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RESEARCH ARTICLE OPEN ACCESS

Prevalence and Sensitivity of Bacterial Urinary Tract Infection among Adult Diabetic Patients in Misan Province, Iraq

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Abstract

Urinary tract infections and their complications causing serious health problems especially in diabetic patients in Misan province, it is essential to determine the causative agent for appropriate treatment of this disease. One hundred and fifty urine samples were taken from adult patients with diabetes. The microbial growth appeared in 106 (70.6%) samples. Axenic culture was 83% versus 17% mixed growth. UTI prevalence was 65% with females comparing 35% of males and it was 56.6% in age group 35-49 years. Furthermore, UTIs were more incident in patients without antibiotics use and diabetic period 5-10 years (82% and 64%) respectively. *Escherichia coli* was identified as the most common causative agent of UTIs (52.9%), followed by *Klebsiella pneumoniae* and *Enterococcus faecalis* (9,1% and 6.6%, respectively). *Streptococcus agalactiae* and *Klebsiella aerugenes* were (4.2%, for each). Moreover, *Pseudomonas aeruginosa*, *Klebsiella oxytoca* and *Staphylococcus haemolyticus* were 2.48% for each. Additionally, the frequency of *Proteus mirablis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Serratia marcessens*, *Staphylococcus epidermidis* and *Staphylococcus warnerii* was 1.65% for each, while *Acinetobacter baumani* and *Bacillus subtilis* were 0.82% for each. Most bacterial isolates had a high sensitivity to imipenem (78.8%) followed by amikacin (61.9%), but low sensitivity to ceftriaxone, tetracycline and Co-trimaxazole (36.4%, 29.7% and 26.3%, respectively) whereas, highly resistance to ampiclox and nitrofurantoin (98.3% and 87.3%, respectively). High rate of multidrug resistance observed among bacterial isolates.

Keywords: UTIs; diabetes; bacteria; antibiotic susceptibility; imipenem.

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INTRODUCTION

Urinary tract infections (UTIs) are one of the most common infectious diseases encountered in medical practice affecting humans of all ages[1]. Globally, UTIs incidence was estimated about 150 million persons per year[2].

Diabetes mellitus is a group of chronic metabolic disorders characterized by increased blood glucose level resulting from defects in secretion, action of insulin or both[3]. The chronic hyperglycemia is associated with long term damage, dysfunction and failure of different organs especially the eyes, urinary system, nerves and cardiovascular system[3]. Over time, diabetic patients may develop cystopathy, nephropathy and renal papillary necrosis complications that predispose them to UTIs[4]. Higher severity of UTI can be cause many complications, ranging from dysuria to pyelonephritis[5]. Moreover, diabetic patients encounter further urinary urgency and incontinence during night[6]. Further more, those patients frequently suffer from bacterial cystitis with higher prevalence in diabetic women including higher prevalence of both asymptomatic bacteriuria and symptomatic UTI added to recurrent complications comparing with healthy women[7,8].

In diabetic and non- diabetic patients, about 80% of UTIs cases were caused by gram-negative bacteria mainly *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa* and *Proteus* spp., while only 15% were caused by gram- positive including *Enterococcus* spp., *Staphylococcus* spp. and *Streptococcus* spp.[9-11].

Susceptibility to antibiotics is usually variable among species and strains, therefore, the resistance to different antibiotics making it difficult to treat in some infections[12]. The widespread and indiscriminate use of broad-spectrum antibiotics led to the emergence of multi-drug resistant bacteria[13].

Briefly, this study was designed to determine the prevalence of causative microbial agents of urinary tract infections in diabetic adult patients and their susceptibility to antibiotics.

PATIENTS AND METHODS

Study design

A descriptive study was conducted at the Microbiology Laboratory, Department of Clinical Laboratories Sciences, College of Pharmacy, University of Misan, Misan city, South of Iraq over the period from May to August in 2018. This study was approved by Ethical Committees of College of Pharmacy of Misan University with written consent withdrawn from all the patients.

Sample collection

One hundred and fifty midstream urine samples were obtained from 150 diabetic adult outpatients between 20-64 years old attending to the Microbiological Examinations Unit at Al-Sadar Teaching Hospital in Misan city with written consent. The urine samples were collected by sterilized disposable containers (BIOZEK, Netherlands) after necessary precautions[14]. Samples were brought to the laboratory within one hour. Questionnaires had completed covering the information pertaining to sex, age, period of diabetes and antibiotics used.

