

*Original Article*

**Antimicrobial Susceptibility Profile of Bacterial Pathogens Isolated From Pregnant Women with Asymptomatic Bacteriuria at Tertiary Hospital in Northeastern Nigeria**

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**Abstract**

Asymptomatic urinary tract infection among pregnant women is a common clinical episode that is frequently undiagnosed. A total of 200 clean-catch mid-stream urine (150 pregnant women and 50 non-pregnant women) were examined by microscopy and culture methods. Overall, the prevalence of asymptomatic bacteriuria was 59.0% (118/200) and was significantly higher among pregnant (63.3%, 95/150) than non-pregnant (46.0%, 23/50) subjects ( $\chi^2 = 4.66$ ,  $df = 1$ ,  $p = 0.03$ ). Tertiary education (72.6%, OR = 1.45,  $p = 0.014$ ), third trimester (77.6%, OR = 1.39,  $p = 0.042$ ), multi-gravidity (79.0%, OR = 1.41,  $p = 0.0017$ ) and multiparity (75.9%, OR = 1.49,  $p = 0.03$ ) were factors associated with asymptomatic bacteriuria in studied pregnant subjects. Of the 118 bacterial isolates, *Klebsiella* spp accounted for 39.8% (47/118), followed by *S. aureus* 22.9% (27/118), *E.coli* 19.5% (23/118), *Proteus* spp 9.3% (11/118) and *P. aeruginosa* 8.5% (10/118) ( $p < 0.0001$ ). Antibacterial susceptibility test revealed that all bacterial isolates were susceptible to quinolones (ciprofloxacin, pefloxacin and ofloxacin). In addition to this, *S. aureus* was also susceptible to erythromycin. However, all bacterial isolates were resistant to readily available antibacterial drugs including augmentin<sup>®</sup>, cotrimoxazole, penicillin and cephalixin. In conclusion, prevalence of asymptomatic bacteriuria among pregnant women in this study is considered to be high and the bacterial isolates were quinolones sensitive and resistant to other commonly used antibacterial drugs. Considering the clinical implications of untreated urinary tract infection, it is therefore advisable that routine urine culture may be adopted as part of antenatal care.

**Keywords:** Asymptomatic bacteriuria, urinary tract infection, antimicrobial susceptibility, tertiary hospital

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## Introduction

Urinary tract infection (UTI) refers to the proliferation of active micro-organisms that infects the genitourinary system, commonly associated with bacteria pathogens. Bacteriuria, a term used to describe presence of bacteria in freshly voided urine, is indicated by presence of  $1 \times 10^5$  or more colony forming units (CFUs) in mid-stream urine and has been historically used in evaluation of UTI (Kahlmeter, 2000). It is a common clinical episode presented in hospitals and community setting, which affects all age groups. It accounts for approximately 10% of hospital visit by women, 15% of these women will still have a UTI at some time during their life and incidence of UTI could be as high as 8% (Mikhali and Anyaegbunam, 1995)

Predisposing factors of pregnant women to ASB includes anatomical and physiological changes during pregnancy (Al-Dujaily, 2000; Gupta et al., 2001), low socioeconomic status, sickle cell traits, diabetes mellitus, gestational age and parity (Patterson, 1987). The short urethra and its proximity to anus and vagina result in easy contamination of the urinary tract with fecal flora (Gupta et al., 2001). Sexual intercourse and use of condoms with spermicidal jelly has also increased the incidence of UTIs (Fihn, 1985). Clinical presentations are in three clinical forms, asymptomatic bacteriuria (ASB), acute cystitis and acute pyelonephritis. Incidence of ASB has been reported between 2-3% in pregnancy over the world, however, the rate is found to be similar in non-pregnant women of same age group (Krishna et al., 2006). Clinical

consequences of untreated ASB include increased frequency of premature delivery, neonates with low birth weight, neonatal sepsis and likelihood to cause acute pyelonephritis at rate of 15-30% (Razz et al., 2004). Untreated UTI may produce progressive kidney damage, development of renal scarring and renal dysfunction (Ahmed, 1998). Most causative agents of UTIs are bacterial pathogens and majority belongs to the large bacterial family "Enterobacteriaceae".

