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Research Article
Bacterial Quality of Urinary Tract Infections in Diabetic and Non Diabetics of the Population of Ma'an Province, Jordan

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Abstract

Background and Objective: The patients with Diabetes Mellitus (DM) have malfunction in bladder which prompt urine accumulation in its pool which serves a decent situation to the microbes to be develop and cause Urinary Tract Infection (UTI). The UTI is the most infectious disease that affects both males and females. This study was designed to detect the bacterial species responsible for UTI in both diabetic and non-diabetic patients in Ma'an province, Jordan. **Materials and Methods:** One hundred sixteen urine samples were investigated to determine UTI-causing bacteria. These samples distributed unequally between diabetic male (12) and diabetic female (25) and also non-diabetic male (13) and non-diabetic female (66). **Results:** It was observed that *E. coli* is responsible for large proportion (44.8%) of UTI in both diabetic (15.5%) and non-diabetic (29.3%) patients. This study showed inequality in the bacterial species that were isolated from both diabetic and non-diabetic samples. However, five bacterial species including *E. aerogenes, E. cloacae, C. freundii, A. baumannii* and *B. subtilis* did not exist in all diabetic samples. Treatment of UTI in both diabetic and non-diabetic patients with chloramphenicol (30 µg), ciprofloxacin (5 µg) and vancomycin (30 µg) resulted in more favorability than other antibiotics. At the same time cephalothin (30 µg) was not recommended. **Conclusion:** *Escherichia coli* was the prevailing bacterial infections among those which were isolated from patients with UTI. Certain forms of bacterial infections inclined to be extra common in diabetic patients than others and other infections may be more severe in people with diabetics than in non diabetics.

Key words: Urinary tract infections, non-diabetic, UTI pathogens, diabetes mellitus, *E. coli*, prevalence

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Urinary Tract Infections (UTIs) are recognized as one of the most widely recognized infectious diseases which are caused by various microorganisms. It's the second most infectious source in medicinal practice groups. Around the world, about 150 million individuals are diagnosed with UTI every year[1-5]. The most widely recognized microscopic organisms that cause UTI are gram negative microbes as *Escherichia coli* and Gram positive microorganisms as *Staphylococcus aureus*.

Diabetes Mellitus (DM) is a metabolic disorder that is described by rising of blood glucose because of incomplete or missing of insulin hormone. The patients with DM have malfunction in bladder which prompt of urine accumulation in its pool which serves a decent situation to the microbes to be develop and cause UTI[2,4,6].

Variable factors that have been proposed as constituting an increased risk for UTIs in diabetics include age, metabolic control, length of time of DM, diabetic cystopathy, more regular hospitalization and instrumentation of the urinary tract, repetitive vaginitis and vascular complexities[3-9].

Furthermore, a higher glucose level in the urine might make a culture medium for pathogenic microorganisms. In spite of the fact that the connection in the middle of diabetes and bacteriuria has been the subject of a few controlled studies, the relationship between diabetes and UTI hazard has not been analyzed until know[4,6,7,10,11].

Diabetes mellitus has for quite some time been thought to be an inclining element for UTI and the urinary tract is the fundamental site of the contamination in diabetics with raised risk of complications of UTI. The important recognized reason for UTI in patients with and without DM is *Escherichia coli*. In non-diabetic patients, the rate of microbes that cause UTI are: *Escherichia coli* 31.4% in males and 58.2% in females, *Enterococcus* spp., 9.4% in males and 6.5% in females, *Pseudomonas* spp. 17.2% in males and 4.7% in females . On other hand, the rate of microbes that cause UTI in diabetic female are 54.1% *Escherichia coli*, 8.3% *Enterococcus* spp., 3.9% *Pseudomonas* spp., while in diabetic male are 32.5, 9.4, 8.5%, respectively[5].

While it is known that acute infection leads to struggle controlling level of blood sugar, continued debate about whether or not diabetic patients are more likely to be subject to infection than age and sex-matched non diabetic control patients[1,2,4]. Adjusting the level of blood sugar in diabetic patients is required to avoid certain bacterial infections and to guarantee care of typical host immune that enables resistance to infection[4-10].

