

## Bacterial isolates and drug susceptibility patterns of urinary tract infection among pregnant women

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**Abstract** Seventy five (75) urine samples were collected and analyzed during the study period of June 2017 and December 2017. Seventeen (17) samples showed significant growth, which amounted to a prevalence of 22.6%. *Escherichia coli* had the highest percentage of isolation with frequency rate 12 (70.59%). Maternal age was not found to be a significant risk factor in this study. In literature, only a significant increasing risk of 1-2% is reported per decade of age which did not become evident in this study, probably due to small sample size, individuals of the age group 26 – 30 years had the highest incidence of infection (52.9%). While the age group 36- 40 years had the lowest incidence of infection (0%). According to prevalence of urinary tract infection in pregnant women in relation to gestational age, There was higher rate of infection in the third trimester (64.70%) compared to second trimester (23.53%) and first trimester (11.76%). Antimicrobial sensitivity testing of our study was done by using Kirby Bauer Disc Diffusion method. Twenty one antibiotics commonly used were tested against the isolated *E. coli*. Amikacin was found to be the most effective drug (100%). *E. coli* which is the predominant cause of UTI, showed high percentage of resistance to ampicillin (91%), but all were sensitive to Amikacin (100%). *Klebsiella* spp which is the second most prevalent pathogen of UTI displayed a similar resistance pattern as of *E. coli* and showed hundred percent resistant to Ampicillin.

**Keywords:** Urine culture, UTI, Antimicrobial susceptibility, Pregnant women.

### أنماط استجابة البكتيريا المعزولة للمضادات الحيوية في التهابات المسالك لدى النساء الحوامل

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**المخلص** في هذه الدراسة تم جمع وتحليل خمسة وسبعون (75) عينة بول خلال فترة الدراسة من يونيو 2017 الى ديسمبر 2017. وظهرت النتائج ان (17) عينة كانت ايجابية النمو، والتي شكلت ما نسبته 22.6% من اجمالي عدد العينات. وكانت بكتيريا *Escherichia coli* أعلى معدل نمو 12 بنسبة (70.59%). وكانت النساء من الفئة العمرية 26-30 سنة أكثر نسبة للتعرض للإصابة (52.9%). تليها الفئة العمرية 21-25 سنة (23.5%)، و 31-35 سنة (17.6%) و 16-20 سنة (5.8%). بينما كانت الفئة العمرية 36-40 سنة لديها أقل نسبة إصابة (0%). وفقا لانتشار عدوى المسالك البولية في النساء الحوامل بالنسبة لفترة الحمل كان الثلث الثالث اكثر تعرض للإصابة (64.70%) مقارنة مع الثلث الثاني (23.53%) والثلث الأول (11.76%). تم إجراء اختبار حساسية المضادات الحيوية لهذه الدراسة باستخدام طريقة Kirby Bauer Disc Diffusion، تم اختبار واحد وعشرون مضاد حيوي شائع الاستخدام ضد بكتيريا *E. coli* المعزولة. حيث كان الأميكاسين Amikacin المضاد الحيوي الأكثر فعالية (100%) يليه الجنتاميسين Gentamicin (91%). بكتيريا *E. coli* التي هي أكثر مسبب لالتهاب المسالك البولية UTI، أظهر نسبة عالية لمقاومة الأمبيسيلين (91%).

**الكلمات المفتاحية:** مزرعة البول، التهاب المسالك البولية، الحساسية لمضادات الميكروبات، النساء الحوامل.

### Introduction

Urinary tract infections (UTIs) are the most commonly encountered infectious diseases by clinicians in developing countries with an estimated annual global incidence of at least 250 million. [1, 2]. UTI affects all age groups, but women are more susceptible than men, due to short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with faecal flora [3].

Urine formed in the kidney is a sterile fluid that serves as a good culture medium for proliferation of bacteria [4].

UTI is evident by the presence of 105 microorganisms or of a single strain of bacterium per ml in two consecutive midstream samples of urine [5].

Eight million women visit a physician annually for evaluation of UTIs [6] at a direct cost of \$659 million [7] and aggregate cost of \$1.6 billion [7,8]. Physiologic changes of pregnancy increase a woman's susceptibility to UTI. Progesterone effects and mechanical compression by the gravid uterus impair emptying of the bladder and lead to

increased bladder residual volume and vesicoureteral reflux. Relative stasis of urine in the ureters results in hydronephrosis. Furthermore, pregnancy-related changes in glomerular filtration rate increases the urinary glucose concentration and alkalinity, thereby facilitating bacterial growth [9]. In addition, alterations in maternal immunologic defense mechanisms occur in pregnancy [10].

