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PREVALENCE OF URINARY TRACT INFECTIONS AND THEIR ANTIMICROBIAL SENSITIVITY AMONG DIABETIC AND NON DIABETIC PATIENTS IN ZAKHO

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ABSTRACT:

Background: Urinary tract infection (UTI) is one of the most common bacterial infections in diabetics and non-diabetics. Worldwide about one hundred fifty million people are infected with UTI each year. Indifference in diabetes management has been shown to increase the risk of complications. This may lead to different microbial infections including UTI. UTI is often treated with various broad-spectrum antibiotics due to concerns about infection with resistant organisms.
Objective: The aim of this study was to determine the prevalence of UTI and antibiotic sensitivity pattern in both diabetic and non-diabetic patients attended the Zakho Emergency Hospital and Chamiskho Health Center at refugee camp in Zakho city.
Methods: Midstream urine sample was collected from 316 patients attended Zakho Emergency Hospital and Chamiskho Health Center from September 2018 to January 2019 in Zakho city.
Results: 316 samples were collected from diabetic and non-diabetics, 195 (61.70%) sample were collected from diabetics and 121 (38.30%) sample were collected from non-diabetic patients. The frequency of male and female was 101 (31.96%) and 215 (68.04%) respectively. The top five dominant bacteria isolated in this study were *Escherichia coli* (43.20%), *Klebsiella pneumoniae* (19.90%), *Proteus mirabilis* (11.65%), *Pseudomonas aeruginosa* (8.73%) and *Staphylococcus aureus* (6.31%). The isolated bacteria were highly sensitive to meropenem (100%) followed by imipenem (97% sensitive) and less sensitive to ciprofloxacine, amikacin and nitrofurantoin. While most of the isolates were resistance to tetracycline (21% sensitive), ampicillin (22% sensitive) and piperacillin (28% sensitive).
Conclusion: Analysis of the results showed that urinary tract infection is more prevalence in diabetic than non-diabetics and in female than male. Also, the most commonly isolated bacterium was *E. coli*. Most isolated pathogens show high resistance to tetracycline and ampicillin.

KEYWORDS: Urinary Tract Infections, Diabetes Mellitus, Susceptibility, Pyuria, Superantigens.

1. INTRODUCTION

Urinary tract infection (UTI) is the most common bacterial infections in diabetics and non-diabetics (Liza & Jonathan, 2006). UTIs is considered as the second most common infection in a medical clinic in the world, and its prevalence rate in Iraq estimated about 23% from all infections (Tektook et al., 2017). UTI can be defined as the presence and active multiplication of microorganisms within the urinary tracts which lead to an inflammatory response of the urothelium that is usually associated with bacteriuria and pyuria (Kunin, 1979). Urinary tract infections are caused by microorganisms usually bacteria that enter the urethra and bladder, causing inflammation and infection (Liza & Jonathan, 2006; Okada et al., 2000). The bacteria may also travel up the ureters and infect the kidneys (Epp et al., 2010; Mueller et al., 2003). The most common UTI is caused by bacterial infections (Boscia et al., 1986; Patterson & Andriole, 1997). Urinary tract infection may affect diabetic and non-diabetic patients, while the infection in diabetic patients are more common (Feleke et al., 2007).
According to WHO diabetes mellitus is the 9th most leading cause of death worldwide. Indifference in diabetes management has been shown to increase the risk of complications (Gul et al., 2005). Such complications may lead to microvascular and macrovascular damage as well as microbial infections especially urinary tract infection (Lewis, 1989). There are four groups of diabetes mellitus, insulin dependent (DM type I) or juvenile diabetes mellitus, noninsulin dependent (DM type II), gestational and diabetes mellitus secondary to other causes (Schlievert, Salgado-Pabón, & Klingelhutz, 2015).
Worldwide one hundred fifty million people are infected with urinary tract infection each year (Gupta, Hooton, & Stamm, 2001). Urinary tract infection can be uncomplicated or complicated (Unachukwu, Obunge, & Odia, 2005). UTI may affect both the upper and the lower tract. Cystitis is a term used to clarify the syndrome involving dysuria, suprapubic tenderness (Liza & Jonathan, 2006). There are many divergences between patients with diabetes mellitus and those without diabetes mellitus, which are important to consider when evaluating the association between diabetes mellitus and the risk of infection (Nicolle, 2000). The pathogenesis of UTI in patients with diabetes have been studied, and the increased susceptibility is attributed to several impairments of host defence mechanisms such as leukocyte adherence, chemotaxis, and phagocytosis that can be impaired in diabetic patients (Geerlings et al., 2000). The increased risk of infection in diabetic patients can be partially explained by a reduced T-cell mediated immune response and impaired neutrophil function among diabetics (Zhanel, Nicolle, & Harding, 1995). Other factors such as local complications related to neuropathy including impaired bladder emptying and higher glucose concentrations in urine may also play a role in increased