This study aimed to assess the occurrence of UTI in diabetic patients in Ma'an Governorate population of Jordan referred to the type of microbiologically confirmed UTI and pattern of the antimicrobial drugs susceptibility were assessed.

MATERIALS AND METHODS

Collection of samples: One hundred sixteen patients were subjected to study from different medical centers from Ma'an province, Jordan. These patients have symptoms of urinary tract infection. All the samples were collected within 3 months from 5 to 51 years old patients of both male and female and diabetic or non-diabetic. All these patients did not receive any antimicrobial therapy for several weeks before sampling. The samples were collected at morning using sterile urine containers which were opened just in the sampling process to prevent any contaminations. The samples were transported to the laboratory to culture them on a suitable media for 24 h under aseptic techniques and were stored at 4°C for further study.

Isolation of bacteria: Upon arrival to the laboratory the samples were cultured on the nutrient agar. The pure colony that resulted from the first inoculation was cultured into 4 plates of MacConkey agar, mannitol salt agar, eosin methylene blue and blood agar to selective and differentiate the resulted colony. These entire five agars were incubated aerobically at 37°C and checked after 24 and 48 h.

Morphological identification: The isolated pure colonies from nutrient agar were examined under dissecting microscope (model SMZ, Nikon, Tokyo) to detect the morphological shape.

Identification of isolated bacteria: All samples of urine culture were verified within an hour of sampling. They were inoculated on blood agar as well as MacConkey agar and incubated at 37°C for 24 h and for 48 h in negative cases. A positive specimen was considered for UTI if a single organism was cultured at a concentration of $\geq 10^5$ CFU mL^{-1}, or when a single organism was cultured at a concentration of 10^4 CFU mL^{-1} and ≥ 5 leukocytes per high-power field were observed on microscopic examination of the urine. Bacterial identification was based on standard culture, morphological and biochemical characteristics of isolates[3,7,8,12].

Oxidase test: Oxidase test was used to determine if a bacterium produces certain cytochrome c and the enzyme cytochrome oxidases as a part of their respiratory chain. These

bacteria can therefore utilize oxygen for energy production with an electron transfer chain. This test can be performed within second using a specific strips impregnated with N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride, cytochrome oxidase oxidizes cytochrome c which in turn oxidizes the N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride producing a dark blue/violet colored product. Gently touch the colonies that will be tested with oxidase detection strip or remove a colonies to clean slide by a sterile loop and touch the strip to them. After that the result shown within 30 sec, the development of dark blue/violet color indicates an oxidase positive otherwise oxidase negative[13].

Catalase test: Catalase is the enzyme that breaks hydrogen peroxide (H_2O_2) into H_2O and O_2. It is easy to test for this enzyme in bacteria. A test culture is exposed to 3% H_2O_2. If catalase is present, H_2O_2 is broken down to H_2O and O_2 .The oxygen is detected as a steady evolution of gas bubbles from the culture. Firstly transfer a large isolated colony from the culture dish to a microscope slide. After that two drops of (3%) hydrogen peroxide reagent (H_2O_2) were added to the colony. If the air bubbling (gas) was produced, the bacteria is catalase positive, if there were no bubbles, the bacteria is catalase negative[14].

Coagulase test: This test is used to differentiate *Staphylococcus aureus* from other genus of coagulase negative *Staphylococcus*. The purpose of the coagulase test is to determine whether a bacterium produces coagulase, an enzyme capable of coagulating liquid plasma into a solid clot. This test can be performed by two methods, slide method (for bounded coagulase enzyme) and tube method (for free coagulase enzyme). In the tube method, lapful of tested organism was taken from broth culture and added to the tube which contain a plasma, here should be have a negative control tube by adding a culture to the empty tube or normal saline to plasma tube. Any degree of clot formation was considered to constitute a positive test. In slide method, two drops were mixed with a bacteriological needle and observed for clumping. Any degree of clumping was considered to constitute a positive reaction. The test can be summarized by taken one drop of sterile saline into each circle, after that emulsifying the tested colonies in saline in the first circle while another circle used as free of culture. Few drops of plasma (undiluted) were added to each circle and the results were seen within few minutes. If a clotting is seen in the form of plasma clumping so coagulase test is positive otherwise coagulase test is negative[15].