Urinary tract infections (UTIs) represent the most common bacterial infection in pregnant and nonpregnant women [11].

Pregnant women are more susceptible to UTI due to a number of factors including ureteral dilatation, increased bladder volume and decreased bladder tone, along with decreased ureteral tone which contributes to increased urinary stasis and ureterovesical reflux [12]. Development of glycosuria seen in 70% of pregnant women encourages bacterial growth in the urine [13].

It has been recognized for sometime that asymptomatic bacteriuria is common in pregnancy thus women are at increased risk of UTIs. [14] reported that except for a short period immediately after birth (infant period), females far exceed males in the prevalence of asymptomatic bacteriuria [15, 4].

the anatomical relationship of the female urethra to the vagina makes it liable to trauma during sexual intercourse as well as bacteria being massaged up the urethra into the bladder during pregnancy or childbirth; the moist environment of the females perineum favours microbial growth and predisposes females to bladder contamination [15, 16, 17]. Other factors including improper cleaning of the perineum, the use of napkins and sanitary towel together with pregnancy and sexual intercourse contribute to the higher incidence of UTIs in various women [18].

In addition, urine of females was found to have more suitable pH and osmotic pressure for the growth of *Escherichia coli* than urine from males [18, 19]. Also, bacterial colonization of the distal 4 cm of the urethra predisposes females to UTIs. Increase in the concentration of amino acids and lactose during pregnancy are believed to encourage the growth of *E. coli* in urine [15]. In boys, UTI is a disease of infancy while in girls; a disease of school age with 3 - 5% of girls having asymptomatic bacteriuria ([20].

It is estimated that 2 to 10% of pregnant woman suffer from any form of UTIs [21]. These infections complicate up to 20% of pregnancies and are responsible for the majority of antepartum admissions to the maternal-fetal medicine units [22].

The prevalence of asymptomatic forms of UTIs has remained constant across countries, and most of the recent observational studies report similar rates, ranging from 2 to 10% - similar to that of nonpregnant women [23, 24]. Acute

cystitis is prevalent in 1 to 4% of pregnant women [25].

Despite the relatively low prevalence of pyelonephritis during pregnancy (0.5 to 2%), it is estimated that 20% to 40% of pregnant women with asymptomatic bacteriuria will develop this condition later in gestation [26]. A study showed that if UTI is left untreated, 30% of mothers will develop acute pyelonephritis compared with 1.8% of nonbacteriuric controls. Many studies have reported that pyelonephritis is more common during the second half of pregnancy, with an incidence peak during the last two trimesters of pregnancy [27, 28, 29]. Acute pyelonephritis may lead to adverse outcomes for the baby and the mother, such as premature delivery, low birth weight infants, preeclampsia, hypertension, renal failure and fetal death [28]. The prevalence of UTI in pregnancy is closely related to socioeconomic factors [30]. Predictors of UTIs' asymptomatic forms include: welfare status, increasing maternal age, multiparity, risky sexual behavior, history of childhood UTIs and history of recurrent UTIs. UTI before pregnancy is a predictor for the diagnosis of asymptomatic bacteriuria at the first prenatal visit [31]. Risk factors for developing cystitis and pyelonephritis in pregnancy include those stated before, as well as a history of *Chlamidia trachomatis* infection, illicit drug use, and having less than 12 years of education ([32].

Antibiotic resistance in uropathogens is increasing worldwide. It varies according to geographic locates and is directly proportional to the use and misuse of antibiotics. Understanding the impact of drug resistance is of the critical importance as the changing rate of antibiotic resistance has a large impact on the empirical therapy of UTIs [33].

This study therefore focuses on the detection and incidence of UTI among pregnant women. It also aimed to isolate and identify the organisms that causing UTI among pregnant women, and determined their susceptibility patterns to commonly used antimicrobial agents.

#### **Material and methods.**

Seventy five (75) samples of urine (5 ml) were collected from pregnant women at the aged ranges from 16 to 40 years attending to the Gynaecology and Obstetrics clinics in Brak Al-Shati, Libya. Clean, dry chemically clear and sterilized containers were used for collection of urine. Midstream of urine was preferred for bacteriological examinations and collected aseptically.

With a calibrated micro-loop, 0.001 ml. of urine was cultured on to a Blood agar, MacConkey agar, EMB agar, Nutrient agar and Muller Hinton Agar plates. After overnight incubation at 37° C for 24 hours, colony counts a bacterial growth of  $\geq 10^5$ /ml was taken as being significant in both symptomatic and asymptomatic pregnant women.