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incidences of UTI in diabetics (Al-Daghistani & Abdel-Dayem, 2002).

Patients with diabetes mellitus react with the health care system more frequently and more intensively than the general population (Hvidberg et al., 2000). Because the outcome of infection may be more significant in a person with diabetes mellitus, they may also be more likely, after looking medical attention, to be assigned a diagnosis of infection or to receive a higher level of care for an infectious illness, compared with a person without diabetes mellitus. Ultimately, persons with diabetes mellitus may have other comorbid factors, such as renal failure or functional disability, which are associated with an increased risk of infection (Al-Rubeaan et al., 2013).A perfect and rapid diagnosis is necessary to cut short the treatment and to reduce the rate of the rise of infection to the upper urinary tract (Okada et al., 2006).

UTI is often treated with various broad-spectrum antibiotics because of concerns about infection with resistant organisms (Al-Rubeaan et al., 2013). Extensive and inappropriate use of antimicrobial agents has constantly caused the occurrence of resistance which has developed a main problem worldwide (Feleke et al., 2007). In most series case of UTI patients are required to start antibiotic treatment before results of antibiotic susceptibility test is conducted. To ensure the right treatment, knowledge of the organisms causing UTI and their antibiotic susceptibility should be presented. As both temporal and local variables can change these data, they must be re-assessment continual (S. V. O. order) to obtain a full clinical response before antibiotic susceptibility of the isolate is established. No data was available on the frequency of urinary tract infection among diabetics and non-diabetics in Zakho. The objectives of this study were the detection of pathogens causing UTI and to determine their antibiotic susceptibility in patients referred to Zakho Emergency Hospital and Chamishko Health Center in Chamishko camp in Zakho city/Kurdistan Region, Iraq.

2. MATERIAL AND METHODS

2.1 Sample collection:

The total of 316 midstream urine samples were collected from patients admitted to Zakho Emergency Hospital and Chamishko Health Center at refugee camp in Zakho city from September 2018 to January 2019. After collection, samples were kept in clean sterile tubes with full labels and transported to the microbiology laboratory in ice box within two hours.

2.2 Specimen cultivation:

Samples were checked for physical characteristics microscopically and cultured on different culture media. For isolation and identification of bacteria, urine samples were streaked on 5% sheep blood agar, MacConkey agar, CLED agar and sabouraud dextrose agar medium using a standard loop. Plates were incubated at 37°C for 18-24 hours. Pure colonies were identified through their colony characteristics, gram staining, oxidase, catalase, coagulase, urease enzymes, indole production test, methyl red test, Voges-Proskaur test, citrate utilization test, and triple sugar iron test.

2.3 Antibiotic susceptibility test:

Antimicrobial susceptibility of bacteria was evaluated according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI) using Muller Hinton Agar medium by Kirby Bauer disk diffusion technique (Wayne, 2011).

