5].

Drug resistance of pathogens is a serious medical problem, because of very fast arise and spread of mutant strains that are insusceptible to medical treatment and the knowledge of etiological agents of UTI and their sensitivities to available drugs is of immense value to the selection and use of antimicrobial agents and to the development of appropriate prescribing policies [6].

The aim of the study is to determine the causative organism, antimicrobial susceptibility, and, to provide epidemiological data about UTI among diabetic patients.

Materials and methods:

This study was carried out in the period March - November 2014. Included(134) patients attending the diabetes center of AL-Yarmuk hospital (71 men and 63 women),their ages were (35-70 yr) ,the mean age of men was(58.8±13.3) and for women was(53.3± 19.1)

All study participants of women patients were non-pregnant individuals. Patients already known to have diabetes were involved in this study, and were diagnosed as diabetic using the World Health Organization criteria [7]. Informed consent was received from participants before inclusion in the study, data of socio-demographic characteristics were collected using pre-tested questionnaires. Every patient was asked about symptoms suggestive of UTI (dysuria, urinary frequency, loin pain, and nausea) and history of other medical disorders, such as hypertension and, for men, prostate enlargement.

Participants were asked to provide a mid-stream urine .All the urine samples were collected in sterile containers, and were processed within one hour after arriving at the laboratory and the reagent strips were used for leukocyte and nitrite test. Depending on the chemical reactions, the reacted strips were matched to a color chart, the intensity of color was proportional to the amount of enzyme presented as appositve result.

A sample of well mixed urine 5-10 ml was centrifuged in a test tube at relatively low speed for 5 minutes until moderately cohesive bottom sediment was produced to be examined microscopically, the supernatant fluid was poured off and sediment re suspended in few drops of urine remaining, a drop of urine was examined under 40x. A minimum of 10-15 high power fields were scanned for WBCs, results were reported in terms of number of cells/HPF. Standard calibration wire loop delivering 0.01 ml of urine was inoculate on nutrient agar, blood agar and Mac- Conkey agar, these plates were incubated aerobically at 37C° for 24hr. All samples positive for one or both leukocyte and nitrite test were inoculated on blood and Mac-Conkey agar [8].

Bacterial colonies were identified on the basis of Gram's stain, morphology, biochemical characteristic and cultured characteristics. API 20E and API-staph were used for identification of enteric bacteria and *Staphylococcus spp.* *Candida albicans* isolated by using Modified Sabouraud 's Dextrose agar , the plats incubated for 48 h at 35C° then the isolates diagnosed according to morphological characters [9]. The antimicrobial screening were performed using Muller Hinton agar disk diffusion assay , the microorganisms from growth on nutrient agar incubated at 37C° for 18h were suspended in saline solution 0.85% NaCl and adjusted to turbidity of 0.5 McFarland standards (10⁸ cfu/ ml) [10].The suspension was used to inoculate the Petri-plates with sterile non toxic cotton swab on a wooden applicator , the antibiotic discs were directly placed on to the bacterial culture , after 24h incubation at 37C° ,antimicrobial activities were assayed by measuring the inhibition zone diameter around the disc using a caliper, The following antibiotics were tested : Ampicillin, Amoxicillin,

Cefotaxim, Erythromycin, Nalidixic acid, Norfloxacin, Ciprofloxacin Gentamicin Trimethoprim-sulfamethoxazole and Cloramphenicol .

Statistical analysis:

Results were analyzed by using X^2 and t test to find the differences between the study groups.

Results:

From (134) samples tested for UTI, there were (84) positive samples depending on the culturing and biochemical results, represented (62.7%) from the total samples, the number of the positive samples for women was 38 (45.3%) and for men were 46 (54.7%) . A symptomatic samples were represented (60.5%) 23 and(58.7%) 27 from the total positive samples for women and men respectively, the differences was not significant between asymptomatic and symptomatic bacteruria with diabetic patients sex , as shown in Table-1.

Table 1-Symptomatic and a symptomatic bacteruria in diabetic patients

variables	A symptomatic	symptomatic
Diabetic women	23(60.5%)	15(39.5%)
Diabetic men	27(58.7%)	19(41.3%)

$X^2=0.03$ $p<0.05$

The patients who had a history of UTI were 22 (58%) for women and 19(41%) for men , the Clinical characteristics of patients were dysuria which represented(26%) in women and (15%) in men , urinary frequency and loin pain represented (21%) in women and (11%), (30%) in men , while the nausea represented (10.5%) in women and (13%) in men. Other factors associated with UTI were hypertension (45%) and (26%) in both women and men and prostate enlargement (35%) in men as shown in Table-2.