Antibiotics sensitivity pattern was performed by Kirby Bauer method (disc diffusion method). Overnight broth culture of the isolated organism was scabbed over the surface of Mueller Hinton agar plates. The antibiotics discs were placed over

the surface of the plate and incubated for 24 hours at 37°C. Two groups of antibiotic were included in the study, as follows:-

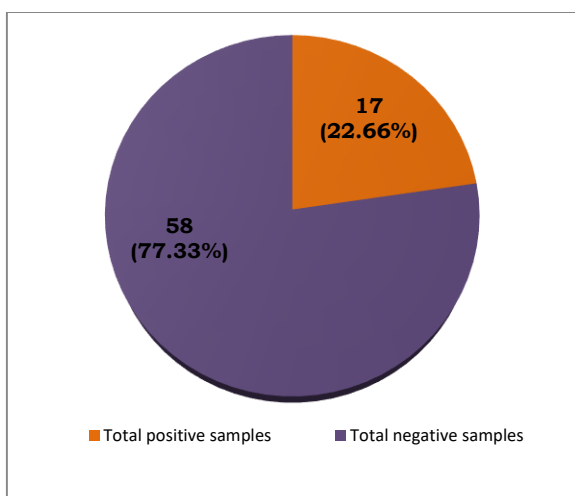
- 1- For gram negative bacteria
- 2- For highly resistant UTI & systemic isolates.

**Results**

Seventy five (75) urine samples were collected and analyzed during the study period of June 2017 and December 2017. Seventeen (17) samples showed significant growth, which amounted to a prevalence of 22.6 % (Table 1 and Figure 1).

**Table 1: Distribution of samples positive and negative cases**

Total no of samples	Total no of samples positive	Total no of samples negative
75	17 (22.66%)	58 (77.33%)

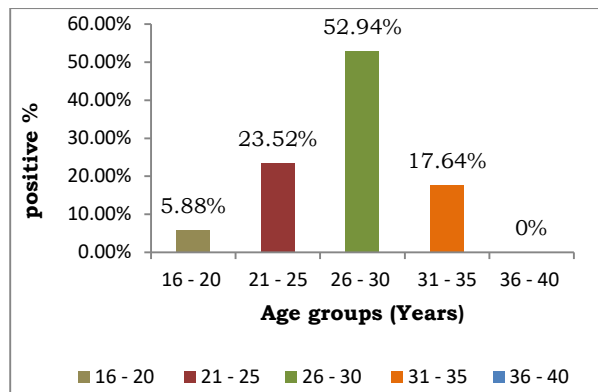


**Figure 1:** Distribution of samples positive and negative cases

The prevalence of infection in relation to age are also shown in (Table 2 and Figure 2), individuals of the age group 26 – 30 years had the highest incidence of infection (52.9%). Followed by age group 21 – 25 years (23.5 %), 31-35 years (17.6%) and 16 -20 years (5.8 %). While the age group 36-40 years had the lowest incidence of infection (0%).

**Table 2: Prevalence of urinary tract infection in pregnant women in relation to age.**

Age groups (Years)	Number examined	Number positive	Positive%
16 – 20	11	1	5.88 %
21 – 25	14	4	23.52 %
26 – 30	21	9	52.94%
31 – 35	16	3	17.64%
36 – 40	13	0	0
<b>Total</b>	<b>75</b>	<b>17</b>	<b>100%</b>

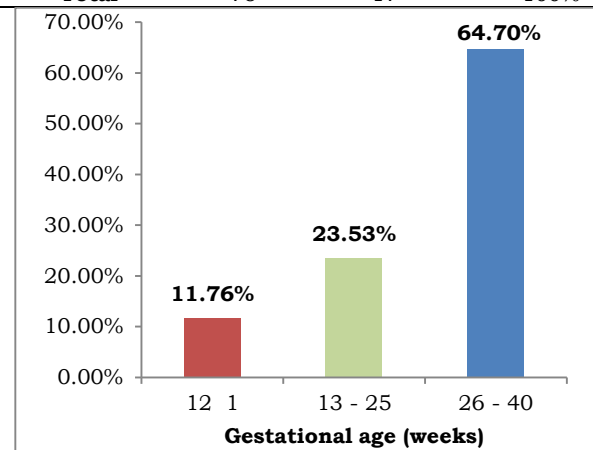


**Figure 2:** prevalence of urinary tract infection in pregnant women in relation to age

The prevalence of urinary tract infection in pregnant women in relation to gestational age shows that the third trimester was the highest (64.70%) compared to second trimester (23.53%) and first trimester (11.76%) (Table 3 and Figure 3).