Urine culture

A 0.1 ml of urine sample was inoculated separately onto Blood agar (LAB, UK) and MacConkey agar (LAB, UK) plates, and incubated aerobically at 37°C for 24 h. The grown colonies were calculated colony forming unit (CFU) per ml. The plates containing more than 10 CFU/ml were selected as a significant growth, then gram stain for initial identification[15]. All grown colonies from primary cultures were subcultured onto Nutrient agar (LAB, UK) plates for the following confirm assays.

The confirm diagnosis for bacterial isolates was accomplished by analytical profile index (API) system (API 20E, API 20 STREP and API STAPH) add to motility, catalase, coagulase and oxidase tests described by Cheesbrough[12].

Antibiotic susceptibility testing

Antimicrobial susceptibility patterns for bacterial isolates were performed by the standard disc diffusion method employing Muller-Hinton (LAB, UK) plates based on the guidelines of Clinical Laboratory Standards Institute[16] using the following antibiotics disc: Amikacin (AK) 30 µg, Ampiclox (APX) 30µg, Ceftriaxone (CTR) 30 µg, Ciprofloxacin (CIP) 30µg, Co-trimoxazole (SXT) 25µg, Gentamicin (CN) 10µg, Imipenem (IMP) 10 µg, Nitrofurantoin (F) 300µg, Tetracycline (TE) 30µg (Titan, Biotech., India) and Cefexime (CFX) 5µg (Oxoid, UK). The inhibition zones of bacterial isolates for antibiotics were measured in mm by

applying ordinary ruler.

Statistical analysis

Statistical analysis was achieved with the Chi-square test by Statistical Package for Social Sciences (SPSS) version 18. *P*-value less than 0.05 was considered as statistically significant and *P*-value less than 0.01 considered as highly significant.

RESULTS

Growth and Gram's staining

Out of 150 urine samples examined in this study, only 106(70.6%) samples yielded growth with a significant difference (P≤ 0.01). Axenic culture was appeared in 88(83%) samples than mixed growth at P≤ 0.01, as in Table 1. Gram-negative bacteria were 85 (70.2%), while Gram-positive were 36(29.8%) at P≤ 0.01 as in Table 2.

Table 1. Growth of uropathogens isolated from urine samples in adult diabetic Patients

Microbial Culture	Number (%)
Axenic growth	88(83)*
Mixed growth	18(17)
Total growth	106 (70.6)
No growth	44(29.4)
Total samples	150(100)

*: P≤0.01.

Table 2. Gram-staining of microbial isolates from urine samples in adult diabetic patients

Gram's Staining	Number (%)
Gram- Positive	36(29.8)
Gram- Negative	85(70.2)*
Total isolates	121(100)

*: P≤0.01.

Prevalence of UTIs in diabetic patients according to some factors

A total of 150 urine samples enrolled in this study, 84(56%) were of females and 66(44%) were of males. Out of 106 positive cultures, the prevalence of UTIs was high in females 69 (65%) than males with a significant difference at P≤0.01. Furthermore, UTIs were more incidence in patients without antibiotics use and period of diabetes 5-10 years (82% and 64%, respectively), while it was 56.6% in age group 35-49 years without signification, as in Table 3.

A total of 121 microbial isolates were obtained, *Escherichia coli* was the most common causative agent of UTIs with 64 isolates, followed by *Klebsiella pneumonia*, and *Enterococcus faecalis* (11 and 8, respectively). *Streptococcus agalactiae* and *Klebsiella aerugenes* were 5 for each. Moreover, *Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Staphylococcus haemolyticus* and *Candida albicans* were 3 for each. Nevertheless, the frequency of *Proteus mirablis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Serratia marcessens*, *Staphylococcus epidermidis* and *Staphylococcus warnerri* was 2 for each, while *Acinetobacter baumani* and *Bacillus subtilis* were 1 for each.

Antimicrobial susceptibility pattern

Antibiotic susceptibility test for urinary bacterial isolates[118] showed highly sensitive to imipenem (78.8%) with significance differences at P≤0.01, and Amikacin (61.9%) at P≤0.05. Furthermore, they were 56.5%, 50% and 44.9% for cefexime, ciprofloxacin and gentamicin respectively, but without signification. Moreover, they were low sensitivity to ceftriaxone, tetracycline and Co-trimaxazole (36.9%, 29.7% and 26.3%, respectively). On the other hand, they were a highly resistance to ampiclox and nitrofurantoin (98.3% and 87.3%, respectively) at P≤0.01, as in

