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The Prevalence Of Uropathogenic Escherichia Coli Strains Among Outpatients With Urinary Tract Infection In Zakho City, Iraq

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The Prevalence of Uropathogenic Escherichia coli Strains among Outpatients with Urinary Tract Infection in Zakho City, Iraq

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ABSTRACT

Uropathogenic *Escherichia coli* (UPEC) is the most common strain of *Escherichia coli* that causes urinary tract infection (UTI) in human. This study involved the prevalence of UPEC among outpatients with UTIs attending three major hospitals in Zakho city. Four hundred urine samples were collected from patients with UTIs of both sexes and different ages (≤ 1 year to over 50 years), during the period from July 2018 until January 2019. All urine samples were analyzed by conventional bacteriological method for the presence of *E. coli*, while molecular method was used for the presence of species-specific *uidA* gene of the isolated *E. coli*. From the enrolled patients, 35.25% (141/400) of them were infected with UPEC. The rate was higher in females than males (90.78% vs 9.22%), respectively. Among both sexes, the age group 41-50 years showed the highest rate (46.67%) of infection, furthermore, among all ages, married patients showed slightly higher prevalence than un-married one (38% vs 32.5%), respectively. The rate of UTI was higher among urban inhabitants (40.56%) than others. During the months of the year, the peak (90.48%) of infection in both sexes was during December while the lowest rates (13.64%) was during January. The study highlights that, the UPEC is one of the most common bacteria that causes UTI in humans and various risk factors contribute in its spreading in the community.

MSC:

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1. Introduction

Urinary tract infection (UTI) is one of the most common bacterial infections in humans and medically is the second most bacterial infection after respiratory tract infection,[38,25] this infection is acquired in all ages from newborn to geriatric. [39] The Gram negative bacteria, especially *Escherichia coli* (*E. coli*) is considered as one of the most causative agents causing UTIs. [8] *Escherichia coli* is a member of family Enterobacteriaceae. [34] It has been believed that within a short time after birth it became the main constituent of the normal intestinal flora of both humans and animals, which in turn take a mutual benefit from each other. [35] Besides *E. coli* is a harmless pathogen, there is an alternative face to be a highly adapted pathogen, which mostly through gene gaining cause several infections ranging from intestinal to extra-intestinal diseases including meningitis, septicemia and infection of the urinary tract. [11]

Various risk factors increase the chances for developing UTIs, such as sex, age, immune deficiency, primary diseases such as renal failure and the effect of urological catheter despite to the facts of the microbial etiology. [41] The recorded rates of complicated UTIs were increased worldwide, especially in developing countries. [10] Approximately 10% of humans have UTI sometime during their lifetime. [15] It is estimated that around 150 million cases of UTIs occur annually worldwide, [24,40] resulting in over 6 billion dollars in direct health care expenditures.[24] The UPEC is responsible for about 80-90% of all UTI in all patients regardless of age with higher prevalence in females than males, [25] yearly about 11% of women were infected by UTI, [14] and more than 50% of them were infected at least one time, as many as 20-40% of them are reinfected within one year. [38] The higher prevalence of UTIs among females is mostly due to the anatomical and physiological characters that facilitate the infection. Therefore, UTIs is mostly a female disease. [15] Untreated asymptomatic bacteriuria (ASB) is responsible for the development of symptomatic bacteriuria approximately 30% of cystitis and up to 50% of pyelonephritis. [25] In developing countries, the prevalence of ASB is three times higher than in developed countries. [3]

Several studies have been performed in Kurdistan Region-Iraq. Such as in Zakho city, a rate of 43.20% isolates of *E. coli* was detected among UTIs patients. [22] While in Duhok city, a much higher rate of UPEC infection which was 74.4% (276/371) was reported in women. [20] Furthermore, in Erbil and Sulaimania cities, high prevalence (42% vs 30%, respectively) of the UPEC were isolated from pure and mixed cultures were. [4] Another study in Erbil city, also,

reported a high prevalence of UPEC accounting for 58.57% in UTIs outpatients. [23] Therefore, due to limited studies in this aspect the present study was adapted to investigate the prevalence of UPEC among the UTI cases referred to the laboratories in three major hospitals of Zakho city, Duhok governorate, Kurdistan-Iraq, using biochemical tests and molecular identification by species-specific gene of isolated UPEC. Furthermore, to correlate between the UPEC and some risk factors and seasonal variations.