However, *Escherichia coli* accounts for most UTIs rating to about 80-90% (Linda et al., 2002). Other bacterial pathogens such as *Pseudomonas* species, *Proteus* species, *Klebsiella* species and other Gram positive bacteria are also implicated.

In most cases, asymptomatic urinary tract infection in pregnant women may go undiagnosed due to non-presentation of obvious clinical symptoms. It may thereby progress to other clinical conditions like cystitis, kidney dysfunction and complications in pregnancy. Apart from clinical implication in mothers, neonatal infection due to ASB has been reported responsible for cases of early onset of disease condition that result in high morbidity and mortality cases. Available data on ASB in pregnancy have been documented in some parts of Nigeria. However, there is paucity of information on ASB among pregnant women in this geographical zone especially in the study area. Information derivable from this study would provide necessary information, especially on prevalence and the impact of demographic variables on ASB, with the view of adopting preventive approach. In view of this, the study was designed to

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examine the prevalence of asymptomatic bacteriuria among pregnant women seen at the antenatal clinic of a tertiary hospital in Northeastern Nigeria.

### Materials and Methods

This cross-sectional study was conducted in the Department of Obstetrics and Gynecology, University of Maiduguri Teaching Hospital (UMTH) between the months of March and May 2009. Pregnant women recruited for the study were those attending antenatal clinic of UMTH, while non-pregnant were staff/students of the hospital. The hospital is a major referral health institution in the north-eastern zone of Nigeria and also served the republic of Chad, Niger, and Cameroon. It is about 500 bed sized capacity hospital, with multi-disciplinary and specialty in medicine, orthopedics, pediatrics, surgery, obstetrics and gynecology, pathology and in the training of health care professionals and research. Ethical clearance for the study was sought from Ethics Committee, UMTH.

The inclusion criteria included pregnant women without apparent signs and symptoms of UTI, and those that were not on antibiotic therapy. Verbal consent of the subjects was sought before the administration of the study questionnaires. Demographic information contained in the questionnaire included age, educational background, weight, parity, gestational age and previous obstetric history. A total of 200 subjects were recruited, 150 pregnant women and 50 non-pregnant women. The subjects were properly instructed on collection of clean-catch mid-stream urine. The mid-stream urine sample was mixed

and 5ml was dispensed into a clean labeled centrifuge tube, and centrifuged at 5,000 rpm for 5 minutes. The supernatant was discarded and the urine deposit was re-suspended by the use of vortex mixer. A drop of the well mixed deposit was placed on a clean, grease-free slide and examined microscopically using X10 and X40 objective lens, respectively. For each uncentrifuged urine sample, a drop (0.001ml) was streaked onto surface of blood agar and Cystine-Lactose Electrolyte Deficient (CLED) agar using standard wire loop.

The plates were incubated aerobically at 37°C for 24 hours; bacterial colonies were expressed in colony forming units (CFUs) per milliliter. A count of  $\geq 10^5$  CFU/ml was considered significant and indicative of bacteriuria. Bacterial growth on the CLED plate was identified based on colonial/morphological appearance using Gram's reaction and motility testing. The bacterial isolates were further confirmed using standard bacteriological methods.

Antibiotic susceptibility testing was carried out on bacterial isolates using Kirby-Bauer disc-diffusion method. The multi-disc included the following antibiotics: peflacin (30µg), ofloxacin (10µg), ciprofloxacin (10µg), gentamycin (10µg), streptomycin (30µg), erythromycin (30µg), flucloxacillin, augmentin® (30µg), cephalixin (10µg), penicillin (30µg), cotrimoxazole (30µg), nalidixic acid (30µg) and lincomycin (30µg). The zone of growth inhibition was measured using calibrated ruler to determine the sensitive and resistant isolates. Data were analyzed using SPSS version 15.0, and results expressed in

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mean and percentage. Significance was inferred if  $p < 0.05$ .