Microgen GN -ID system: The Microgen GN-ID comprises of only 12 substrates which are specifically selected to optimize the identification of the most commonly encountered oxidase negative Bacilli including the family Enterobacteriaceae and *Acinetobacter* spp. Combination of Microgen GN A+GN B identification systems was used for the identification of the commonly encountered Enterobacteriaceae from urinary samples that were oxidase-positive gram negative Bacilli. The bacterial culture was examined by gram stain and oxidase test prior to use of the Microgen GN-ID System[11,14].

Microgen *Bacillus*-ID system: Microgen *Bacillus*-ID has been developed for the identification of *Bacillus* spp. and related genera. It is simple and easy-to-use 24 reaction system and the results were examined in 48 h. The bacterial culture was examined by oxidase test prior to use of the Microgen *Bacillus*-ID System.

Microgen Strep-ID: Microgen Strep-ID is a biochemical test system which utilizes a 12 well (12 test) microwell test strip and 3 off-strip tests; hippurate hydrolysis (provided), alpha-hemolysis and beta-hemolysis for the identification of Streptococcal and Enterococcal species. Substrates that used were selected specifically to differentiate between *Streptococcus*, *Enterococcus* and related species by simple and easy method and obtained the results in 24 h.

Microgen Staph-ID: Microgen Staph-ID has been developed for the identification of commonly encountered *Staphylococcus* spp. Gram stain (positive), catalase (positive) and latex agglutination/coagulase tests are performed as pre-tests on the isolate. Substrates that used were selected specifically for *Staphylococcus* and related species by simple and easy method and get the results in 24 h.

Sensitivity test: Antibiotic Susceptibility Testing (AST) is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection. This test was performed according to disc diffusion method. By using series of antibiotic-disk, that are placed on the mueller-hinton agar media and inoculated to form a bacteria lawn. The plate was incubated with bacteria at 37°C for 24 h. If the organism is susceptible to antibiotic, a clear zone appears around the disk where growth has been inhibited. The inhibition zone depends on the sensitivity of the bacteria to the specific antibiotics and also the antibiotic diffusion through the agar[6-14]. The following antibiotics were used: ciprofloxacin,

gentamicin, nalidixic, ampicillin, amoxicillin, chloramphenicol, tetracycline, vancomycin, cefuroxime and cephalothin.

Statistical analysis: Data were analyzed using statistical package SPSS version 16. The percentages in different categories were compared using Chi square test[1,11].

RESULTS

Attempts were made to determine whether there was any difference in the bacterial quality and quantity of UTI and the antibiotic sensitivity patterns of the pathogens related between diabetic and non-diabetic patients. This study showed dissimilarity in the bacterial species that were isolated from both diabetic and non-diabetic samples. All the information regarding to the 116 patients is shown in Table 1 and 2.

Morphological identification: The identification of UTI-causing bacteria was made using standard morphological characteristics. The microscopically examination and the stain procedures of all samples (Table 3-5) showed that samples include both Gram positive and Gram negative bacteria.

Biochemical characteristic: All the samples were divided into 5 groups depending on the results shown in Table 6. First group include gram positive bacilli bacteria that was treated with Microgen *Bacillus*-ID Kit. Second group is gram negative oxidase negative bacteria that was treated with Microgen GN A -ID Kit. Third group is gram negative oxidase positive bacteria that was treated with Microgen GN A+B-ID Kit. Fourth group is gram positive cocci in chain that was treated with Microgen Strep-ID Kit. The last group is gram positive cocci in cluster that was treated with Microgen Staph-ID Kit (Table 7).