Table 3. Prevalence of UTIs in diabetic patients according to some factors

Total *n(%)	Sex n(%)		Age Group (year)n (%)			Diabetes period (year) n(%)			Antibiotics use n(%)	
106 (100)	Male 37(35)	Female 69(65)**	20-34 15(14.2)	35-49 60(56.6)	50-64 31(29.2	< 5 25(23.6)	5-10 68(64.1)**	> 10 13(12.3)	Yes 19(16)	No 87(82)**

*n: Number of the patients with positive culture **: P≤0.01.
Frequency of uropathogens in diabetic patients

Table 4. *E. coli* (64) appeared sensitive to imipenem and amikacin (89% and 76.6%, respectively) at P≤ 0.01. *K. pneumoniae*[11] was sensitive to amikacin (72.7%) at P≤0.01, followed by imipenem, cefexime and ciprofloxacin (54.5%, for each), but without signification. *K. aerugenes*[5] was sensitive to imipenem (80%) at P≤0.01, while it was 60% for amikacin, ciprofloxacin, gentamicin and ceftrixone at P≤0.05. *S. agalactiae*[5] was sensitive to imipenem (100%), amikacin and ciprofloxacin (80%, for each) at P≤0.01, followed by cefixime, gentamicin and tetracycline (60%, for each) at P≤0.05. *K. oxytoca*[3] was sensitive to imipenem, amikacin, cefexime, and ceftriaxone (66.7% for each) at P≤0.01. *S. haemolyticus*[3] was sensitive to imipenem (100%), cefixime and ciprofloxacin (66.7%, for each) at P≤ 0.01. *S.pyogenes* and *S. warnerii* (2, for each) were sensitive to imipenem and cefexime (100%, for each). *Serratia marssecens*[2] was sensitive to cefexime and ciprofloxacin (100%, for each). *S. epidermidis*[2] was sensitive to imipenem (100%). Finally, *B. subtilis*[1] was sensitive to imipenem,

Table 4. Antibiotic sensitivity profile of bacterial species isolated from adult diabetic patients with UTI

		Antibiotic sensitivity n(%)									
Bacterial species	*n	IMP	AK	CFX	CN	CIP	CTR	TE	SXT	F	APX
Escherichia coli	64	57(89)	49(76.6)	35(54.7)	37(57.8)	34(53.1)	27(42.2)	21(32.8)	25(39)	9(14)	0(0)
Klebsiella pneumoniae	11	6(54.5)	8(72.7)	6(54.5)	3(27.2)	6(54.5)	4(36.3)	2(18.2)	3(27.2)	3(27.2)	0(0)
Enterococcus faecalis	8	4(50)	3(37.5)	3(37.5)	2(25)	3(37.5)	3(37.5)	2(25)	0(0)	0(0)	0(0)
Klebsiella aerugenes	5	4(80)	3(60)	3(60)	3(60)	3(60)	3(60)	2(40)	1(20)	1(20)	0(0)
Streptococcus agalactiae	5	5(100)	4(80)	3(60)	3(60)	4(80)	2(40)	3(60)	1(20)	0(0)	0(0)
Klebsiella oxytoca	3	2(66.7)	2(66.7)	2(66.7)	1(33.3)	1(33.3)	2(66.7)	1(33.3)	0(0)	0(0)	2(40)
Pseudomonas aeruginosa	3	1(33.3)	0(0)	1(33.3)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Staphylococcus haemolyticus	3	3(100)	1(33.3)	2(66.7)	1(33.3)	2(66.7)	0(0)	0(0)	0(0)	0(0)	0(0)
Proteus mirablis	2	1(50)	0(0)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Staphylococcus aureus	2	1(50)	0(0)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Streptococcus pyogenes	2	2(100)	0(0)	2(100	1(50)	1(50)	0(0)	0(0)	0(0)	0(0)	0(0)
Staphylococcus epidermidis	2	2(100)	0(0)	1(50)	0(0)	1(50)	0(0)	1(50)	0(0)	0(0)	0(0)
Staphylococcus warnerii	2	2(100)	0(0)	2(100)	0(0)	1(50)	0(0)	1(50)	0(0)	1(50)	0(0)
Serratia marssecens	2	1(50)	1(50)	2(100)	1(50)	2(100)	1(50)	1(50)	1(50)	1(50)	0(0)
Acinetobacter baumanii	1	1(100)	1(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Bacillus subtilis	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	0(0)	0(0)	0(0)
Total	118	93 (78.8)**	73 (61.9)***	65 (56.5)	53 (44.9)	59 (50)	43 (36.4)	35 (29.7)	31 (26.3)	15 (12.7)	2 (1.7)

*n: Number of isolates, **:P≤0.01, ***:P≤0.05.
IMP: Imipenem, AK: Amikacin, CFX: Cefixime, CN: Gentamicin, CIP: Ciprofloxacin, CTR: Ceftriaxone, TE: Tetracycline, SXT: Co-trimoxazole, F: Nitrofurantoin, APX: Ampiclox

amikacin, cefexime, ciprofloxacin, gentamicin, ceftriaxone and tetracycline (100%, for each). While *A. baumanii*[1] was 100% sensitive for imipenem and amikacin.

