

# Prevalence, Antimicrobial Susceptibility Profile, and the Associated Risk Factors of Uropathogenic *E. coli* among Pregnant Women in Zakho City, Kurdistan Region, Iraq

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## Abstract

**Background:** *Escherichia coli* (*E. coli*) are known to be a major cause of uropathogen among women globally. Urinary tract infection in pregnant women continues to pose clinical problems, which is a big burden for physicians. The emergence of antibiotic resistance to *E. coli* is inevitable and one of the critical health-associated problems. The present study aimed to determine the *E. coli* isolates, risk factors, and antibiotic resistance profile of the UTI among pregnant women.

**Methods:** We conducted this cross-sectional study on pregnant women who attended maternity hospital in Zakho City, Kurdistan Region, Iraq. From October 2020 to January 2021, 196 subjects were recruited in the present experiment. Uropathogenic *E. coli* were detected from mid-stream urine of the participants. Subsequently, we performed antibiotic sensitivity test using microbiological standard tests. Through the use of designed questionnaires, the required data were collected, processed, and analysed via the Fisher's exact test and Chi-square.

**Results:** Out of a total of 196 subjects, 34 (17.34%) were culture positive for *E. coli* infection among pregnant women. A high rate of infection was detected in the age group of between 25-34 years (21.6%). Additionally, the highest rate of *E. coli* belonged to illiterate people (19.4%), followed by those with higher education (18.9%). The assessment of the risk factors associated with *E. coli* indicated that diabetes ( $P=0.028$ ), a history of UTI infection ( $P=0.049$ ), and the presence of symptoms during infection ( $P=0.001$ ) were statistically significantly different. The antibiotics sensitivity profile in urine samples of pregnant women showed that *E. coli* were extremely sensitive to Nitrofurantoin (82.4%), Meropenem (79.4%), and around 67.6% for both Amikacin and Ciprofloxacin.

**Conclusion:** The prevalence rate of *E. coli* infection is quite lower among pregnant women compared to that in non-pregnant ones. The major risk factors associated with *E. coli* during pregnancy were diabetes mellitus, a history of UTI, and the presence of symptoms during infection. *E. coli* isolated from urine samples seem to be different concerning antibiotic sensitivity pattern. During their regular follow-up, pregnant women should also be examined for major risk factors and antibiotic susceptibility.

**Keywords:** *E. coli*, Risk factors, Uropathogen, Antibiotic sensitivity, Pregnant women, Iraq

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## 1. Introduction

*Escherichia coli* (*E. coli*) are one of the major causes of urinary tract infections (1). Several studies have reported *E. coli* a major cause of UTI amongst Gram-negative bacteria and *Staphylococcus* species have been found to be the most common among Gram-positive bacteria (2-4). Therefore, this pathogen is clinically important for physicians and can be isolated from different clinical specimens. In pregnant women, UTIs, including *E. coli*, continue to pose clinical problems and are a major burden for physicians. Generally, it has been reported that uropathogens in pregnant women are extensively higher than those in non-pregnant female population (5). Both symptomatic and asymptomatic UTIs among pregnant women are predominant, which commonly lead to side effects on the mother, the fetus,

and the new born (6). During pregnancy, the substantial changes in the urinary tract and the immune system can surge the frequency rate of bacteriuria, causing side effects for both mother and fetus. Parity, sickle cell anaemia, higher age, diabetes, a history of UTI, and urinary tract disorders could further augment the risk of UTIs in pregnant women (7).