Sensitivity test: All isolated bacteria were tested using different discs of antibiotic using a procedure which is previously discussed to check antibiotic resistance profile for diabetic-UTI and non-diabetic-UTI patients (Table 8, 9).

Table 1: Sex and number of diabetic and non-diabetic patients with UTI

Gender	Male (%)	Female (%)
Non diabetic	13 (16.5)	66 (83.5)
Diabetic	12 (32.4)	25 (67.6)
Total	25 (21.5)	91 (78.5)

DISCUSSION

This study demonstrated that both gram positive and gram negative bacteria may cause UTI with different percentages (Table 7). The samples that have a bacterial count; $\geq 10^5$ cells mL^{-1} were considered as confirmed UTI and they required suitable antibiotic treatment[16]. Female patients exhibit a high percentage of UTI-causing bacteria, 56.9 and 21.6% for non-diabetic and diabetic females, respectively.

These results probably attributed to a short urethra and the closed vagina opening to the anal region[10-14]. However, 11.2 and 10.3% for non-diabetic and diabetic male constituting a 21.5% of all patients[1]. In diabetic and non-diabetic samples, *E. coli* has a higher incidence than another UTI-causing pathogenic. *Escherichia coli* as a member of Enterobacteriaceae with a 44.82% indicated the presence of fimbriae which facilitate them to invade epithelial cells of urinary tract causing UTI[7-23]. As in the most previous results including this study, *E. coli* is the most prevalent causative bacteria of UTI[3-6]. This case occur because *E. coli* have different Virulence Factors (VFs) that enable them to origin infection. One of these VFs is the presence of adherence organelles;

Table 2: Age and sex distribution of diabetics and non-diabetic patients group with UTI

	Diabetic patients				Non-diabetic patients			
	Male		Female		Male		Female	
Patients age groups (year)	No.	%	No.	%	No.	%	No.	%
5-10	3	25.0	0	0.0	0	0.0	6	9.09
11-20	0	0.0	0	0.0	5	38.46	10	15.15
21-30	0	0.0	6	24.0	4	30.77	19	28.79
31-40	2	16.67	2	8.0	3	23.08	27	36.36
41-50	7	58.33	11	44.0	1	7.69	7	10.61
51-60	0	0.0	6	24.0	0	0.0	0	0.0
Total	12	1034	25	21.55	13	11.21	66	64.9

fimbriae (Type 1 fimbriae, S fimbriae, P fimbriae and afimbrial adhesion) that increase the chance of adherence of *E. coli* to uroepithelial tissue which is a required step of UTI[10-14]. Other virulence factors are aerobactin, cytotoxic necrotizing factor and hemolysin that differ in the action which lead to UTI[11].

Table 3: Bacterial species were isolated from patients affected with UTI

Bacterial species	Percentages
E. coli	44.82
P. aeruginosa	7.76
K. pneumonia	6.90
E. aerogenes	3.45
E. cloacae	1.72
P. mirabilis	10.34
S. marcescens	1.72
C. freundii	0.86
A. baumannii	0.86
S. aureus	5.17
S. pyogenes	2.59
E. faecalis	6.03
S. epidermidis	3.45
B. subtilis	1.72
S. saprophyticus	2.59

Proteus mirabilis represented 10.34% of UTI-infected samples with which prevail in female rather than male. *Proteus mirabilis* is the second type of bacteria that cause UTI due to expression of four types of fimbriae (as in *E. coli*) that lead to adherence step and being peritrichous that enable bacterial cells to swim and attacking uroepithelial tissue. These flagella-mediated motility is required to ascend the ureters to the kidney and cause UTI[17]. *Pseudomonas aeruginosa* represented a third UTI-causing bacteria with 7.76% in both sex. The resistance of *P. aeruginosa* for antiseptic techniques in the hospitals helps these bacteria to cause UTI-infection[18]. Another reason that cause UTI by *P. aeruginosa* is the presence of capsule that protect them from phagocytosis and peritrichous flagella that move toward uroepithelial tissue[18]. *Klebsiella pneumoniae* represented 6.90% of infected samples, which is in agreement with the results of Daza *et al*.,[19]. These bacteria have the same way for infection as *E. coil* due to the presence of capsule and fimbriae, which facilitate the invasion and entrance to urinary