Table 2-Clinical characteristics of patients and factors associated with UTI among diabetic patients

variables	Women n=38 (100%)	Men n=46 (100%)
History of UTI	22 (58%)	19 (41%)
Dysuria	10 (26%)	7 (15%)
Urinary frequency	8 (21%)	5 (11%)
loin pain	8 (21%)	14 (30%)
Nausea	4 (10.5%)	6(13%)
hypertension	17 (45%)	12 (26%)
Prostate enlargement	-	16 (35%)

Samples were analyzed for pyuria microscopically and leukocytes results in urine samples as seen in Table-3. The presence of leukocytes >10 WBC/HPF in the urine predict a positive urine culture and hence indicates urinary tract infection and a combination of leukocytes and bacteruria measured by HPF appears to be very useful marker in diagnosis of UTI [11].

Table 3-Leukocytes counts resulted by microscopic examination pyuria

WBC/HPF	N= 84
0-4	17
5-15	26
16-25	33
26-35	3
abundant	5

The bacterial diagnosis depend on the gram stain and the general morphological characterization on Mac-Conkey and blood agar , as they were pink and had a special Oder , dray, medium size and convex colonies which relatively related to *Escherichia spp* and other colonies were mucoid big size, more regular and pink which may related to *Klebsiella spp*. Catalase test was positive and oxidase test was negative for all bacterial isolation, and the other isolates which grow only on blood agar, they appeared yellow, smooth, middle in size and there was a heamolysis .

The predominant bacterial isolates were *E. coli* (24) isolates represented (28.6%) followed by *Klebsiella spp*_(17) which represented (20%) , *S. aureus* represented (14) isolates represented (16.7%) , *Proteus spp*(13) isolates represented (15.5%) , *Streptococcus fecalis* (11) isolates represented

(13%) and *Candida albicans* (5) isolates represented (6.2%). As shown in Table -4 and Figure-1. There was no significant difference between the bacterial infections and the sex of the patients.

Table 4-Distribution and percentage of pathogens among diabetic patients

Pathogens	NO. of isolates	Women	Men
<i>E coli</i>	24(28.6%)	16 (66.7%)	8(33.3%)
<i>Klebsiella spp</i>	17(20%)	10(59%)	7(41%)
<i>S. aureus</i>	14(16.7 %)	9(64.3%)	5(36%)
<i>Proteus spp</i>	13(15.5%)	6(46%)	7(54%)
<i>Streptococcus fecalis</i>	11 (13%)	6(54.5%)	5(45.5%)
<i>Candida albicans</i>	5(6.2%)	4(80%)	1(20%)
Total	84(100%)	38(45.3%)	46(54.7%)