The emergence and spread of antibiotic resistance to several pathogens are a major public health issue worldwide. The overuse of these drugs without medical prescription and their low cost considerably increase the risk of antibiotic resistance (8). It was previously reported that the increase in the resistance rate to the most commonly used antibiotics may have played a role in the existence of recurrent infections rate (9). In addition, the differences in antibiotic resistance rate

was reported among different geographical regions (2, 10). Thus, periodic evaluation of the prevalence rate of *E. coli* and antibiotic resistance profile is recommended in order to achieve optimal outcomes and avoid side effects of drugs (11). There is a variety of infection-causing microorganisms along with high resistance patterns of such microorganisms in Kurdistan Region of Iraq (1, 4, 12, 13). During pregnancy, women should be assessed and detected for major causes and risk factors associated with UTI during their antenatal follow-up. The sensitivity of antibiotics and their adverse effects associated with pregnancy should also be considered. The objectives of the present study included the followings: identification and isolation of *E. coli* causing UTIs among pregnant women in Zakho city, Kurdistan, Region, Iraq; assessment of the UTI-related risk factors and the pattern of urinary complaints during pregnancy; investigation of antibiotic susceptibility profile of *E. coli* isolates and determination of the antibiotic resistance patterns.

## 2. Methods

We conducted this cross-sectional research among the pregnant women who attended the maternity hospital of Zakho City, Kurdistan Region, Iraq, for antenatal care from October 2020 to January 2021. During this period, 196 pregnant women who had urinary tract problems and all the patients attended this maternity hospital, were recruited. There were 50 non-pregnant subjects with an age ranging from 16-45 years (the mean age of 25.65 years $\pm$ 5.4 SD). The sample size was measured on the basis of the number of pregnant women attending the centre during the previous six months using systematic random sampling method and taking as reference in Menoufia Governorate, Egypt (14).

The pregnant women who participated in the present study were assessed with a designed questionnaire. This questionnaire was divided into three parts; the first one included sociodemographic characteristic, namely age, educational status, residence, occupation of the pregnant woman, and family income. The second part comprised the risk factors associated with UTI including the gestational age, number of pregnancies, diabetes mellitus, abortion, previous treatment for UTI during the current pregnancy, a history of UTI, and the presence of symptoms.

The inclusion criteria were pregnant women who agreed to participate in the study. The pregnant women who were not willing to participate and were

taken antibiotic agents within 7 days at the time of the enrolment were excluded from this work.

### 2.1. Sample Collection and Processing

Primarily, the subjects passed a small amount of urine; clean-catch midstream urine samples (196) were then collected from each subject into a sterile specific container (5 mL) on the same day of enrolment in order to avoid contamination. Afterwards, the samples were sent to the laboratory for further analysis and the majority of the samples were directly analysed within one hour after collection.

Subsequently, 10  $\mu$ l of well-mixed urine samples were inoculated on MacConkey and blood agar (Oxoid Ltd. Hampshire, UK), using a disposable loop according to the standard culture procedures. The plates were then incubated overnight at 37°C and bacterial growth was detected. The identification of morphological colony and biochemical tests were used to confirm the *E. coli* pathogen. We incubated the cultures without growth colony after 24 h of incubation for another 48 h. The cultures were inspected and more than 10<sup>5</sup> CFU/ml of urine was considered to be positive for *E. coli* infection.

### 2.2. *E. coli* Isolation and Sensitivity Test

We initially recognised *E. coli* pathogens according to the characteristics of the bacteria morphologically on MacConkey agar and Eosin Methylene Blue (EMB) agar following microbiological cultures and biochemical tests based on the CLSI guidelines (15).

We measured antibiotic susceptibility of the *E. coli* isolates utilising agar disc diffusion method. The isolated single colonies were normally suspended in normal saline (5 ml) to standard 0.5 McFarland in order to standardise the inoculated size of bacteria. Using sterile swab, these suspensions of bacteria were streaked on the surface of Muller Hinton agar. Thereafter, the antibiotic discs were placed on the inoculated plates with a sterile forceps, and incubated for 18-24 h at 37 °C. The tested antibiotic discs (Oxoid Ltd, UK) were Ceftriaxone (20 $\mu$ g), Nalidixic acid (30  $\mu$ g), Amikacin (10  $\mu$ g), Gentamycin (10  $\mu$ g), Nitrofurantoin (100  $\mu$ g), Ciprofloxacin (10  $\mu$ g), Doxycycline (10  $\mu$ g), Cefotaxime (30  $\mu$ g), Meropenem (10  $\mu$ g), and Cefixime (30  $\mu$ g). The diameter of the antibiotic inhibition zone surrounding the disc was evaluated via a digital metal calliper and the isolates were then categorised as resistant and sensitive following the CLSI.