Thirteen different types of antibiotics were used to carry out the sensitivity of isolated bacteria to antibiotics, and the zone of inhibition was estimated and compared to the standard one of each antibiotic. Antibiotics used in this study include amikacin (AK 10 mcg), ampicillin (AM 25 mcg), ceftriaxone (CRO 10 mcg), chloramphenicol (C 10 mcg), ciprofloxacin (CIP 10 mcg), gentamicin (CN 10 mcg), imipenem (IPM 10 mcg), meropenem (MEM 10 mcg), nitrofurantoin (F 100 mcg), piperacillin (PRL 100 mcg), tetracycline (TE 10 mcg), sulfamethoxazole-trimethoprim (SXT 25 mcg) and vancomycin (VA 30 mcg).

2.4 Determination of glucose in blood and urine:

There are many methods used to determine blood glucose levels. To check the glucose level in blood (Blood Glucose Meter) used, prepared by (AccuChek Active, Germany). To determine the glucose in urine, uripath strip (Plasmatace Co, UK) was used.

Diabetes was diagnosed as patients who had a history of diabetes or fasting blood sugar above 7 mmol/L (126 mg/dl) or in a patient with classic symptoms of hyperglycemia and random blood sugar above 11.1 mmol/L (200 mg/dl) (Marti et al., 1998).

2.5 Biostatic analysis:

Statistical Package for Social Sciences (SPSS) version 22.0 and Microsoft Office Excel were used to analyze the results of the current study. Chi-square test was used and $P < 0.05$ was considered to be statistically significant (Sokal and Rohlf, 2009).

2.6 Ethical issues:

Local approval was sought from (Directorate General of Health Duhok/Research Ethics Committee). Permission to collect samples from Zakho hospital registries and laboratory records was obtained from (Directorate of Health Zakho/Head of Laboratory Medicine). While the permission to collect samples from Chamishko Health Center was obtained from both (Directorate General of Health Duhok/Research Ethics Committee) and (Board of Relief and Humanitarian Affairs B.R.H.A.).

3. RESULTS

From a total 326 midstream urine samples collected from diabetic (195, 61.70%) and non-diabetic (121, 38.30%) patients ($p<0.003$), a total of 206 of 206 (65.18%) positive cultures have been obtained. The frequency of male and female was 101 (31.96%) male and 215 (68.04%) female as shown in table 1 and figure 2. The frequency of positive cultures (206, 65.18%) was of 140 (67.96%) from diabetic and 66 (32.04%) were from non-diabetic's case. The source of samples and gender distributions are shown in table 2.

Table 1: diabetic frequency in male and female.

Categories		Patient state		Total	
		Non-diabetic	Diabetic	No.	%
Gender	Female	85	130	215	68.04%
	Male	36	65	101	31.96%
Total	No.	121	195	316	100%
	%	38.30%	61.70%	100%	

In the present study the most dominant bacteria isolated were *Escherichia coli* (43.20%), *Klebsiella pneumoniae* (19.90%), *Proteus mirabilis* (11.65%), *Pseudomonas aeruginosa* (8.73%) and *Staphylococcus aureus* (6.31%) details described in figure 3 and table 4.

The main isolated bacteria in this study *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Pseudomonas aeruginosa* were identified according to their colony and

culture characteristics on Cystine Lactose Electrolyte Deficient (CLED) agar medium. Lactose fermenting bacteria produce yellow small colonies (*E. coli)* and large yellow extreme mucoid colony (*K. pneumoniae)*, while non-lactose fermenting bacteria, the translucent blue colonies that's smaller than *E. coli* were (*Proteus spp.*) and green colonies, rough periphery and sweet odor on CLED ager medium were (*P. aeruginosa*) as shown in figure 1. Beside Gram-negative bacteria other microbes can grow on CLED agar, like gram positive cocci such as *Enterococcus spp*, *Staphylococcus aureus* and *Staphylococcus saprophyticus*. It also supports the growth of yeasts like *Candida albicans*.