2. Materials and Methods

2.1. Sample Collection

In this study 400 clinical midstream urine samples were collected from the enrolled outpatients of both sexes and different ages (≤ 1 to above 50 years) referred by the urologestis to the laboratories of the three major hospitals in Zakho city, during the period from July 2018 until January 2019. During the sample collection period, patients were informed about the right way how to collect the urine sample and each patient was provided with a sterile labelled disposable screw-capped container. The collected samples were cultured on different culture media in the Microbiology Laboratory of Zakho General hospital and both Maternity and Emergency hospitals, then plates with pure colonies of uropathogenic *E. coli* were subcultured and the positive plates were subjected in the microbiology laboratory of the Medical Technology Department-Zakho Technical Institute for performing biochemical tests. The molecular study of the isolats was performed in the Molecular research laboratory of Biology Department-Faculty of Science-University of Zakho.

2.2. Laboratory Diagnosis

Identification of the isolated *E.coli* was performed by the morphological characterization of the isolated UPEC colonies on the culture media according to their colony morphology on selected media (MacConkey and blood agar). [13] The isolated colonies were tested by gram stain according to the protocol supplied with the kit (Atom scientific-UK). Furthermore, some biochemical tests were performed, for further confirmation of the isolated bacteria, such as indole tests, methyl red test, citrate utilization test and triple sugar iron agar (TSI). [27] For *E. coli* preservation, 0.5 ml of overnight incubated nutrient broth containing *E. coli* was added to sterile tubes containing 0.5 ml sterile glycerol, mixed well, and stored at -20°C, for 6 months. [9]

2.3. Molecular Diagnosis

The genomic DNA of the *E. coli* was extracted according to the protocol supplied with the Kit (PrimePrep™ Genomic DNA Extraction Kit/GeNet Bio-Korea). The concentration and purity of the genomic DNA of each sample was determined using a Nanodrop spectrophotometer 2000 (Thermo scientific, USA), then used for PCR amplification. The *uidA* primer was used as a species-specific primer for *E. coli* identification and for amplifying the target *uidA* gene as shown in Table (1). The PCR amplification reaction of each sample is shown in Table (2). The prepared reaction tubes were inserted in the thermal cycler for amplification, the amplification condition is shown in Table (3). After amplification, the PCR product was confirmed by running in gel electrophoresis for one hr using 1.2% (w/v) of agarose prepared in 1x TBE buffer.

Table 1: Primers used for detection of species-specific gene in UPEC

Primer	DNA sequence 5'- 3' (forward and reverse)	Amplified product (bp)	Reference or source
uidA	F-CATTACGGCAAAGTGTGGGTCAAT R-CCATCAGCACGTTATCGAATCCTT	658 bp	[1]

Table 2: Components of the PCR reaction

Components	Volume (µl)
Master mix	5 µl
Forward primer (10 pmol/µl)	2 µl
Reverse primer (10 pmol/µl)	2 µl
Genomic DNA (25-50 ng/µl)	2 µl
PCR grade water	9 µl
Grand Total	20 µl

Table 3: The amplification condition of species-specific PCR analysis for *uidA* primer

Initial denaturation	Denaturation	Annealing	Extension	Final extension	Reference
94 °C	92 °C	58 °C	72 °C	72 °C	[1]
10 min	1 min	1min	30 sec.	5 min	
1 cycle		35 cycles		1 cycle	

2.4. Statistical Analysis

Graph Pad Prism 8.1 software was performed for statistical analysis. The chi-square test was used to find out the significant differences between categories and P-value ≤ 0.05 considered significant. Krona Excel template was used to compare the communication of two or more groups (https://sourceforge. net/p/krona/home/krona/?version =8).

3. Results

During the present study, 35.25% (141/400) of enrolled outpatients were infected with UPEC, with a higher rate in females than males (90.78% vs 9.22%), respectively, as shown in Table (4).