2.3. Ethics

The study protocols, questionnaire, and consent forms were approved by the Ethics and Science Committee of the College of Medicine, University of Zakho, Duhok City, Iraq. Written informed consent was obtained from all the subjects prior to the sampling.

2.4. Statistical Analysis

We employed the GraphPad Prism software package, version 8, for the analysis of our findings. The results were stated as frequency (%) and mean±standard deviation. Comparisons between the variables and pregnant and non-pregnant women were made with Chi-square and Fisher’s exact test. The level of significance was set at P value of <0.05.

3. Results

3.1. Characteristics of the Participants

Characteristics of the participants associated with the *E. coli* UTI infection among pregnant women are presented in Table 1. During the study, a total of 196 participants were recruited, the majority of whom were aged between 25-34 years 111(56.6%) and 15-24 years 75 (38.3%). About 31 (15.8%) of the participants were unable to write and read while 68 (34.7%) had a higher

educational level. Around 185 (94.4%) of them were housewives whereas the rest were government workers. Out of the total study population, 172 (87.8%) lived in urban areas and 157 (80.1%) had an intermediate level of family income.

3.2. Frequency Rate of *E. coli* among Pregnant Women

The frequency rate of infection among different demographic variables are presented in Table 1. Out of 196 subjects, 34 (17.34%) were culture positive for *E. coli* infection among the pregnant women. There were no associations between the studied sociodemographic variables and the prevalence of *E. coli* (Table 1). However, a high infection rate was found in the age group of between 25-34 years 24 (21.6%). The highest rate of *E. coli* infection belonged to the illiterate cases 6 (19.4%), followed by those with a higher level of education 7 (18.9%). Additionally, around 18% of *E. coli* infection was found for both the employed and housewives. The subjects living in rural areas showed the highest rate of infection 6 (20.8%). The highest prevalence rate was found among those with higher family income 2 (33.3%), followed by those with lower income 8 (24.2%).

3.3. Risk Factors Associated with Uropathogenic *E. coli*

Herein, we investigated 196 pregnant women and evaluated the risk factors correlated with uropathogenic

**Table 1:** Sociological and demographic characteristics of the pregnant women referred to the maternity hospital of Zakho (n=196)

Variables	Total Frequency (%)	Culture results for <i>E. coli</i> infection		*P value
		Frequency (%) of positive infection	Frequency (%) of negative infection	
<b>Age group (Year)</b>				
16-24	75 (38.03)	8 (10.07)	67 (89.03)	0.51
25-34	111 (56.06)	24 (21.06)	87 (78.04)	
35-45	10 (5.01)	2 (20)	8 (80)	
<b>Educational status</b>				
Illiterate	31 (15.08)	6 (19.04)	25 (80.06)	0.91
Primary school	60 (30.06)	11 (18.03)	49 (81.07)	
High school	68 (34.07)	10 (14.07)	58 (85.03)	
Higher education	37 (18.09)	7 (18.09)	30 (81.01)	
<b>Occupation</b>				
Worker	11 (5.06)	2 (18.02)	9 (81.08)	0.6
House wife	185 (94.04)	32 (17.03)	153 (82.07)	
<b>Residence</b>				
Urban	172 (87.08)	29 (16.09)	143 (83.01)	0.41
Rural	24 (12.02)	5 (20.08)	19 (79.02)	
<b>Family income</b>				
Low	33 (16.08)	8 (24.02)	25 (75.08)	0.27
Intermediate	157 (80.01)	24 (15.03)	133 (84.07)	
High	6 (3.01)	2 (33.03)	4 (66.07)	

\*Chi-square test (Fisher’s exact test) was used to measure P value.