Table 4: Sex, cell shape, colony appearance and colony color for bacterial cells isolated from diabetic and non-diabetic UTI samples

		Colony appearance				
Bacterial species	Morphology	Form	Elevation	Margin	Appearance	Colony color
A. baumannii	Rods	Circular	Mucoid	Entire	Smooth	Slightly pink
B. subtilis	Rods	Circular	Flat	Undulate	Rough	Milky
C. freundii	Rods	Circular	Convex	Entire	Rough	Slightly pink
E. aerogenes	Rods	Circular	Convex	Entire	Rough	Pinkish
E. cloacae	Rods	Circular	Convex	Entire	Rough	Slightly pink
E. coli	Rods	Irregular	Convex	Circular	Rough	Pinkish
E. faecalis	Cocci in clusters	Circular	Convex	Entire	Smooth	Milky
K. pneumonia	Rods	Circular	Mucoid	Entire	Rough	Slightly pink
P. aeruginosa	Rods	Circular	Convex	Entire	Rough	Green
P. mirabilis	Rods	Circular	Convex	Entire	Rough	Slightly pink
S. aureus	Cocci in clusters	Circular	Convex	Entire	Smooth	Yellow
S. epidermidis	Cocci in clusters	Circular	Convex	Entire	Smooth	White
S. marcescens	Rods	Circular	Convex	Entire	Rough	Red
S. pyogenes	Cocci in chain	Circular	Convex	Entire	Rough	Grayish-white
S. saprophyticus	Cocci in clusters	Circular	Convex	Entire	Smooth	White

Table 5: Gram stain, presence of capsule, spore and flagella of isolated bacteria from diabetic and non-diabetic UTI samples

Bacterial species	Gram stain	Capsule stain	Endospore stain	Flagella stain
A. baumannii	Gram negative	Capsulated	Non-spore former	Un-flagellated
B. subtilis	Gram positive	Capsulated	Spore former	Monotrichous
C. freundii	Gram negative	Non-capsulated	Non -spore former	Peritrichous
E. aerogenes	Gram negative	Capsulated	Non -spore former	Monotrichous
E. cloacae	Gram negative	Capsulated	Non -spore former	Peritrichous
E. coli	Gram negative	Capsulated	Non -spore former	Peritrichous
E. faecalis	Gram positive	Capsulated	Non -spore former	Peritrichous
K. pneumonia	Gram negative	Capsulated	Non -spore former	Peritrichous
P. aeruginosa	Gram negative	Capsulated	Non -spore former	Peritrichous
P. mirabilis	Gram negative	Capsulated	Non -spore former	Peritrichous
S. aureus	Gram positive	Capsulated	Non -spore former	Un-flagellated
S. epidermidis	Gram positive	Capsulated	Non -spore former	Un-flagellated
S. marcescens	Gram negative	Capsulated	Non -spore former	Peritrichous
S. pyogenes	Gram positive	Capsulated	Non -spore former	Un-flagellated
S. saprophyticus	Gram positive	Capsulated	Non -spore former	Un-flagellated

Table 6: Oxidase test, catalase test, coagulase test, urease test and hemolysis pattern of bacterial isolated from diabetic and non-diabetic UTI samples

Bacterial species	Oxidase test	Catalase test	Coagulase test	Hemolysis pattern	Urease test
A. baumannii	Negative	Positive	Negative	γ	Negative
B. subtilis	Positive	Positive	Negative	β	Negative
C. freundii	Negative	Positive	Negative	γ	Positive
E. aerogenes	Positive	Positive	Negative	γ	Positive
E. cloacae	Negative	Positive	Negative	γ	Negative
E. coli	Negative	Positive	Negative	γ	Negative
E. faecalis	Negative	Negative	Negative	γ	Negative
K. pneumonia	Negative	Positive	Negative	γ	Positive
P. aeruginosa	Positive	Positive	Negative	β	Negative
P. mirabilis	Negative	Positive	Negative	γ	Positive
S. aureus	Negative	Positive	Positive	β	Positive
S. epidermidis	Negative	Positive	Negative	γ	Positive
S. marcescens	Negative	Positive	Negative	γ	Positive
S. pyogenes	Negative	Negative	Negative	β	Negative
S. saprophyticus	Negative	Positive	Negative	γ	Positive