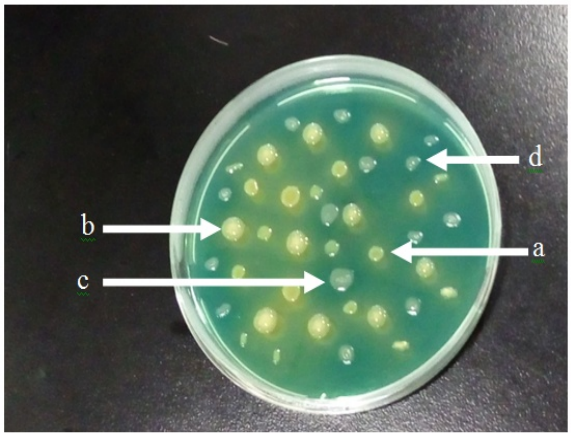

Figure 1. Lactose fermenting bacteria a- *Escherichia coli* b-*Klebsiella pneumoniae*, and non-lactose fermenting c- *Pseudomonas aeruginosa*d- *Proteus mirabilis* on CLED ager medium.

Urinary tract infected patients in this study were divided into seven groups according to their age and diabetic status (Table 3). This study showed that the prevalence of urinary pathogens was not consistent across all age groups. Generally, it was found that the high percentage of isolates (23.30%) was in those patients grouped in 51-60 year, especially diabetic patients in the same group. Statistical analysis showed that the incidence of infection was significant ($p < 0.05$) among age group of 51-60 year old.

Table 2: Sample source and gender distribution

Categories		Sample source				Total	
		Zakho Emergency Hospital		Chamishko Health Center			
		No	%	No.	%	No.	%
Sex	Female	121	38.30%	94	29.75%	215	68.05%
	Male	64	20.25%	37	11.70%	101	31.95%
Total		185	58.55%	131	41.45%	316	100%

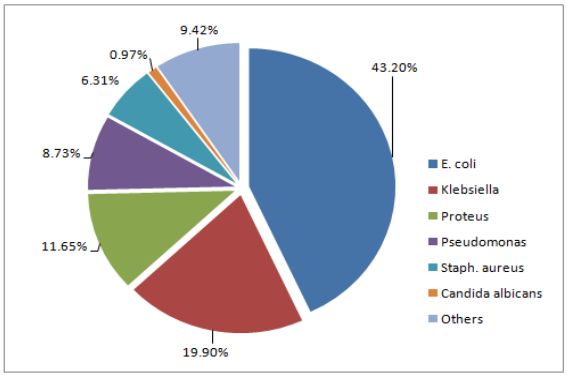

Figure 3. Pie chart showing distribution of patients with urinary tract infection according to causative agent.

The frequency of isolated pathogenic microbes in Zakho Emergency Hospital was *Escherichia coli* (28.86%), *Klebsiella pneumoniae* (21.64%), *Staphylococcus spp.* (20.61%), *Proteus mirabilis* (13.40%), *Streptococcus spp.* (8.24%), *Pseudomonas aeruginosa* (6.18%), and *Candida albicans* (1.03%).
While the frequency of isolated microbes in Chamishko Health Center was *Escherichia coli* (55.96%), *Klebsiella pneumoniae* (18.34%), *Pseudomonas aeruginosa* (11.00%),*Proteus mirabilis* (10.09%), *Staphylococcus* spp. (3.66%) and *Candida albicans* (0.91).

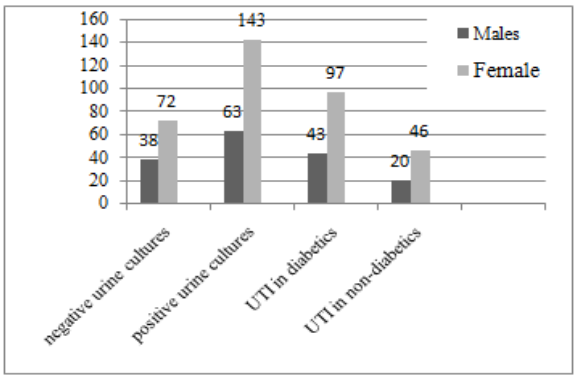

Figure 2. Negative and positive urine samples cultures and gender ratio in both cases.