**Table3: Prevalence of urinary tract infection in pregnant women in relation to gestational age.**

Gestational age(weeks)	Number examined	Number positive	Positive%
1 – 12	19	2	11.76%
13 – 25	25	4	23.53%
26 – 40	31	11	64.70%
<b>Total</b>	<b>75</b>	<b>17</b>	<b>100%</b>



**Figure 3:** prevalence of urinary tract infection in pregnant women in relation to gestational age.

Among the significant isolates, Escherichia coli had the highest percentage of isolation with frequency rate 12 (70.59%) followed by Klebsiella spp. was 3(17.64%), while the lowest was Proteus spp. was 2(11.76%) (Table 4)

**Table 4: Percentage of Isolation of various significant pathogens in urine of pregnant women.**

Pathogens	Number isolated	Percentage (%)
Escherichia coli	12	70.59%
Klebsiella spp	3	17.64%
Proteus spp	2	11.76%
<b>Total</b>	<b>17</b>	<b>100.00%</b>

Antimicrobial sensitivity testing of our study was done by using Kirby Bauer Disc Diffusion method, according CLSI Clinical and Laboratory Standards Institute (2015). At the end of incubation period, the diameter of the zones of inhibition around each disc was measured with vernier calipers on

the back of plate, with reflected light against a dark non-reflected background. Twenty one antibiotics commonly used were tested against the isolated E. coli. Amikacin was found to be the most effective drug (100%) followed by Gentamicin (91.6%). (Table 5).

**Table 5: Antibiotic sensitivity pattern of isolation**

Antimicrobial agents	<i>E.coli</i> ( n=12)		<i>Klebsiella spp</i> ( n=3)		<i>Proteus spp</i> ( n=2)	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Ampicillin (AS)	1 (8.33%)	11(91.6%)	0	3 (100%)	0	2 (100%)
Cotrimoxazole (BA)	2 (16.6%)	10(83.3%)	1(33.3%)	2(66.6%)	0	2(100%)
Cefotaxime (CF)	5(41.6%)	7(58.3%)	2 (66.6%)	1(33.3%)	1(50%)	1(50%)
Piperacillin (PC)	6(50%)	6(50%)	1(33.3%)	2(66.6%)	1(50%)	1(50%)
Chloramphenico(CH)	1(83.3%)	2 (16.6%)	1(33.3%)	2 (66.6%)	1(50%)	1(50%)
Ciprofloxacin (RC)	10(83.3%)	2 (16.6%)	3 (100%)	0	2 (100%)	0
Ceftizoxime (CI)	7(58.3%)	5(41.6%)	1(33.3%)	2(66.6%)	1(50%)	1(50%)
Tetracycline (TE)	3(25%)	9(75%)	1(33.3%)	2(66.6%)	0	2(100%)
Ofloxacin (ZN)	4 (33.3%)	8 (66.6%)	0	3 (100%)	0	2(100%)
Gentamicin (GM)	11(91.6%)	1 (8.33%)	3 (100%)	0	2 (100%)	0
Amikacin (AK)	12(100%)	0	3 (100%)	0	2 (100%)	0
Gatifloxacin (GF)	4 (33.3%)	8 (66.6%)	1 (33.3%)	2 (66.6%)	1 (50%)	1 (50%)
Azithromycin (AZ)	5 (41.6%)	7 (58.3%)	1 (33.3%)	2 (66.6%)	0	2 (100%)
Meropenem (MP)	3 (25%)	9 (75%)	1 (33.3%)	2 (66.6%)	2 (100%)	0
Pipracillin (PT)	6 (50%)	6 (50%)	1 (33.3%)	2 (66.6%)	1 (50%)	1 (50%)
Ticarcillin (TT)	10(83.3%)	2 (16.6%)	2 (66.6%)	1 (33.3%)	1 (50%)	1 (50%)
Cefoperzone (CM)	4 (33.3%)	8 (66.6%)	1 (33.3%)	2 (66.6%)	2 (100%)	0
Cefpirome (CG)	7 (58.3%)	5 (41.6%)	0	3 (100%)	1 (50%)	1 (50%)
Teicoplanin (TF)	6 (50%)	6 (50%)	0	3 (100%)	2 (100%)	0
Aztreonam (AC)	5 (41.6%)	7 (58.3%)	2 (66.6%)	1 (33.3%)	1 (50%)	1 (50%)
Netilmicin (NT)	8 (66.6%)	4 (33.3%)	0	3 (100%)	0	2 (100%)