Table 4: Distribution of UPEC among outpatients with UTI

No. of examined urine samples	Total No. of UPEC Infections		Sexes			
			Females		Males	
	No.	%	No.	%	No.	%
400	141	35.25	128	90.78	13	9.22

All UPEC isolates were identified by the cultural characters on MacConkey and blood agars,[13] in addition to biochemical tests, that confirmed the isolated bacteria are *E. coli* strains which included; positive result for both indole and methyl red tests, negative reaction with Gram's stain rod-shaped, that is unable to utilize citrate and the production of acids in both slant and butt, with gas production and without H_2S production in TSI agar. [34] The amplified isolated *E. coli* produced a single band of a target *uidA* gene with a molecular weight of 670 bps that confirmed its presence as shown in Figure (1).

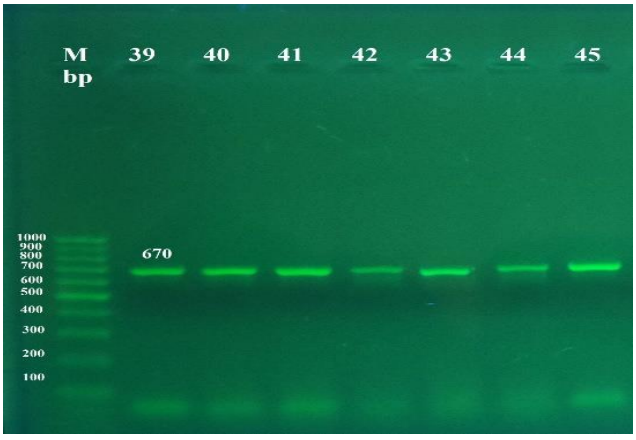

Figure 1: The PCR amplification for species identification of *E. coli* using specific-species *uidA* primer (670bps) as indicated in lanes 39-45. Amplicons were separated on 1.2% agarose gel electrophoresis for 1 hr. Lane M=DNA marker [100-1000 bps].

Table (5) and Figure (2) show that the rate of infection in females over all ages was higher than that of males with the highest being among the ages ≤1-10 and 11-20 years which was 95.45%. While in males, the highest rate of the infection was reported among the ages 51≤ which was 16.67%. In general, the highest rate of infection with UPEC among both sexes was recorded among the age group 41-50 years, which is accounted for 46.67% (21/45). Statistically this rate was significant (p< 0.013) among both sexes and various ages.

Table 5: Distribution of the *E. coli* according to sex and age

Age groups (years)	No. of examined samples	Total No. of infections		Sex			
				Females		Males	
		No.	%	No.	%	No.	%
≤ 1-10	54	22	40.74	21	95.45	1	4.55
11- 20	73	22	30.14	21	95.45	1	4.55
21- 30	121	44	36.36	40	90.91	4	9.09
31- 40	70	20	28.57	18	90.00	2	10.00
41- 50	45	21	46.67	18	85.71	3	14.29
51≤	37	12	32.43	10	83.33	2	16.67
Total	400	141	35.25	128	32.00	13	3.25