T test=0.6 p < 0.01

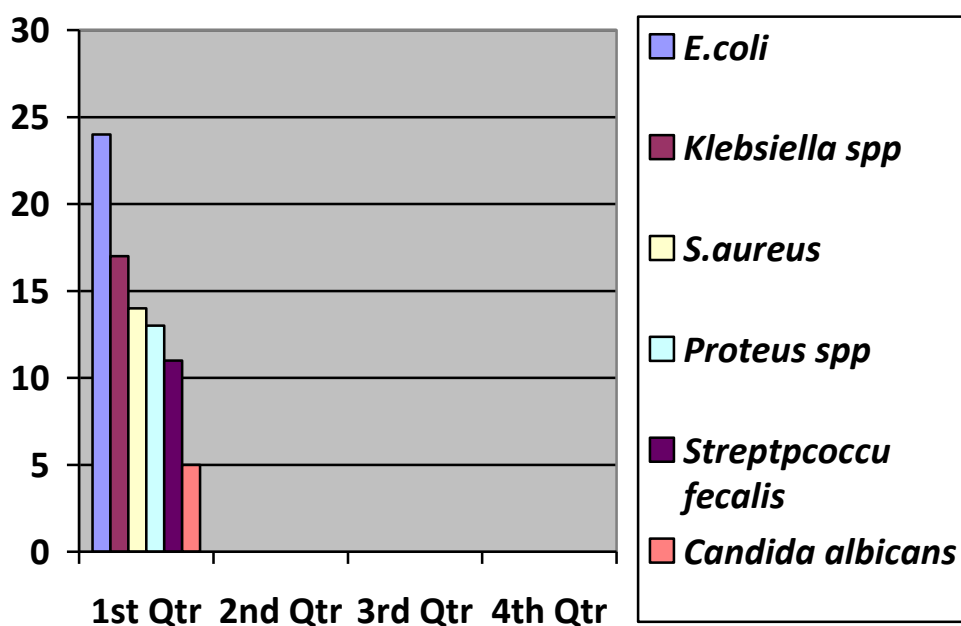


Figure 1-The incidence of pathogenic isolation among diabetic patients

The most common microorganisms were tested for their susceptibilities to several antimicrobial used in the study, Table-5 shows the resistance and sensitivity rate of gram positive cocci. *S.aureus* has developed high resistance to ampicillin and amoxicillin (71.4% and 57%) respectively but *streptococcus fecalis* remained susceptible to ampicillin and amoxicillin (73% and 64%). Both *S.aureus* and *Streptococcus fecalis* have resistance to erythromycin (64% and 82%). Three gram negative bacteria were tested for their susceptibility and resistance to the antibiotics, *E. coli*, *Klebsiella spp* and *Proteus spp* were resistance to ampicillin (54 % , 88% and 80%) and amoxicillin (58%,82% and 80%), and sensitivity to trimethoprim-sulfamethoxazol was common among these microorganisms as shown in Table-6.

Table 5-Ratio of sensitive and resistant gram positive isolated bacteria

Antibiotics	<i>S. aureus</i> n=14(100%)		<i>S. fecalis</i> n=11(100%)	
	sensitive	Resistant	sensitive	Resistance
Ampicillin	4(28.6%)	10(71.4%)	8(73%)	3(27%)
Amoxicillin	6(43%)	8(57%)	7(64%)	4(36%)
Cefotaxim	4(28.6%)	10(71.4%)	3(27%)	8(73%)
Erythromycin	5(36%)	9(64%)	2(18%)	9(82%)
Nalidixic acid	-(0%)	14(100%)	-(0%)	11(100%)
Norfloxacin	-(0%)	14(100%)	-(0%)	11(100%)
Ciprofloxacin	-(0%)	14(100%)	-(0%)	11(100%)
Gentamicin	3(21%)	11(79%)	1(9%)	10(91%)
Trimethoprim-sulfamethoxazole	-(0%)	14(100%)	-(0%)	11(100%)
Chloramphenicol	-(0%)	14(100%)	-(0%)	11(100%)

Table 6- Ratio of sensitive and resistant gram negative isolated bacteria.

Antibiotics	<i>E.coli</i> n=24(100%)		<i>Klebsiella spp</i> n=17 (100%)		<i>Proteus spp</i> n=13 (100%)	
	sensitive	resistant	sensitive	resistant	sensitive	resistant
Ampicillin	11 (46%)	13 (54%)	2 (12%)	15 (88%)	3 (20%)	10 (80%)
Amoxicillin	10 (42%)	14 (58%)	3 (18%)	14 (82%)	3 (20%)	10 (80%)
Cefotaxim	19 (79%)	5 (21%)	13 (76%)	4 (24%)	11 (85%)	2 (15%)
Erythromycin	11 (46%)	13 (54%)	5 (29%)	12 (71%)	3 (20%)	10 (80%)
Nalidixic acid	17 (71%)	7 (29%)	15 (88%)	2 (12%)	10 (80%)	3 (20%)
Norfloxacin	19 (79%)	5 (21%)	17 (100%)	0 (0%)	12 (92%)	1 (8%)
Ciprofloxacin	20 (83%)	4 (17%)	17 (100%)	0 (0%)	9 (69%)	4 (31%)
Gentamicin	19 (79%)	5 (21%)	15 (88%)	2 (12%)	11 (85%)	2 (15%)
Trimethoprim-sulfamethoxazole	16 (67%)	8 (33%)	15 (88%)	2 (12%)	9 (69%)	4 (31%)
Chloramphenicol	10 (42%)	14 (58%)	8 (47%)	9 (53%)	11 (85%)	2 (15%)

Discussion:

The main findings of the present study were that the prevalence of asymptomatic bacteruria among diabetic patients was 60.5%, 58.7% in women and men respectively. Age of the diabetic patients was not associated with the prevalence of UTI, but the investigated clinical characteristics of patients and other factors were associated with UTI among diabetic patients. Similar findings were reported for diabetic patients in Saudi Arabia and India [12,13]. Diabetic patients are at increased risk of infection in general and, in particular, to UTI and the susceptibility of diabetic patients to UTI could be explained by diminished neutrophil response, lower urinary cytokines, and leukocyte concentrations, which might facilitate the adhesion of microorganisms to uroepithelial cells [14].