**Discussion**

Seventy five (75) urine samples were collected and analyzed during the study period of June 2017 and December 2017. Seventeen (17) samples showed significant growth, which amounted to a prevalence of 22.6%. The isolation rate of pathogens in this study is similar to Sabrina J. Moyo et. al., 2010 . however higher compared to previous studies such as R Marahatta et. al., 2011 and R. Alijahan et. al., 2012. And on other hand lower than that reported from Nigeria Obiogbolu et. al.,2009 and Saudi Arabia Nawal Salim ., 2011. This difference may be due to the inclusion of both symptomatic and asymptomatic pregnant woman in this study or as a result of difference socioeconomic status of the pregnant women.

Maternal age was not found to be a significant risk factor in this study. In literature, only a significant increasing risk of 1- 2% is reported per decade of age which did not become evident in this study, probably due to small sample size, individuals of the age group 26 – 30 years had the highest incidence of infection (52.9%). Followed by age group 21 – 25 years (23.5 %), 31-35 years (17.6%) and 16 -20 years (5.8 %). While the age group 36- 40 years had the lowest incidence of infection (0%). The aforementioned age groups having the highest was also observed in previous studies. The reason could be due to the fact that many women within this age group are likely to have had many children before the present pregnancy and it has been reported that

multiparity is a risk factor for acquiring bacteriuria in pregnancy.

In this study, the frequency of urinary tract infection was higher in the third trimester compared to the first and second trimester. This is in agreement with Leigh et. al .,1989 who reported an increased frequency of urinary tract infection in the third trimester compared to the first and second trimester of pregnancy. However, this report does not agree with Onuh et. al., 2006 who reported a higher prevalence of urinary tract infection in the second trimester compared to the third trimester. This difference may be as a result of either change in urinary stasis and vesicoureteral reflux or decrease in urinary progesterones and oestrogens in the various trimester of pregnancy.

Among the significant isolates, Escherichia coli had the highest percentage of isolation with frequency rate 12 (70.59%) followed by Klebsiella spp. was 3(17.64%), while the lowest was Proteus spp. was 2(11.76%). these isolates were identified by routine cultural, morphological characteristics and biochemical tests in the laboratory. This study finds similarity with that of Obiogbolu et. al., 2009 who reported Escherichia coli (37%) was the commonest offending bacterial pathogen isolated followed by Klebsiella spp (20.4%), Proteus mirabilis (16.7%), and K. Parveen et. al .,2011 who reported E.coli had the highest percentage of isolation (86,1%) followed by Klebsiella spp (7.7%)and Proteus spp (4.6%).and does not agree with Nawal Salim., 2011 who

reported that *Streptococcus agalactia* was the most predominant organism closely followed by *Escherichia coli*.

Twenty one antibiotics commonly used were tested against the isolated *E. coli*. Amikacin was found to be the most effective drug (100%) followed by Gentamicin (91.6%).

*E. coli* which is the predominant cause of UTI, showed high percentage of resistance to ampicillin (91%), cotrimoxazole (83%), Tetracycline (75%), and low resistance to Ciprofloxacin (16.6%), Ticarcillin (16.6%), Gentamycin (8.33%), but all were sensitive to Amikacin (100%). *Klebsiella* spp which is the second most prevalent pathogen of UTI displayed a similar resistance pattern as of *E. coli* and showed hundred percent resistant to Ampicillin however, *Proteus* spp was similarly resistant to most of the antibiotics as that of *E. coli* and *K. pneumonia*.

This study agrees with the work of Kebira et al., 2009 in Kenya which revealed that Amikacin was 100% sensitive to *E. coli* isolated from urine specimens, ciprofloxacin 95% sensitive. and also agree with M.M Ali., 2011 which reported that Ciprofloxacin and Gentamicin were very effective against urinary isolates.

In this study Amikacin, Ciprofloxacin, Ticarcillin and Gentamycin were the most effective against urinary isolates.

### Conclusion

In this study out of seventy five (75) urine samples of pregnant women, seventeen (17) 22.6% samples were positive for urinary pathogens. Among the significant isolates, *Escherichia coli* had the highest percentage of isolation with frequency rate 12 (70.59%) and was most isolated bacterium, *Klebsiella* spp. was 3(17.64%), while the lowest was *Proteus* spp. was 2(11.76%).

This suggests a need for continuous monitoring to organisms causing urinary tract infection in all pregnant women, and testing its resistance to the different antimicrobial agents before antibiotic prescription in order to ensure adequate treatment of urinary tract infection.

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