*P<0.013

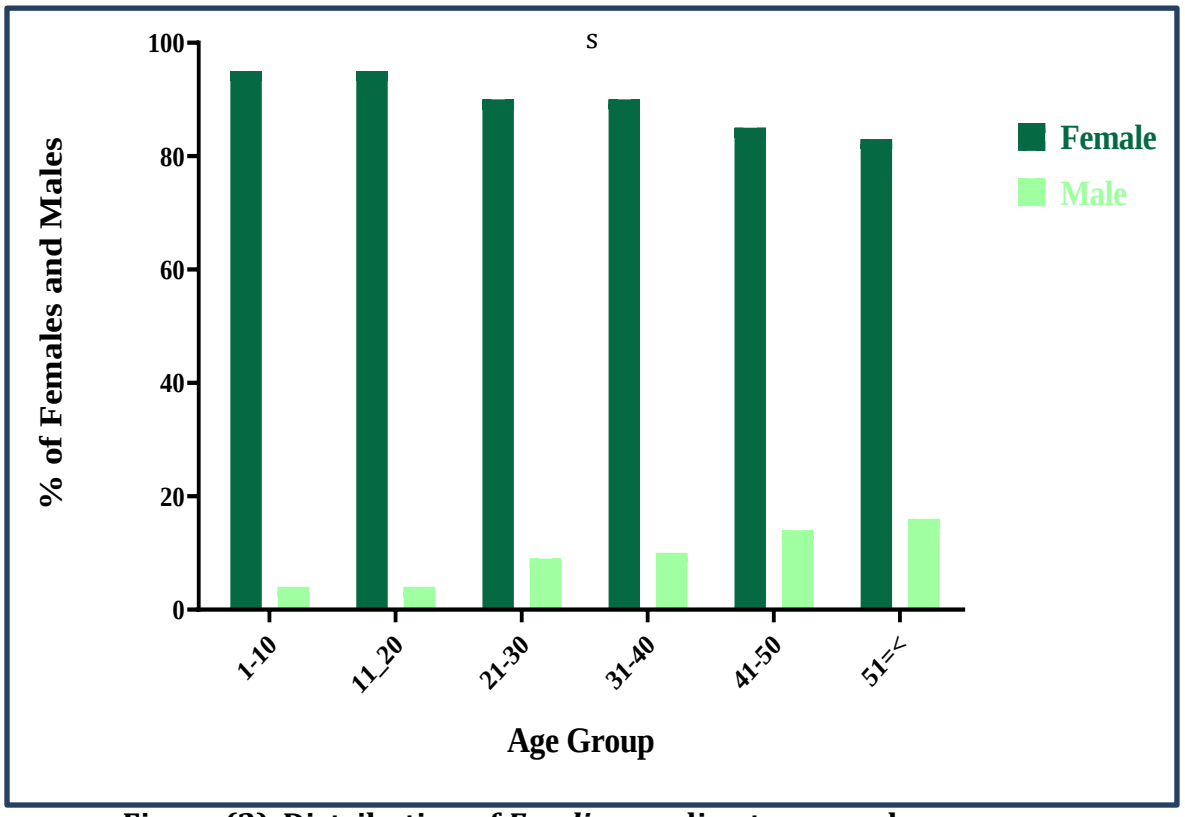

Figure (2): Distribution of *E. coli* according to sex and age

Table (6) refelect the distribution of *E. coli* according to marital status, among which the highest rate of UPEC cases was recorded which was 38.00 %(76/141) as compared to umarried people, but statistically, this difference was non-significant (P>0.05).

Table 6: Distribution of *E. coli* according to marital status

Marital Status	No. of examined samples	Total No. of Infections		Sex			
				Females		Males	
		No.	%	No.	%	No.	%
Married	200	76	38.00	67	88.16	9	11.84
Single	200	65	32.50	61	93.85	4	6.15
Total	400	141	35.25	128	32.00	13	3.25
P>0.05							

The occurrence of UPEC among both married and single patients of different ages was higher in females than males, as shown in Figure (3), with the highest rate (31.34%) being within the ages 21-30 years. On the other hand, the highest rate (33.33%) of UPEC among single females, was in ages of ≤1-10 years. Regarding males, the highest rate in married males was

recorded among the ages 41-50 years which was 33.33%, while in single males, the highest rate of infection was 50% in ages of 21-30 years.

