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Antibiotic Susceptibility Pattern of Uropathogenic Bacteria in Pregnant Women with and Without Diabetes in Kirkuk City, Iraq.

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Abstract

In a cross-sectional survey conducted at Gynecological and Pediatric Hospital in Kirkuk City, Iraq. One hundred urine samples from pregnant women were collected including both diabetic and nondiabetic women, aged between 18-40 years, from July 2023 to December 2023. The results revealed that out of 100 participants, 14 were diagnosed with diabetes mellitus (DM), 22 with gestational diabetes mellitus (GDM), and 64 with no diabetes. However, only 35% of them exhibited bacterial growth. The recent study indicates that women with diabetes are at a higher risk of developing urinary tract infections (UTIs). Specifically, 42.9% of samples from women with diabetes (DM) and 41% of samples from women with gestational diabetes mellitus (GDM) showed evidence of bacterial growth, compared to 31% of samples from women without diabetes. Two Gram-negative bacteria (*Escherichia coli*, *Klebsiella* spp) and two Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus* spp) obtained. *Escherichia coli* was the most frequent bacteria (60%), followed by *Klebsiella* spp at 20%, *Staphylococcus aureus* (14.3%), *Enterococcus* spp (5.7%). The isolates showed a high susceptibility rate to levofloxacin 100%, 91.5% to nitrofurantoin, 91.4% to imipenem, 88.5% to amikacin, 85.7% to both gentamicin and ceftriaxone, respectively. Conversely, 71.4%, 68.5% and 65.7% of the isolates exhibited high-level resistance towards azithromycin, amoxicillin, and ceftazidime respectively, while 37.2%, 28.5%, and 25.8% of the isolates demonstrated resistance to cefixime, ciprofloxacin, and cefoxitin respectively.

1. Introduction:

There are two types of diabetes mellitus (DM) that occur during pregnancy: pre-existing diabetes, referred to as DM, which the patient had diabetes prior to pregnancy, and gestational diabetes mellitus (GDM), which is newly diagnosed diabetes during pregnancy. GDM is defined by an inability to properly process glucose, which may arise either during pregnancy or become apparent for the first-time during pregnancy [1], [2], [3]. Among pregnant women, urinary tract infections (UTIs) are the most frequent bacterial disease, especially with those have exhibit concurrent health conditions. These may include diabetes, congenital malformations, sickle cell anemia, polycystic kidney disease, and chronic UTIs. *Escherichia coli* and other Gram-negative bacteria, such as *Klebsiella* spp, *Acinetobacter*, and *Proteus* spp, represent 70–80% of UTIs infections during gestation. Conversely, about 10% of UTI infections in pregnant women are caused by Gram-positive bacteria such as group B *streptococcus* and *Enterococcus faecalis* [4], [5]. Moreover, the prevalence of symptomatic UTIs during pregnancy ranges from (3-10.1)% in non-diabetic women, while it can reach 27.6 % in diabetic pregnant women [5], [6]. Physiology and structure of the bladder and kidneys could really change during pregnancy, which could raise

the chance of developing acute cystitis or pyelonephritis in addition to asymptomatic bacteriuria. Due to immune system malfunction, bacterial adherence to the uroepithelium, and glycosuria, pregnant women with diabetes may be more susceptible to infection [7], [8].

When treating urinary tract infections (UTIs), patients with gestational diabetes mellitus (GDM) often follow the same protocol as pregnant patients without diabetes, administering antibiotics based on antibiotics susceptibility as determined by urine culture. UTIs are frequently treated with ampicillin, amoxicillin, trimethoprim-sulfamethoxazole, nitrofurantoin, and cephalosporins. UTIs must be rapidly diagnosed and treated since, if ignored, they can result in consequences such kidney abscess, urosepsis, and emphysematous cystitis/pyelonephritis. Pregnancy-related urinary tract infections (UTIs) are linked to an increased risk of preeclampsia and newborn birth abnormalities [2].Aside from effectively managing UTIs, it's imperative to uphold optimal blood sugar levels during pregnancy. DM raises the possibility of difficulties during pregnancy, such include premature birth, low birth weight, and cesarean delivery, preeclampsia, and the birth of babies with macrosomia [8]. During pregnancy, untreated UTIs can lead to severe complications. Treating UTIs can decrease the likelihood of pregnancy-related issues. Therefore, screening for early detection and treatment of UTIs in expectant mothers is crucial to avoid difficulties [9]. Therefore, this study aimed to examine the prevalence of UTIs among both diabetic and non-diabetic pregnant women, identify the predominant bacterial species responsible for these illnesses, and evaluate their sensitivity to commonly prescribed antibiotics. The anticipated outcomes of this research are to provide valuable insights that can assist physicians in selecting the most effective antibiotics for treating pregnant patients, particularly those with diabetes mellitus (DM).