The results of antibiotic susceptibility showed that the isolated bacteria were highly sensitive to meropenem (100%) followed by imipenem (97%) and less sensitive to ciprofloxacine, amikacin and nitrofurantoin.
Most of the isolates were resistance to tetracycline (21%), ampicillin (22%) and piperacillin (28%). The antibiotic sensitivity of UTI pathogens isolated in the present study described in table 5.

Table 3, urinary infected patients grouping according to age and gender.

Age group (Year)	Gender				Total	
	Female		Male			
	D.M	Non-D.M	D.M	Non-D.M	No.	%
< 10	2	3	1	5	11	5.33%
11-20	3	2	0	1	6	2.91%
21-30	9	18	2	3	32	15.53%
31-40	28	5	9	1	43	20.87%
41-50	21	9	11	4	45	21.84%
51-60	23	6	14	5	48	23.30%
≥ 61	11	3	6	1	21	10.19%
Total	97	46	43	20	206	100%

D.M=diabetes mellitus, Non-D.M=non-diabetes mellitus

Table 4: The distribution of isolated microbes in diabetic and non-diabetics.

Pathogens	Health state				Total	
	Non-diabetic		Diabetics		No.	%
	ZEH	CHC	ZEH	CHC		
Escherichia coli	7	17	21	44	89	43.20%
Klebsiella pneumoniae	4	9	17	11	41	19.90%
Proteus mirabilis	4	3	9	8	24	11.65%
Pseudomonas auroginosa	2	5	4	7	18	8.73%
Staphylococcus aureus	5	1	6	1	13	6.31%
Staphylococcus saprophyticus	3	1	0	0	4	1.95%
Staphylococcus epidermidis	2	0	4	1	7	3.39%
Streptococcus pneumonia	2	0	5	0	7	3.39%
Streptococcus pyogenes	1	0	0	0	1	0.48%
Candida albicans	0	1	1	0	2	0.97%
Total	30	37	67	72	206	100%

ZEH; Zakho Emergency Hospital, CHC; Chamishko Health Center.

Table 5: Antibiotic sensitivity in UTI isolated pathogen and its percentage sensitivity to antibiotics.

Antibiotic	Pathogens					
	E. coli	*Klebsiella*	*Proteus*	*Pseudomonas*	*Staph. spp.*	*Strep. spp.*
	n=35	n=21	n=14	n=10	n=12	n=6
AK	80%	0%	28.57%	100%	66.67%	66.67%
AM	22.85	0%	0%	0%	100%	33.33%
CRO	25.71%	ND	42.86%	100%	0%	66.67%
C	25.71%	100%	14.29%	0%	83.33%	100%
CIP	68.57%	100%	28.57%	0%	50%	100%
CN	54.28%	0%	28.57%	100%	66.67%	66.67%
IPM	100%	100%	85.71%	100%	100%	100%
MEM	100%	100%	100%	100%	100%	100%
F	77.15%	90.47%	0%	100%	50%	33.33%
PRL	17.14%	0%	42.86%	20%	100%	33.33%
TE	14.28%	42.85%	0%	0%	50%	16.67%
SXT	51.42%	0%	0%	0%	83.33%	33.33%
VA	ND	ND	ND	ND	100%	66.67%

n=number of samples. ND= not done.