Table 7: Distribution of isolated bacteria in the diabetic and non-diabetic UTI samples for both sexes and its identification kit

	Female		Male			
Bacterial isolates	Diabetic	Non-diabetic	Diabetic	Non-diabetic	Total	Percentage
Gram negative						
E. coli	12	28	6	6	52	44.82
P. aeruginosa	2	4	3	0	9	7.76
K. pneumonia	1	3	1	3	8	6.90
E. aerogenes	0	4	0	0	4	3.45
E. cloacae	0	2	0	0	2	1.72
P. mirabilis	2	8	1	1	12	10.34
S. marcescens	1	0	0	1	2	1.72
C. freundii	0	1	0	0	1	0.86
A. baumannii	0	0	0	1	1	0.86
Gram positive						
S. aureus	2	4	0	0	6	5.17
S. pyogenes	2	1	0	0	3	2.59
E. faecalis	1	6	0	0	7	6.03
S. epidermidis	1	2	1	0	4	3.45
B. subtilis	0	2	0	0	2	1.72
S. saprophyticus	1	1	0	1	3	2.59
	21.6%	56.9%	10.30%	11.2%	99.98	

tract of both male and female and cause UTI. Another Enterobacteriaceae species exhibit a low percentage of infected samples; *E. aerogenes* (3.45%), *E. cloacae* (1.72%), S. *marcescens* (1.72%), C. *freundii* (0.86%) and *A. baumannii* (0.86%). It's worth mention that all these bacterial species are pathogenic due to presence of capsule of all except *C. freundii*.

As previously reported by Daoud and Afif[2] and Althunibat *et al*.[16], *E. coli* is highly responsible for UTI (53.24%) followed by *E. faecalis* and *P. mirabilis* (24.05 and 19.537%, respectively). While in this study *E. faecalis* occupied fifth position but in the first line related to gram positive bacteria. The inequalities in the type and distribution of UTI-causing bacteria may result from different environmental conditions, host factors and practices such as healthcare and education programmers, socioeconomic standards and hygiene practices in each community. In this study, diabetic samples (37) (31.9%) distributed among 12 males (32.4%) and 25 females (67.6%). These bacterial species that were isolated from diabetic samples represent parts of bacterial species that were isolated from non-diabetic samples.

Escherichia coli presented equally in diabetic males (50%) and diabetic females (48%) of samples. This is probably as a result of UTI-causing bacteria with the same pattern. *Pseudomonas aeruginosa* is the second bacterial species that were isolated from diabetic samples (8% of diabetic females and 25% of diabetic males). The higher presence of *P. aeruginosa* in urine samples of diabetic male compared with that of non-diabetic male (0.0%) probably due to the immune suppression occurred by opportunistic UTI[20]. *Enterobacter aerogenes, E. cloacae, C. freundii, A. baumannii*

Table 8: Antimicrobial inhibition zone diameter (mm) against isolated bacteria from diabetic and non-diabetic UTI samples

Bacteria species	C_{30}	Na_{30}	Cip_5	Va_{30}	TE_{30}	Ceu_{30}	Cep_{30}	Am_{75}	G_{10}	Amp_2
E. coli	21	22	33	24	9	17	-	22	7	8
P. aeruginosa	-	-	14	9	-	-	-	-	18	7
K. pneumonia	11	7	21	15	8	-	-	24	8	7
E. aerogenes	7	-	25	15	7	-	12	9	17	9
E. cloacae	8	5	10	11	7	-	-	7	7	4
P. mirabilis	8	23	35	32	-	11	-	18	20	9
S. marcescens	7	7	18	12	4	15	4	8	4	7
C. freundii	9	5	15	11	8	4	5	5	5	-
A. baumannii	10	4	10	10	3	5	7	10	4	-
S. aureus	29	19	33	22	10	12	5	18	15	-
S. pyogenes	24	10	29	18	5	8	4	9	12	19
E. faecalis	20	12	27	12	14	-	4	4	9	3
S. epidermidis	30	12	17	22	11	-	-	2	9	7
B. subtilis	24	11	14	11	9	5	4	7	9	10
S. saprophyticus	19	16	19	12	9	-	-	4	12	4