**Table 2:** Risk factors associated with uropathogenic *E. coli* among the pregnant women referred to Maternity General Hospital in Zakho City (n=196)

Risk factors	Total frequency (%)	Culture results for <i>E. coli</i> infection		*P value
		Frequency (%) of positive infection	Frequency (%) of negative infection	
<b>Gestational age</b>				
First Trimester	52 (26.05)	12 (23.01)	40 (76.09)	0.44
Second Trimester	71 (36.02)	11 (15.05)	60 (84.05)	
Third Trimester	73 (37.03)	11 (15.01)	62 (84.09)	
<b>Number of pregnancies</b>				
1 to 2	100 (51)	17 (17)	83 (83)	0.99
3 to 4	73 (37.03)	13 (17.08)	60 (82.02)	
> 5	23 (11.07)	4 (17.04)	19 (82.06)	
<b>Diabetes mellitus</b>				
Yes	9 (4.06)	4 (44.04)	5 (55.06)	0.028
No	187 (95.04)	30 (16.01)	157 (83.09)	
<b>Abortion</b>				
Yes	50 (25.05)	5 (10)	45 (90)	0.08
No	146 (74.05)	29 (19.09)	117 (80.01)	
<b>Previous treatment for UTI during pregnancy</b>				
Yes	43 (21.09)	0 (0)	43 (100)	0.001
No	153 (78.01)	34 (22.02)	119 (77.08)	
<b>Past history of UTI</b>				
Yes	151 (77.01)	30 (19.09)	121 (80.01)	0.049
No	45 (22.09)	4 (8.09)	41 (91.01)	
<b>Presence of symptoms</b>				
Yes	31 (15.08)	20 (64.05)	11 (35.05)	0.001
No	165 (84.02)	14 (8.05)	151 (91.05)	

\*Chi-square test (Fisher's exact test) was used to measure P value.

*E. coli* (Table 2). Our results indicated that diabetes mellitus (P=0.028), a history of UTI infection (P=0.049), and the presence of symptoms during infection (P<0.001) were significantly associated with *E. coli* UTI. However, gestational age (P=0.44), number of pregnancies (P=0.99), and abortion (P=0.08) were revealed to be insignificantly associated with UTI.

### 3.4. Comparison between Pregnant and Non-pregnant Women Concerning the Frequency of *E. coli*

Out of 196 samples, 34 (17.34%) were culture positive for *E. coli* infection in the pregnant women (Table 3). However, the frequency of *E. coli* infection among the non-pregnant subjects was 34.69% (n=17). Based on the results of Chi-square (Fisher's exact test), surprisingly, the highest prevalence rate of infection belonged to the non-pregnant cases compared to the pregnant women,

with significant differences (P=0.032) (Table 3). Overall, 49/245 (20%) urine samples were culture positive for *E. coli* infection.

### 3.5. *E. coli* Susceptibility to Antibiotics

The antibiotics susceptibility profile of pregnant women showed that isolates were enormously sensitive to Nitrofurantoin (82.4%) and Meropenem (79.4%), and around 67.6% for both Amikacin and Ciprofloxacin (Table 4). However, in the non-pregnant women, *E. coli* isolates from the samples were extremely sensitive to Amikacin (100%), followed by Meropenem (94.1%) and Gentamicin (82.4%) (Table 4). Table 4 demonstrates the results of the statistical analysis between the pregnant and non-pregnant subjects regarding the test of antibiotics susceptibility to *E. coli* isolates.

**Table 3:** Comparison between the pregnant and non-pregnant women concerning the frequency rate of *E. coli* infection in Zakho City, Iraq

	Total frequency in the samples	Frequency (%)	*P value
Pregnant women	196	34 (17.34)	0.032
Non-pregnant	49	17 (34.69)	

\*Chi-square test (Fisher's exact test) was used to measure P value.