2. Materials and Methods:

2.1 Study Area and Population:

The current study was conducted at microbiology laboratory of Gynecological and Pediatric Hospital in Kirkuk City, Iraq. During the period from July to December 2023. One hundred urine samples were taken from pregnant women at various stages of gestation who had UTI symptoms with or without diabetes mellitus. The age of patients ranged between 18-40 years.

2.2 Inclusive and Exclusive Criteria:

Pregnant women who had signs of urinary tract infection, including dysuria, urinary urgency, and increased frequency of urination, were selected for this study. However, pregnant women who had used antibiotics in the two weeks before to the research and those who declined to participate were excluded.

2.3 Sample Collection

Participants were provided with sterile screw-capped universal containers to collect clean mid-stream urine samples on their own while urinating, samples were individually labeled [10].

2.4 Sample Processing:

Urine samples underwent culturing on plates of various media, including Blood, MacConkey, Mannitol salt, and Eosin methylene blue, to isolate diverse bacterial species. The inoculated plates were incubated aerobically at37°C for 24 hr. using a 4 mm calibrated loop platinum wiring [10].

2.5 Bacterial Identification:

All significant isolates underwent examination using standard microbiological methods, including gram staining and various biochemical tests. To verify the identity of the isolates, diverse biochemical tests had been applied [11], [12].

2.6 Antibiotic Susceptibility Test:

The antibiotic susceptibility test was conducted using Kirby-Bauer 1966 diffusion techniques. Briefly, isolated colonies were spread onto Muller-Hinton agar, and antibiotic paper discs were placed on the surface of plate. After incubating the colonies at 37°C for 24 hours, the zones of inhibition around the discs were examined and compared according to the guidelines provided by the Clinical and Laboratory Standards Institute to determine susceptibility or resistance [13]. Twelve antibiotics have been used in the study: amoxicillin (AX) - 10 μg, cefixime (CFM) - 5 μg, ceftazidime (CAZ) - 30 μg, ceftriaxone (CRO) - 10 μg, cefoxitin (CX) - 30 μg, ciprofloxacin (CIP) - 10 μg, levofloxacin (LEV) - 5 μg, amikacin (AK) - 10 μg, gentamycin (CN) - 10 μg, azithromycin (AZM) - 15 μg, nitrofurantoin (F) - 100 μg, and imipenem (IPM) - 10 μg. The selection of these antibiotics was based on their frequent prescription and usage patterns.

2.7 Analysis of Results:

The statistical analysis was conducted using Microsoft Excel and SPSS software (IBM SPSS Statistics 25).

3. Results and Discussion:

3.1 Study Population and Prevalence of Bacteriuria:

In this study, 100 pregnant women were enrolled, and significant bacteriuria was identified in 35 of them. The age group 18-29 years had the highest incidence of bacteriuria at 71.4%. This was followed by the 30-34 years age group with 22.8%, and the 35-40 years age group at 5.7%. The mean age was 26.7$\pm$5.7 and 59%of the women were in the second trimester. Regarding diabetes status, around 14%, 22%, and

64% of individuals had DM, GDM, and no DM, respectively. Table 1.

These results indicate that the prevalence rates align with a recent study conducted in Baghdad, Iraq, where the prevalence UTIs among pregnant women reached 31% [14]. While, in another study conducted in Karbala, Iraq, the rate was 62% [15]. Similarly, in Dhaka, Bangladesh, the recorded prevalence UTIs among pregnant women was 37.3 % [16]. In contrast, another study within the same country reported a significant increase in the rate of prevalence UTIs approaching 76.0% [17]. In India, less prevalence UTIs was recorded, which was 46.6% [17]. Moreover, the revealed result in the current investigation, surpasses the reported prevalence of Sudan, Ethiopia, Tanzania and Kirkuk- Iraq, which were 14%, 7.8%, 14.6 and 10%, respectively [18], [19], [20], [21]. Discrepancies in prevalence could be attributed to various factors such as geographic locations, variations in sample sizes, differences in the sensitivity of the test protocol, the efficiency of healthcare systems , levels of personal hygiene among pregnant women in different countries, overall prevalence rates, and the degree of microbial contamination in the environment, among other factors[22].