3. DISCUSSION

The incidence of urinary tract infection and its clinical impact are depend on both sexes and health status. The present study show higher prevalence rate of UTIs was detected in diabetics (67.96%) compared to non-diabetics (32.04%) and the relationship was highly significant ($p<0.003$). Also in terms of gender, the prevalence rate was higher in females (68.04%) compared to males (31.96%). These results are in agreement with other researches which showed that UTIs are more prevalent in diabetic than in non-diabetic patients, and are more common occurrence in women than men (Douri, 2008). Many researches showed that diabetic women more likely to have UTI than non-diabetic women (Tektook *et al,* 2017). The higher incidence of urinary tract infections in females is due to unique anatomical features of the female genitourinary tract,

which includes a shorter urethra and the more proximal location of the urethral meats to the anus contribute to easy colonization of the pre-urethral region with enteric bacteria (Al-Rubeaan et al., 2013). Add to that, following sexual intercourse during which bacteria may inadvertently be introduced to the urethra. Overall, the patients suffering from diabetes mellitus are more vulnerable to microbial invasion from their own microbiom. Bacteria may also play a role in developing diabetes mellitus, through producing exotoxins (superantigens) that lead to develop hallmark symptoms of type II diabetes (Schlievert *et al.,* 2015). Antimicrobial sensitivity to antibiotics is very important to physicians for treating UTIs by selecting the effective antibiotic against isolated bacteria. In general the susceptibility of bacteria to antibiotics is changing as there is an increasing resistance to antibiotics reported worldwide.

In our study in Zakho, the prevalence of Gram negative bacteria was higher when compared with the prevalence of Gram positive bacteria. This result was not agreed with that reported by Al-Berfkani *et al,* (2016). Microorganisms belonging to *Enterobacteriaceae* family were the most common bacteria isolated from UTI in the current study. For instance *E. coli* was the most predominant bacteria and similar result was reported by Assafi *et al*, (2016). The results in this study showed that the urine cultures revealed no bacterial growth even in the case of presence hallmark of UTI. This may be due to another pathogens rather than bacteria, like viruses and parasites.

E.coli was the main isolated bacteria in this study at a rate of 43.20% of isolation. In the study of Al-Berfkani *et al* (2016), the most frequently isolated microorganism was *E. coli* with a rate of 51.70%. Similar results were reported by Alhamdany (2018) 55.0%, and by Angoti *et al* (2016) 55.38%. In the present study *E. coli* isolated from diabetic patients was 46.76%, this result slightly agrees with Douri (2008) results that showed *E. coli* rate was 66.6% in diabetic patients and 100% in non-diabetics. Consistent with the results of the other studies, the present study demonstrated that *E. coli* was the most common pathogen isolated in diabetics and non-diabetic patients.

K. pneumoniae was the second most common isolated bacteria, it was 19.90% of isolation, this result agreed with Tektook *et al,* (2017) study with rate 24.50%, and in Alsohaili *et al* (2015), was 11.70%, while this result disagreed with Angoti *et al* (2016), results that showed less isolated bacteria with the rate 0.48%.
In our study the isolation of *K. pneumoniae* from diabetic patients was 20.14% and in non-diabetic was 19.40%, in Sewify *et al* (2016), was 19.60 in diabetic patients, while in Hadi *et al* (2014), was 12.22%.

The prevalence of *P. mirabilis* isolates was 8.73% (10.44% in non-diabetic 12.23% in diabetics). While the rate of isolated *P. mirabilis* in diabetic patients in Douri (2008), was 22.22%. Javed *et al* (2015), and Tektook *et al* (2017), demonstrated that the first three commonly isolated bacteria were *E. coli*, *K. pneumoniae* and *P. mirabilis*.

The frequency of *P. aeruginosa* in the present study was 8.73%, this percentage ratio disagree with results showed by AL-Barwary (2007), with the rate 4% while in Tektook *et al* (2017), was 11.0%.

Gram positive bacteria were less prevalence in the present study compared with gram negative bacteria, in both diabetics and non-diabetic patients. *Staphylococcus spp.* and *Streptococcus spp.* rate were 11.65% and 3.88% respectively. The study reported by Douri (2008) in Baghdad showed that the fewer occurrences of isolated bacteria from diabetics were *Staphylococcus spp.* with a rate of 11.1%. While in Zakho city Al-Berfkani *et al* (2016), demonstrated that the second most common isolated bacteria from non-diabetic patients was *Staphylococcus spp.* with a rate of 48.0%. On the other hand, the prolong exposure to exotoxins produced by *Staphylococcus spp.* bacteria lead to develop the hallmark symptoms of type II diabetes. According to Koch's postulates Schlievert and his team (2015) prove that increasing of *Staphylococcus aureus* in microbiom lead to more superantigen productions. These exotoxins interact with fat cells and the immune system to cause insulin resistance, glucose intolerance, and systemic inflammation (Schlievert et al., 2015).