DISCUSSION

The present study showed that axenic culture was 83% versus to mixed growth, this result in agreement with Abd Al Abbas and Jasim[17] founding 82% of samples gave axenic growth. Axenic refer to pure colonies from the primary culture, as in Table 1. Furthermore, this study showed that a large proportion of uropathogens were gram-negative comparing to gram-positive isolates as in Table 2. This result is consistent with previous studies[10,18,19].

Table (3) shows the prevalence of UTI according to some factors. The results revealed a high prevalence of UTI in females (65%), in agreement with other previously reported studies[9-11,19]. Females are more prone to have UTI than males because the urethra is shorter and closer to the anus adding to a sexual intercourse[20]. Nevertheless, this study showed that the occurrence of UTI in diabetes was more frequently in the age group (35-49 years), followed by the age group(50-64 years) in agreement with study done by Adeyeba *et al*.[21]. Generally, engagement in sexual activity and increasing age of diabetics make them vulnerable to urinary tract infection[22]. Moreover, UTI occurrence was more frequent in patients without antibiotics use and diabetic period (5-10 years). Indiscriminate and uncontrolled use of antibiotics for long period with chronic glucosuria encouraging the microbial growth and thereby increase the risk of UTIs[23,24].

The present study showed that *E. coli* was the most common bacteria with 64 isolates (52.9%), followed by *Klebsiella spp.* 19(15.7%) isolates then *E. faecalis* 8(6.6%) isolates and *S. agalactiae* 5(4.1%) isolates. Several studies reported that *E. coli* and *Klebsiella* spp. were the most frequent uropathogens in diabetic patients, while the other species were different[10,18,19,25]. Relatively, UTIs occur when gastro-intestinal bacteria can be entering to the urethra and then colonization, once these bacteria gain access to the urinary bladder they may multiply and transmitting up through ureters to kidneys[14]. *E. coli* is normally habitat in the intestinal tract, it is most predominant bacterium causing UTI in both diabetic and non- diabetic patients[11,25,26]. *E. coli* have numerous virulence factors which enable to colonize the urethra such as P-fimbria to bind the epithelium, other factors contribute in its pathogenicity like α and β-hemolysins, colicins and cytotoxic necrotizing factor[27]. *Klebsiella* spp. are opportunistic pathogens and they are the etiological agents for urinary tract infection in both community- acquired and hospital- acquired infections. Capsule production, siderophore activity and biofilm formation are an important virulence factors in pathogenesis of *Klebsiella*[28]. *Enterococcus faecalis* is also opportunistic bacterium and it's the causative agent for UTI in the general population[26]. This bacterium has many virulence factors playing an important role in its pathogenicity such as aggregation substance, Enterococal surface protein and hemolysins[29]. On the other hand, *S. agalactiae* has been isolated from patient with poorly glucosuria[10]. Although, the bacterium form a part of urogenital flora, but became opportunistic, particularly in patient with poor immunity.

Table (4) showed that the uropathogenic bacteria were highly sensitive to imipenem (78.8%), followed by Amikacin (61.9%). These results are consistent with other previous studies[18,19,25]. In contrast, all bacterial species appeared a complete resistance to ampiclox (100%) with the exception of *S. agalactiae* (60%). This is due to the acquisition of *mec A* gene by plasmid transferring[30,31]. On the other hand, many bacterial species appeared resistant to three or more antibiotics which may be due to plasmid carrying drug resistant genes, a biofilm formation inside the urinary bladder increases the chance of multi-drug resistance bacteria to colonize the urinary tract and thus to infection[32,33]. Furthermore, MDR efflux – system contributes to the emergence of multi- drug resistance strains, especially in gram – negative bacteria[34,35]. Additionally, the exposure of bacterial pathogens to high concentration of antibiotics for long times creates severe selection pressure leading to emergence of resistance[36].

CONCLUSION

The prevalence of UTIs was higher in females comparing of males, and the age group (35-49) years in adult diabetic patients. *Escherichia*

coli was identified as the most common causative agent of UTIs in diabetes in Misan province. Although, the high rate of multi – drug resistance among bacterial isolates, but imipenem is a very effective antibiotic.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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