The study indicate that *E. coli* is still the most common cause of UTI between our diabetic patients (28.6%) Followed by *Klebsiella. spp* (20%), *Staph aureus*(16. 7%) and this corresponds with the data obtained by other study in Turkey as from 860 urine positive samples *Escherichia coli* and *Klebsiella* species were the most frequently isolated bacteria from both outpatients and hospitalized patients [15]. And in other Turkish study, twenty-four bacterial species were identified, with *Pseudomonas aeruginosa* (5%) *Escherichia coli* (4%), and *Staphylococcus epidermidis* (3%) being the most frequent [16].

In Egypt, The identified bacterial species were arranged in a descending order according to their frequency percentage viz. *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Proteus vulgaris*, *Streptococcus pneumoniae*, *Staphylococcus saprophyticus* [17].

Enterobacteria were the most frequent (85%) and especially *Escherichia coli* (44.7%). Gram positive bacteria (11.6%) were more frequently *Staphylococcus saprophyticus* (3.1%) in other study from UK [18]. In Karachi *E.coli* (43.1%) was most frequent, followed by *Klebsiella pneumoniae* (22.4%) and *Staphylococcus aureus* (15.5%) [19].

This study showed that *Candida albicans* represented 6.2% from the total isolates, 80% in women and 20% in men, other study showed that the epidemiology of 54 episodes of candiduria, the isolation of *Candida* in urine were studied in an observational survey over 3 months. *Candida* spp. was isolated from 4.7% of positive urine cultures [20].

Another study in teaching hospital of Ahvaz 744 urine samples, the prevalence of candiduria in subjects were 16.5% included 65.1% women and 34.9% men, and the most common isolates were *candida albicans* 53.3% [21]. The antibiotic resistant calculated for *E.coli* was (54%) resistant to ampicillin and erythromycin, and (58%) resistant to amoxicillin, which was correlate with other study found that *E. coli* was highly resistant to erythromycin, ampicillin and amoxicillin (90%, 80%, and 69%) [22].

In another study [23] showed *E.coli* as the commonest cause of UTI and antibiotic resistant was high among the strains which emphasize the need for judicious of antibiotics. Certain virulence factors like hemolysin production and presence of fimbriae in the *E.coli* may be associated with urovirulence [24]. The susceptibility studies showed 37% *E. coli* strains resistant to amoxicillin+clavulanate 33% to cotrimoxazole and 22% to ciprofloxacin [25]. Other study showed that thirteen percent of *E.coli* strains isolated from UTI were resistant to cefotaxime but only (1%) to fosfomycin. *Klebsiella pneumoniae* was resistance to nitrofurantoin 38%, *Proteus mirabilis* showed 52% resistance to cotrimoxazole and 13% *Staphylococcus aureus*, were methicillin-resistant [26]. In Nigeria out of 150 samples, 28 (18.7%) yielded *S. aureus* with the highest isolation from urine (8.7%), the highest sensitivity is recorded for chloramphenicol (36%) while the least was for lincocin (7%), erythromycin, rifampin, gentamicin and ciprofloxacin recorded 18%, 14%, and 11% respectively. Resistance is recorded for norfloxacin (100%), streptomycin (100%), floxapin (100%) and ampiclox (100%) [27].

Streptococcus fecalis remained susceptible to ampicillin and amoxicillin (73% and 64%) respectively but it has developed high resistance to Ciprofloxacin and gentamicin and this was recorded in other studies [28].

Conclusion:

In both symptomatic and asymptomatic diabetic patients with UTI in the study, *E. coli* was the most frequent isolate followed by *Klebsiella spp.* Gram-positive bacteria were sensitive to amoxicillin, while all Gram-negative organisms showed higher sensitivity to ciprofloxacin.

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