The less identified gram positive bacteria in this study was *Streptococcus spp.* with rate of 3.88% this result slightly agreed with that found by Hadi *et al* (2014), who reported that the rate of *Streptococcus spp.* was 1.0%, while much higher result was found by Tektook *et al* (2017) who reported 13.7% of *Streptococcus spp.*

Fungi causing UTIs, like *Candida albicans*, was reported in the present study with the rate of 0.97%. Mansoor *et al* (2015), demonstrated that *C. albicans* shows higher percentage compared with our study with the rate of 5.6%. No significant difference was determined between the diabetic patients and non-diabetic patients regarding the isolated bacteria species.

In this study the result of most isolated uropathogens had very high degree of resistance to tetracycline, ampicillin, piperacillin, sulfamethaxazole-trimethoprime and ceftriaxone. Similar result have been reported elsewhere in Turkey by Arslan *et al* (2014), in Jordan by Alsohaili *et al* (2015), in Iraq by Hadi *et al* (2014) and in Erbil (Kurdistan-Region Iraq) by Mansoor *et al* (2015). The sensitivity to antibiotics is very important to physicians as they need to know the local pattern of microbial susceptibility in urinary tract infections for selecting proper drugs for treatment.

Sensitivity to antibiotics for *E.coli* and *K. pneumonia* was very high to meropenem, imipenem, ciprofloxacin and nitrofurantoin, *E.coli* was less sensitive to gentamicin and sulfamethoxazole-trimethoprim, while *K. pneumonia* was resistance to gentamicin and sulfamethoxazole-trimethoprim. Many studies in neighbour countries reported similar results to our findings regarding antibiotic susceptibility as in Yürüyen *et al,* (2017) in Turkey, Angoti *et al,* (2016) in Iran and in Sewify *et al,* (2016) in Kuwait.

The most effecting antibiotics on *P. mirabilis* were meropenem and imipenem, and *P. mirabilis* was less sensitive to ciprofloxacin and piperacillin, while it was resistant to most other antibiotics.
P. aeruginosa was very sensitive to amikacin, ceftriaxone, gentamicin, imipenem, meropenem and nitrofurantoin, while it was resistance to chloramphenicol, ciprofloxacin, piperacillin, tetracycline and sulfamethoxazole-trimethoprim.

Gram positive bacteria *Staphylococcus spp.*and*Streptococcus spp.* show high sensitivity to vancomycin, imipenem, meropenem, amikacin, chloramphenicol, and gentamicin. *Staphylococcus spp.* showed less sensitivity to ciprofloxacin, nitrofurantoin and tetracycline. Regarding to ampicillin, piperacillin and sulfamethoxazole-trimethoprim. *Staphylococcus spp.* showed higher sensitivity than *Streptococcus spp.*, while *Streptococcus spp.* was more sensitive to ceftriaxone and ciprofloxacin than *Staphylococcus spp.* In this study there is no evidence reported to show antimicrobial sensitivity difference between diabetic and non-diabetic patients.

5. CONCLUSION

From the current study, it can be concluded that urinary tract infections were more incidence in diabetic than non-diabetics and in female than male. The most commonly isolated bacteria was *E. coli* and the most effective antibiotics were meropenem and imipenem, while the most isolated pathogens showed high resistance to tetracycline. Urine culture should be performed in every diabetic and non-diabetic patient before starting empirical treatment, since the selection of antibiotics for the treatment of urinary tract infections is important for both successful treatment and the decrease of resistance development.

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