**Table 4:** Antibiotics susceptibility patterns of *E. coli* between the pregnant and non-pregnant women

Antibiotics	Frequency of isolates recovered from urine (% of sensitivity)		*P value
	Pregnant (n=34)	Non-pregnant (n=17)	
Amikain (AK10)	23 (67.06)	17 (100)	0.006
Cefixime (CFM5)	10 (29.04)	8 (47.01)	0.175
Cefotaxime (CTX30)	12 (35.03)	11 (64.07)	0.045
Cetrixone (CRO10)	10 (29.04)	11 (64.07)	0.017
Ciprofloxacin (CIP 10)	23 (67.06)	10 (58.08)	0.375
Gentamicin (CN 10)	15 (44.01)	14 (82.04)	0.009
Nalidixic acid (NA 30)	16 (47.01)	12 (70.06)	0.097
Doxycycline (DO 10)	7 (20.06)	9 (52.09)	0.022
Meropenem (MEM10)	27 (79.04)	16 (94.01)	0.172
Nitrofurantoin (F100)	28 (82.04)	11 (64.07)	0.147

\*Chi-square test (Fisher's exact test) was used to measure P value.

#### 4. Discussion

*Uropathogenic E. coli* is a major cause of urinary tract infections (2-4). In pregnant women, this pathogen continues to pose significant clinical problems and challenges for physicians. During pregnancy, numerous risk factors have been proposed to surge the frequency of bacteriuria causing serious adverse reactions to the fetus and mother, including gestational age, multiparity, diabetes mellitus, urinary tract abnormalities anatomically, as well as a previous history of UTI (16). Furthermore, anaemia, educational status, socio-economic status, catheterization, and sexual activity increase the risk of UTI among pregnant women (16). Antimicrobial resistance to *E. coli* remains a major threat to public health globally, particularly in developing countries, like Kurdistan Region of Iraq. It also triggers serious health-related problems, such as treatment failure and prolonged hospitalisation (1, 4, 12, 13). The present study aimed to identify the prevalence rate and risk factors associated with *uropathogenic E. coli* among the pregnant women in Zakho city, Kurdistan Region, Iraq. Additionally, we sought to investigate the antibiotic susceptibility profile of *E. coli* isolates among pregnant women.

Herein, the overall infection rate of the isolated *E. coli* was quite lower among the pregnant women compared to that of the control group (non-pregnant women). The infection rate was statistically significantly difference between the pregnant and non-pregnant subjects. The low infection rate in the present study among the pregnant women could be attributed to the fact that pregnancy is a stage when the vaginal microbiota condition by very high oestrogen levels has a blood supply of glycogen and a high concentration of lactobacilli flora, which significantly reduces the development of pathogenic bacteria. This finding is

consistent with other studies performed in Tanzania, showing a similar rate of *uropathogenic E. coli* infection among pregnant women (17). However, the prevalence rate of *E. coli* was quite lower than that in a study conducted in Kirkuk city, Iraq, which detected that the prevalence of *uropathogenic* among pregnant women was 43% (18). Another study performed in Iran indicated that the prevalence of *E. coli* among pregnant women was higher compared to that in our results (19). This difference and inconsistency could be due to variations in the environmental factors and community and social habits, low economic status, awareness and knowledge about personal hygiene, and educational standards. Regular health education provided at health services and public knowledge among pregnant women on antenatal care and follow-up throughout pregnancy in the studied region may be attributable to the lower prevalence of UTI infection identified in this study compared to that in previous research.