In this study, it was found that the prevalence of UTIs was approximately similar during all three trimesters for pregnant women. Throughout pregnancy, hormonal changes and physiological adaptations occur in the urinary tract structure. These changes can affect the susceptibility to UTIs and may remain relatively stable throughout the trimesters. About 36% of study participants are diabetic positive. Complications in diabetic conditions such as high glucose levels in blood and urine, immunocompromised health condition, and retention of urine in the bladder of diabetic patients favor bacterial growth. According to the present study, women who have diabetes are more likely to get urinary tract infections (UTIs). Diabetes could contribute to the infection worse specifically, bacterial growth was observed in 42.9% of samples from pregnant women with diabetes mellitus (DM) and 41% of samples from women with gestational diabetes mellitus (GDM), compared to 31% of samples from non-diabetic women. [15]. Similarly, another study revealed that pregnant women with diabetes had a greater incidence of urinary tract infection (UTI) at 27.6% compared to those without diabetes (3%–10.1%) [2].

3.2 Bacterial Isolates and Biochemical Tests:

Bacterial isolates were initially identified based on their phenotypic characteristics like shape, color, size, and texture of the colonies, as well as the smell resulting from their growth as shown in Figure 1. Gram staining was employed to observe the cells' reaction to the dye, as well as to examine their shapes, sizes, and patterns of aggregation. This study also indicated biochemical tests adopted for identification of isolated strains based on what was stated by [23], [24], [25], as shown in Table 2. The findings from the current investigation revealed that *E. coli* and *Klebsiella* spp yielded negative results in the oxidase test, yet they tested positive in the catalase test. *E. coli* showed positive results in both the methyl red and indole tests, while yielding negative results in the Citrate utilization and Voges–Proskauer tests. Conversely, *Klebsiella* spp tested negative for both the methyl red and indole tests but displayed positive outcomes in the Citrate utilization and Voges–Proskauer tests.*Staph aureus* colonies exhibited a yellow color due to their fermentation of mannitol sugar and their positive identification through the coagulase test. *Enterococcus* spp, on the other hand, demonstrated positivity for methyl red, Voges-Proskauer, and mannitol sugar fermentation. All isolates gave negative results for H_2S production. However, only *E. coli* and *Klebsiella* spp showed positive results for gas production.

Table 1. Characteristics of UTI in pregnant women.

Variables		No. subjects	No. (%) Bacteriuria
Maternal age	18-29 years	61	25 (71.4)
	30-34 years	34	8 (22.8)
	35-40 years	5	2 (5.7)
Gestation age	1^{st} trimester	15	5 (33.3)
	2^{nd} trimester	59	21(35.6)
	3^{rd} trimester	26	9 (34.6)
CO-morbidities	DM	14	6 (42.9)
	GDM	22	9 (41)
	No DM	64	20 (31)
Total		100	35 (35%)

a b c d

Figure 1. a- *Klebsiella* spp growth on MacConkey agar
b -*Staphylococcus aureus* on Mannitol salt agar
c- *E. coli* on Eosin methylene blue agar
d- *Enterococcus* spp on MacConkey agar.

3.3 Distribution of Bacterial Species:

Among pregnant women, the total frequency of UTIs was 35%. Four distinct bacterial species were found and isolated in this investigation. The most prevalent species of bacteria isolated was *E. coli* (60%) followed by *Klebsiella* spp (20%), *Staphylococcus aureus* (14.3%), and *Enterococcus* spp (5.7%), as observed in previous study conducted in Kirkuk , Iraq [26] Table 3. In the first three trimesters, pregnant women with

Table 2. Biochemical tests adapted for the identification of bacterial isolates.

Organisms	Biochemical tests										
	Voges-Proskauer	Methyl red	Catalase	Coagulase	Oxidase	Mannitol	Indole	Urease	Citrate	TSI H2S	TSI Gas
E. coli	-	+	+	-	-	+	+	-	-	-	+
Klebsiella spp.	+	-	+	-	-	+	-	+	-	-	+
Staphylococcus aureus	-	-	+	+	-	+	-	+	+	-	-
Enterococcus spp.	+	+	-	-	-	+	-	-	-	-	-

Table 3. Percentage distribution of the isolated bacteria species.