C_{30} : Chloramphenicol 30 µg, Na_{30} : Nalidixic 30 µg , Cip_5 : Ciprofloxacin 5 µg, Va_{30} : Vancomycin 30 µg, TE_{30} : Tetracycline 30 µg, Ceu_{30} : Cefuroxime 30 µg, Cep_{30} : Cephalothin 30 µg, Am_{75} : Amoxicillin 75 µg, G_{10} : Gentamicin 10 µg, Amp_2 : Ampicillin 2 µg

Table 9: Effectiveness percentage of different antibiotics for isolated bacteria from diabetic and non-diabetic UTI samples

		No. of isolated bacteria		
Antibiotics	Disc code	Sensitive	Resistant	Percentages
Chloramphenicol	C_{30}	89	27	76.72
Nalidixic	Na_{30}	84	32	72.43
Ciprofloxacin	Cip_5	97	19	83.62
Vancomycin	Va_{30}	93	23	80.17
Tetracycline	TE_{30}	47	69	40.52
Cefuroxime	Ceu_{30}	34	82	29.31
Cephalothin	Cep_{30}	31	85	26.72
Amoxicillin	Am_{75}	61	55	52.59
Gentamicin	G_{10}	57	59	49.14
Ampicillin	Amp_2	66	50	56.89

and *B. subtilis* are five bacterial species that were not isolated from all diabetic samples in this study. Although there are several studies represented them in both male and female affected with diabetes mellitus. Because, firstly the number of diabetic samples is low compared with other studies, secondly the percentage of isolation of these species generally low specially Gram negative species (*E. aerogenes, E. cloacae, C. freundii* and *A. baumannii*)[21].

Although the relationship between sugar level and risk of UTI in diabetes is controversial, DM has for a long time been associated with increase in prevalence of bacteria compared with patients without diabetes[20,22]. In women case, the prevalence of bacteria is high if the women is diabetic but the diabetic men are more suspected to UTI than diabetic women[2]. According to morphological studies, immunohistochemistry evaluation and biochemical tests, there are no differences in behavior of bacterial species that were isolated from diabetic and non-diabetic samples as shown in the Table 4, 5 and 6 suggesting that the bacteria causing UTIs in diabetic patients are the same as in UTIs in non-diabetic patients. Many studies had shown that DM increase the risk of UTI through different mechanisms. The mechanism in the pathogenesis of the increased prevalence of UTI in diabetic patients is glucosuria that enhanced bacterial growth through the increase in cells number;[3] suggesting the neutrophils dysfunction[4-6,8-11]. Multi-studies showed that polymorphonuclear cells of patients with DM show decrease in number and function (chemotaxis, phagocytosis and killing) of them[3]. In addition, local cytokine secretion might be of importance. Cytokines are small proteins that play an important role in the regulation of host defenses against bacterial infections. Urinary cytokine excretion IL-8 and IL-6 concentrations have been low in diabetic patients than in non diabetic patients. Lower urinary leukocyte cell count correlated with lower urinary IL-8 and IL-6 concentrations. This might contribute to the increased incidence of UTIs in this patient group[23].

The third suggested mechanism for the increased risk of bacteriuria in patients with DM is an increased adherence of bacteria, which can be due to either a decrease anti-adherence activity of the urine and an enhanced adherence capacity of uroepithelial cells[19]. Anti-adherence

mechanism performed by a glycoprotein called Tamm-Horsfall Protein (THP) which is produced from kidney and prevent bacterial fimbriae (type 1 and S) from attachment with uroepithelial tissue. This protein level in DM patients was low that enhance adherence of bacteria to uroepithelial tissue and caused UTI[3].