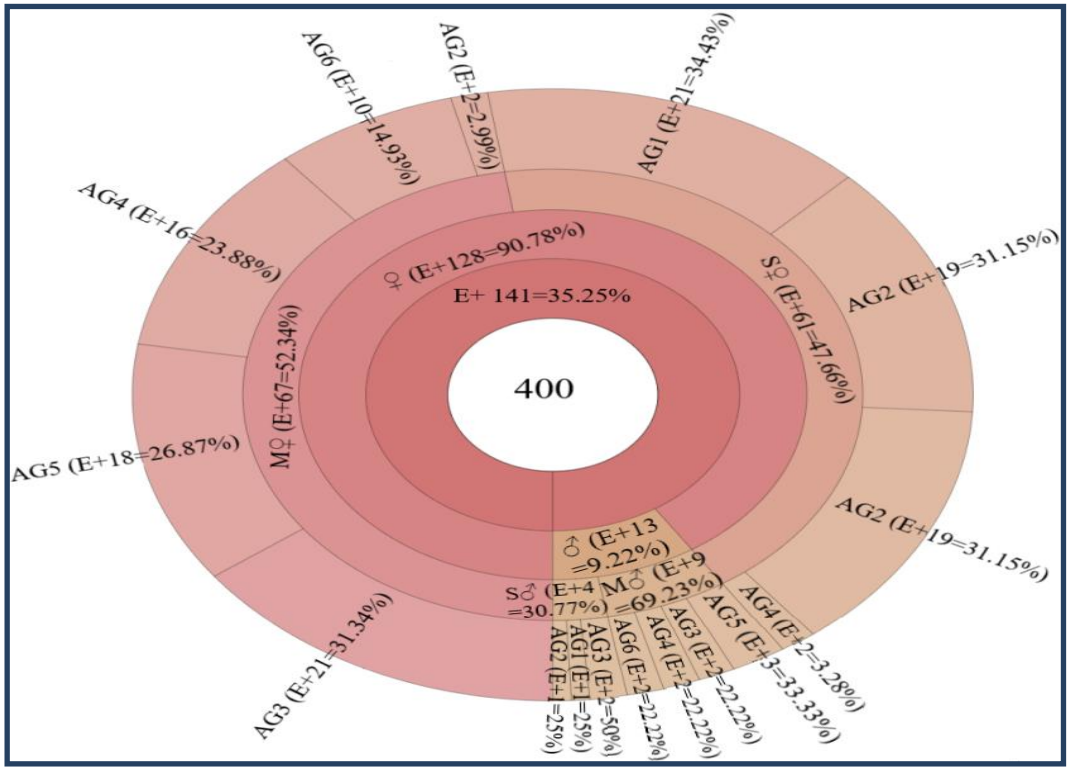

Figure 3: Distribution of *E. coli* according to marital status and age

Keys: 400=total samples examined, E+=positive samples for *E. coli*, ♂=Male, ♀= Female, M=married, S=single, AG1=age group ≤1-10, AG2= age group 11-20, AG3=age group 21- 30, AG4=age group 31- 40, AG5=age group 41-50, AG6=51≤.

The variation of the environmental temperature had positive impact on the rate of UPEC infection as shown in Table (7), which indicate that the number of *E. coli* at average high temprature months (30.7˚C) was higher (39.57%) as compared with that of average low temperature months (13.3˚C) which was 31.46%.

Table 7: Incidence of UPEC during high and low temperature months

Various temperature ˚C and months	No. of examined samples	Total no. of infections	
		No.	%
Jul-Sept (Mean temp. 30.7˚C)	187	74	39.57
Oct-Jan (Mean temp. 13.3˚C)	213	67	31.46
Total	400	141	35.25

With regard to residency, that the highest prevalence of UPEC was among patients living in Zakho city which was 40.56% as compared to the rate of patients living in villages and camps (Table. 8). Statistically the differences between sex and residency was extremely significant (P<0.000001).

Table 8: Distribution of UPEC according to residency

Residence	No. of examined samples	Total no. of infections		Sex			
				Females		Males	
		No.	%	No.	%	No.	%
City	323	131	40.56	120	91.60	11	8.40
Villages	36	5	13.89	3	60.00	2	40.00
Camps	41	5	12.20	5	100	0	0
Total	400	141	35.25	128	32.00	13	3.25
*P<0.000001							

4. Discussion

Uropathogenic *E. coli* is the predominant bacteria that cause UT infections in humans of both sexes and all ages. [25] In the present study, the rate of UPEC among enrolled outpatients was 35.25%. Higher rates of infection than the present study were recorded in Iraq and other countries such as: In Baghdad and Anbar cities-Iraq, *E. coli* was reported at a rate of 40.86%, [18] while in Kerbala city, a higher rate [44.64%] was reported in 110 examined urine samples. [6]