Growth	Frequency	Percentage (%)
Escherichia coli	21	60
Klebsiella spp	7	20
Staphylococcus aureus	5	14.3
Enterococcus spp	2	5.7
Total	35	100

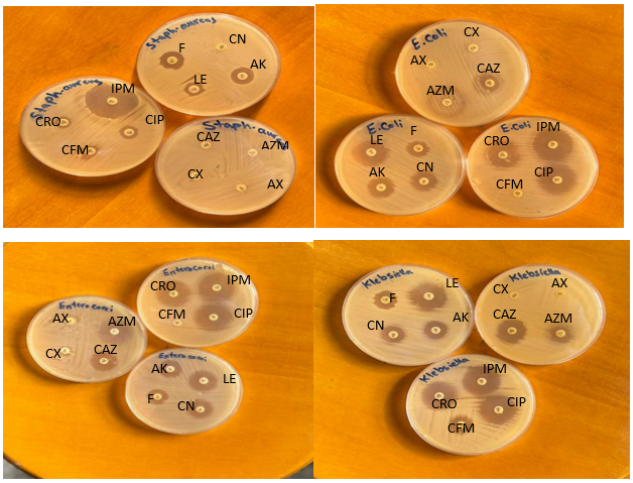

Figure 2. Susceptibility of *S. aureus*, *Escherichia coli*, *Klebsiella* spp, *Enterococcus* spp to antibiotics.

UTIs are most likely to infect with Gram-negative bacteria such as *E. coli* and *Klebsiella* spp. [27]. Indeed, it was reported that Gram-negative bacteria are predominant in UTIs. The most prevalent organism found in this study was *E. coli*, followed by *Klebsiella* spp. Among the Gram-positive organism, *Enterococcus* spp and *Staphylococcus* spp were reported as frequent causes of UTIs. Similar findings have been reported in a study conducted in India [10].

3.4 Antibiotic Sensitivity Test:

The results of the antibiotic sensitivity assay indicated the presence of both single and multiple antibiotic resistance levels against commonly prescribed drugs as indicated in (Table 4 and Figure 2). Among the 35 bacterial isolates, levofloxacin had the highest overall sensitivity (n=35; 100%), nitrofurantoin and imipenem (n=32; 91.5%), Amikacin (n=31; 88.5%), gentamicin and ceftriaxone (n=30; 85.7%). Furthermore, the sensitivity of cefoxitin, ciprofloxacin, and cefixime against various isolates was demonstrated to be 74.2%, 71.5%, and 62.8% respectively. Some tested antibiotics, such as ceftazidime (n=12; 34.3%), and azithromycin (n=10; 28.6%), amoxicillin (n=10; 28.5%), showed less than 40% of sensitivity against various uropathogenic bacteria. Levofloxacin, nitrofurantoin and imipenem are effective against most *E. coli* strains, and similar outcomes were reported in previous studies [10], [21], [28], [29]. Amoxicillin was found more ineffective against multiple organisms such as *E. coli*., *Klebsiella* spp., *Staphylococcus aureus*., and all *Enterococcus* spp, which was supported by the previous report [17]. The antibiotic susceptibility assay also revealed that uropathogenic bacteria such as *E. coli*., *Klebsiella* spp., and *Staphylococcus aureus* exhibited significant resistance to amoxicillin, and azithromycin (beta-lactam group) antibiotics, as observed in previous studies [30], [31]. This may be due to the rise of beta-lactamase-producing bacteria and the widespread usage of these antibiotics. Despite being generally regarded as a safe conventional medication during pregnancy, this result limits the usage of the beta-lactam category of antibiotics. It was found that third-generation antibiotics such as ceftazidime were found poor in effectiveness against uropathogenic bacteria. [32]. On the other hand, amikacin, nitrofurantoin, imipenem and gentamycin were shown to have minimal levels of resistance, indicating that these medications might be utilized as first-line therapy for UTIs in pregnant women. The growth of more resistant bacteria may result from the empirical use of antibi-

Table 4. Antibiotic sensitivity and resistance pattern of different organisms isolated in the study.

Antibiotic		*E. coli*	*Klebsiella* spp	*Staphylococcus aureus*	*Enterococcus* spp	N (%)
Amikacin	S	19	6	4	2	31 (88.5)
	R	2	1	1	0	4(11.5)
Amoxicillin	S	6	2	2	0	10(28.5)
	R	14	5	3	2	24 (68.5)
Azithromycin	S	4	1	3	2	10 (28.6)
	R	17	6	2	0	25 (71.4)
Cefixime	S	14	6	0	2	22 (62.8)
	R	7	1	5	0	13 (37.2)
Ceftazidime	S	8	4	0	0	12 (34.3)
	R	13	3	5	2	23 (65.7)
Ceftriaxone	S	18	6	5	1	30 (85.7)
	R	3	1	0	1	5 (14.3)
Cefoxitin	S	13	6	5	2	26 (74.2)
	R	8	1	0	0	9 (25.8)
Ciprofloxacin	S	15	3	5	2	25 (71.5)
	R	6	4	0	0	10 (28.5)
Gentamicin	S	18	5	5	2	30 (85.7)
	R	3	2	0	0	5 (14.3)
Levofloxacin	S	21	7	5	2	35 (100)
	R	0	0	0	0	0 (0)
Nitrofurantoin	S	20	5	5	2	32 (91.5)
	R	1	2	0	0	3 (8.5)
Imipenem	S	20	6	5	1	32 (91.5)
	R	1	1	0	1	3 (8.5)

otics, complicating the management of UTIs [33].The findings showed that amoxicillin, azithromycin, and ceftazidime were less effective against uropathogenic bacteria, limiting their usage in treating UTIs.