All isolated bacterial species, Gram negative and Gram positive, were treated with various antibiotics in order to select suitable antibiotic for treating the patients in early stage of UTI. This sensitivity profile was checked by disk-diffusion method using different types of antibiotic that belonged to different antibiotic families[16].

The sensitivity and resistance level of commonly used antibiotics (Table 8, 9) were different from one bacterial species to another depending on the mechanism of the antibiotics action. Gram positive bacteria could be sensitive mainly to chloramphenicol, which belong to chloramphenicol family that inhibits the protein synthesis by inhibition of peptidyl transferase enzyme[24]. Ciprofloxacin (fluoroquinolones family) and vancomycin (glycopeptides antibiotic) were found the most effective antibiotic to all isolated bacterial species in both diabetic and non-diabetic UTI, although both of them were differ in its mechanism, ciprofloxacin prevent DNA synthesis by inhibition of gyrase and topoisomerase enzymes, while vancomycin prevents cell wall synthesis by inhibition of N-Acetylmuramic acid (NAM) and N-Acetylglucosamine (NAG) production[21-25] .

Mainly cefuroxime (cephalosporin family), cephalothin (cephalosporin family) and ampicillin (aminopenicillin family) were resistance to the most isolated species, due to presence of β-lactamase enzyme, which attacked with β-lactam ring that found in the structure of these antibiotics. Therefore, to use these antibiotics in UTI treatment, it should be combined with β-lactamase inhibitor such as clavulanic acid[23,26]. Nalidixic acid (fluoroquinolones family), tetracycline (tetracycline family) and gentamicin (aminoglycoside family) can't be used for all isolated species because these antibiotic not sensitive from all isolated species. *Klebsiella pneumoniae* is sensitive to amoxicillin that belong to β-lactam antibiotic family which means that β-lactamase is not effective while amoxicillin stay active[27-29]. Augmentin is a combination between amoxicillin and clavulanic acid that was used widely to treat UTI in both diabetic and non-diabetic, the combination between them to prevent interaction between β-lactamase enzyme and β-lactam[23,24].

This study showed that diabetic and non-diabetic UTI pathogens decrease susceptibility to the most types of antibiotics, so it is very necessary to develop new antimicrobial and therapeutic agents that have high effectiveness with no side effect, easy availability and also less expensive. As conclusion, *Escherichia coli* are the most common bacterial species that cause urinary tract infection in both diabetic and non-diabetic patients[21-25]. Generally, Gram negative bacteria (especially that belong to Enterobacteriaceae family) are the most common UTI-causing bacteria than Gram positive bacteria. Although there is no significant difference between the same bacterial species isolated from the urine sample of diabetic and non-diabetic patients, the bacterial cells in the diabetic sample are more than that from non-diabetic one. The UTI patients can be treated with different types of antibiotic as ciprofloxacin regardless of male or female[30], single or married, diabetic or not. But should be taken in mind if the patients are pregnant or not (if female) and child or adult or aged. Beside it is worth to investigate if there are any growth determinants of the bacterial cells in diabetic urine sample.

CONCLUSION AND RECOMMENDATIONS

It is concluded that *E. coli* was the predominant bacterial isolate among those were isolated from patients with UTI. Certain forms of bacterial infections inclined to be extra common in diabetic patients than others and other infections may be more severe in people with diabetics than in non-diabetics. The next step is to study the immunological defects that may lead to an increase in exposure to infections and assistance in the development of guidelines for the prevention of urinary tract infection. Beside it is worth to investigate if there are any growth determinants of the bacterial cells in diabetic urine sample.

SIGNIFICANCE STATEMENT

The UTIs are more common in diabetic patients than in non-diabetic patients showing *E. coli* as being the most common isolate. Searching for UTI in diabetic patients is important for treatment and prevention of renal complications. Therefore, urine culture should be made in all hospitalized diabetic patients.

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