Different factors, such as gestation age, age, and level of education, have been reported to lead to UTIs, including *E. coli*, among pregnant women (20). In the present study, the pregnant women in their first trimester had the highest rate of *E. coli*, followed by those in their second trimester. These findings were on the contrary to previous works performed in Mosul city, Iraq. They found that the highest rate of infection was among pregnant women in the third trimester followed by the second trimester and then in the first trimester (21). Furthermore, the highest rate of *E. coli* was detected among the women aged between 25-34 years with high parity and its lowest rate belonged to those aged between 16-24 years; this is in agreement with a study conducted in Iraq (22). The frequency rate of infection was shown to be greater in the age group of 25-34 years, which could be due to the fact that women in this age range are more sexually active. A highest

rate of infection was also reported among the pregnant women who lived in rural areas in comparison with those inhabiting urban area. This could be due to the nature of rural sanitation in addition to the lack of concern with personal hygiene. The current work showed a higher rate of *E. coli* among employed subjects compared to housewives; meanwhile, there was no statistically significant differences between them. This may be since housewives can visit the health unit regularly for antenatal care or due to the fact that working women are more exposed to different risk factors. According to the educational level of the participants, the higher rate of *E. coli* infection was found in the illiterate women, followed by those with a higher level of education. This might be on account of pregnant women's lack of information regarding the transmission and prevention of uropathogens, including *E. coli*.

Our results are consistent with those reported by several studies conducted in Egypt and Iraq; they found that the highest infection rate was recorded among women with a lower level of educational status or illiterate women (23). Another study in Turkey found that UTI was significantly greater among women who had secondary level of education (24). On the other hand, a study performed in Pakistan found no significant effects by education on the prevalence of UTI among pregnant women (25). Additionally, the highest infection rate was found among pregnant women who had a history of UTIs compared to those without a history of infection, with statistically significant differences. Our findings are consistent with several studies performed in Iraq and Pakistan, suggesting significant differences between women with a history of UTI and those without it (26). We also found a significant association between the asymptomatic and symptomatic patients. Furthermore, there were significant differences between the diabetic and non-diabetic pregnant women with UTI caused by *E. coli*. This is in contrast with another study, conducted in Ethiopia, which showed no significant differences between diabetic and non-diabetic patients (27). These variances might be attributable to living standards, culture, practice, and the research population's categorisation, as well as the research time and urine screening procedures used.

The isolated *E. coli* from the urine samples of the pregnant women herein revealed a difference in antibiotic sensitivity pattern. The antibiotic susceptibility pattern found that uropathogenic *E. coli* was extremely resistant to ampicillin and highly

susceptible to imipenem and Nitrofurantoin. Our data are in the line with those of other studies, showing that the isolated pathogen from urine samples were vastly resistance to ampicillin and extremely sensitive to imipenem (28). Based on other study performed in Iraq, around 75% of the isolated *E. coli* from urine samples were highly resistant to amoxicillin and clavulanic acid (3). The same authors have shown that around 98% of *E. coli* were sensitive to imipenem and Amikacin (3). Our findings implied the alarming increased resistance of *E. coli* against ampicillin and Gentamycin. This could be due to the overuse and misuse of this antibiotic among general population. Thus, an urgent measure is required in the region for taking this threatening development of antibacterial resistance under control.

## 5. Conclusion

The frequency rate of *E. coli* infection was lower among the studied pregnant women compared to that in the control group. The infection rate was lower in the pregnant women compared to that reported by other studies conducted in Kurdistan Region, Iraq. This variation could be assigned to the differences in the standard of personal hygiene, environment, social habits, and low economic status of the recruited targets. The study design and methodology may also result in differences concerning infection rate among different studies in the region. During pregnancy, the major risk factors associated with *E. coli* were diabetes mellitus, a history of UTI, and the presence of symptoms during infection. The isolated *E. coli* from the urine samples showed differences in antibiotic sensitivity pattern. Nitrofurantoin, Meropenem, Amikacin, and Ciprofloxacin were found to be the major effective antibiotics against isolated *E. coli* from urine samples among pregnant women. It could be recommended that pregnant women be assessed for risk factors and antibiotic sensitivity test be carried out during their regular follow-up.

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**Conflict of Interest:** None declared.

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