The gene *uidA* encodes for *E.coli* which is the inducible β-D-glucuronidase enzyme.[30] Thus, almost all studies have used this gene as a molecular marker for *E.coli* identification. [1] The length of *uidA* gene reported in this study was 670 bps, Similarly in Duhok-Kurdistan region/Iraq, also the sam length (670 bp) for this gene was reported. [32] While a slightly shorter than that reported in the current study (658 bps) using the same primer sequence was recorded.[1] The variation in the length in both studies might be attributed to the fact that genes usually contain several repeats of the coding microsatellite (1-10 bps) and minisatellite (>10 bps), which are very dynamic components of genomes and are subcategories of tandem repeats (TRs) which makes up the genomic repetitive regions. [21, 31] This variation may provide functionally diversion in the cell surface of the antigen that allows the cells for rapid adaptation to the fluctuating environment and/or the evasion of the immune system of the host. [29]

Similarly a comparable occurrence of UPEC among both sexes have been reported in Duhok city-Iraq, in which females showed a much higher rate than males (81.3% vs 18.68%), respectively. [31] Moreover, variable prevalence of UPEC among different ages in this study is somewhat similar to other studies, such as in Duhok city, the highest infection rate [61.96%] with UPEC was recorded among ages of 31 to 45. [20] While other studies reported different results such as: in India they reported the highest rate(51.15%) of *E. coli* infection among females aged 21-40 years, while it was 53.84% among males aged 40 to 80 years. [16]

Over all ages, the risk of UTI infection in females was higher than that of males. [42] This could be attributed to a variety of factors, such as; the anatomical structure of the urinary tract, sexual intercourse, personal hygiene and number of pregnancies. [2] These differences make females more susceptible to pathogens and allow them to access to the bladder. [33] Furthermore, about 40-50% of females during their lives may have at least one symptomatic UTI [2] and about 20-30% of adult females approximately can experience recurrence UTI within 6 months after primary infection by UTI, and about 3% will experience a third infection. [25] This picture is slightly different in elder men, where increasing prostatic hypertrophy may obstruct the urine flow and increase the risk of developing a UTI. Generally, in elder people, UTIs are the most common bacterial infections, which oftenly are asymptomatic. [26] In infants, although breastfeeding reduces the risk of infection with UTI, [42] but several other factors can fascilitate *E. coli* infection such as, weak body structure, incomplete development of the child's immune system, and incorrect methods for cleaning the anal area. [7]

The prevalence of UPEC among both married and single people was higher in females than males, but generally married pepole aged 21 to 50 years showed higher rates, while in single ones the infection was higher at ages between ≤1 to 30 years. The most probabile reason for the higher rate of infection with UPEC among married people at ages of 21-50 years is due to the sexual activities at these ages. [17] While among single people, low rates were recorded among ages of 31-40 years because at this age the females are more mature and can protect themselves from infection. [28]

The variable occurrance of UPEC at different months of the year, with higher rates in warm months is in accordance to the previous study in Zakho city in which the majority of uropathogenic infections occurred at high temperature months than that of low temperature months which were 36.71% vs 29.69%, respectively. [5] The risk of UTIs increases with the increase of the environmental temperature, this might be due to the increase in the rate of

deydration at warm temperature that reduce the protection against UTIs, therefore, the incidence of UTIs is increased by 8-20% during the summer months relative to the winter months. [37] Drinking a high amount of fluid enhances high urine excretion which decreases the incidence or recurrence of UTI while consuming small amounts of water causes a decrease in urine outpouring, which leads to an increased incidence of UTI. [5]

Regarding residency, the rate of UTIs was higher in people living in the city. Similarly in Erbil city/Kurdistan region, the incidence of UTIs among females and males living in urban areas were higher than those living in rural areas, which were 76.1% and 54.7%, and 45.3% and 23.9%, respectively. [19] Furthermore, in Palestine the prevalence of *E. coli* among patients who live in the city was higher than those living in camps and villages which were 33.3%, 20.5% and 18.8%, respectively. [36] This may be due to the contribution of other bacteria besides UPEC in these infections. [12] This is not only due to different living style in the urban compared with the rural but also the high population density, however, more studies are required in this direction.

5. Conclusion

The UPEC strain is the most common pathogen which causes UTIs in humans of both sexes and at all ages. The *uidA* primer gave successful identification of *uidA* gene in the tested *E.coli* isolates, that confirme final diagnosis of *E. coli.* Females over all ages showed higher rates than males. It is worthwhile to mention that various risk factors facilitate the spreading of *E.coli* among the community.

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