The limitation of the study is that it only involves women who attended the healthcare system, and the results do not reflect the entire community. Therefore, a further study including women from the community will be required to validate these findings.

4. Conclusion:

A significant portion of the participants were diagnosed with diabetes mellitus (DM) or gestational diabetes mellitus (GDM), only 35% exhibited bacterial growth in their urine samples. Pregnancy-related UTIs can arise from a variety of risk factors, one of them being diabetes, which can exacerbate the illness. *E. coli* emerged as the prevalent bacteria, followed by *Klebsiella* spp, *Staphylococcus aureus*, and *Enterococcus* spp. Notably, all bacterial isolates demonstrated high susceptibility rates to levofloxacin, nitrofurantoin, imipenem, amikacin, gentamicin, and ceftriaxone. However, resistance to azithromycin, amoxicillin, ceftazidime, cefixime, ciprofloxacin, and cefoxitin was observed in varying proportions. When it comes to treating UTIs, the same treatment is frequently given to pregnant women with GDM as to those without it. Underscoring the importance of antibiotic use and continuous surveillance to combat antimicrobial resistance.

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نمط الحساسية المضادات الحيوية للبكتيريا المسببة لأمراض المسالك البولية لدى النساء الحوامل المصابات وغير المصابات بمرض السكري في مدينة كركوك، العراق

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الخلاصة

تم جمع 100 عينة من الادرار من النساء الحوامل المصابات وغير المصابات بمرض السكري في مستشفى النصر للنسائية و الاطفال في مدينة كركوك واللذين تراوحت اعمارهم بين 18 – 40 سنة وللفترة من (تموز – كانون الاول) 2023. بينت النتائج ان 14 عينة اخذت من المصابات بالسكري نوع *DM* و 22 عينة من المصابات بسكري الحمل *GDM* في حين 64 عينة كانت لغير المصابات بمرض السكري. ظهر النمو البكتيري في 35% من العينات اذ تم عزل نوعين من البكتيريا السالبة للون كرام (*Escherichia coli*, *Klebsiella spp*) ونوعين من البكتيريا الموجبة للون كرام (*Staphylococcus aureus*, *Enterococcus spp*). تشير الدراسة الحالية إلى زيادة خطر الإصابة بالتهابات المسالك البولية لدى النساء المصابات بالسكري. حيث ظهر النمو البكتيري بنسبة 42.9% من عينات النساء المصابات بالسكري من النوع *DM* ، وبنسبة 41% من عينات النساء المصابات بسكري الحمل *GDM* ، بينما كانت نسبة النمو البكتيري 31% من العينات لدى النساء غير المصابات بالسكري. اظهرت النتائج سيادة بكتيريا *Escherichia coli* في اغلب العينات وبنسبة 60% تلتها *Klebsiella spp* وبنسبة 20% وفيما يخص البكتيريا الموجبة للون كرام كانت نتائج العزل 14.3% و 5.7% لكل من *Staphylococcus aureus* و *Enterococcus spp* على التوالي. اظهرت العزلات حساسية عالية 100% تجاه المضاد الحيوية *levofloxacin* و 91.5% تجاه *Nitrofurantoin* 91.4% تجاه *Imipenem* و 88.5% تجاه *Amikacin* و 85.7% لكل من *Gentamicin* و *Ceftriaxone* في حين اظهرت 71.4% و 68.5% و 65.7% و 37.2% و 28.5% و 25.8% مقاومة ضد كل من *Azithromycin* و *Amoxicillin* و *Ceftazidime* و *cefixime* و *Ciprofloxacin* و *Cefoxitin* على التوالي.

الكلمات الدالة: المضادات الحيوية؛ البكتيريا المسببة للأمراض البولية؛ النساء الحوامل؛ السكري؛ مدينة كركوك.

التمويل: لايوجد.

بيان توفر البيانات: جميع البيانات الداعمة لنتائج الدراسة المقدمة يمكن طلبها من المؤلف المسؤول.

اقرارات:

تضارب المصالح: يقر المؤلفون أنه ليس لديهم تضارب في المصالح.

الموافقة الأخلاقية: لم يتم نشر المخطوطة أو تقديمها لمجلة أخرى، كما أنها ليست قيد المراجعة.