### Results

Table I presents the characteristics of the 150 enrolled pregnant subjects, the means  $\pm$  standard deviation of age and weight were  $25.4 \pm 5.0$  years and  $66.8 \pm 8.2$  kg, respectively. Overall, the prevalence of asymptomatic bacteriuria was 59.0% (118/200) and was significantly higher among pregnant (63.3%, 95/150) than non-pregnant (46.0%, 23/50) subjects ( $\chi^2 = 4.66$ ,  $df = 1$ ,  $p = 0.03$ ). Of the 118 bacterial isolates, *Klebsiella spp* accounted for 39.8% (47/118), followed by *S. aureus* 22.9% (27/118), *E.coli* 19.5% (23/118), *Proteus spp* 9.3% (11/118) and *P. aeruginosa* 8.5% (10/118) ( $p < 0.0001$ ). However, similar bacterial isolates was observed among both pregnant and non-pregnant subjects (Table II). The distribution of bacterial isolates based on age group of the subjects is presented in Table III with age group 20-29 years contributing highest proportion of 69.5% (82/118).

**Table I. Characteristics of pregnant subjects enrolled into the study**

Variables	Values (%)
<b>Number enrolled</b>	150
<b>Age (years)</b>	
Mean $\pm$ SD	$25.4 \pm 5.0$
<b>Weight (kg)</b>	
Mean $\pm$ SD	$66.8 \pm 8.2$
<b>Educational background</b>	
Primary	2 (1.3)
Secondary	30 (20.0)
Tertiary	62 (41.3)
Quranic	13 (8.7)
None	43 (28.7)
<b>Gestational age</b>	
First trimester	53 (35.3)
Second trimester	48 (32.0)
Third trimester	49 (32.7)
<b>Gravidity</b>	
Primigravid	58 (38.7)
Secondigravid	30 (20.0)
Multigravid	62 (41.3)
<b>Parity</b>	
None	66 (44.0)
Single	26 (17.3)
Multiple	58 (38.7)

SD Standard deviation.

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**Table II. Prevalence of bacteriuria and isolated bacterial pathogens in pregnant and non-pregnant subjects**

Bacterial Isolates	Pregnant subjects (N = 150)	Non-pregnant subjects (N = 50)	Total (N = 200)	p values
<i>Klebsiella spp</i>	39 (26.0)	8 (16.0)	47 (23.5)	0.15
<i>S. aureus</i>	23 (15.3)	4 (8.0)	27 (13.5)	0.19
<i>E. coli</i>	15 (10.0)	8 (16.0)	23 (11.5)	0.25
<i>Proteus spp</i>	10 (6.7)	1 (2.0)	11 (5.5)	0.21
<i>P. aeruginosa</i>	8 (5.3)	2 (4.0)	10 (5.0)	0.71
<b>Overall</b>	<b>95 (63.3)</b>	<b>23 (46.0)</b>	<b>118 (100.0)</b>	<b>0.03</b>

N Number

**Table III. Distribution of bacterial pathogens based on age group of subjects studied**

Age group (years)	<i>Klebsiella</i>		<i>S. aureus</i>		<i>E. coli</i>		<i>Proteus spp</i>		<i>P. aeruginosa</i>		Total
	PS	NPS	PS	NPS	PS	NPS	PS	NPS	PS	NPS	
<20	2	1	0	1	1	3	1	0	1	0	10 (8.5)
20-29	28	4	17	3	14	1	8	1	4	2	82 (69.5)
30-39	9	3	5	0	0	4	1	0	3	0	25 (21.2)
40-49	0	0	1	0	0	0	0	0	0	0	1 (0.8)
Sub-total	39	8	23	4	15	8	10	1	8	2	-
Total	47 (39.8)		27 (22.9)		23 (19.5)		11 (9.3)		10 (8.5)		118 (100.0)

PS Pregnant subjects  
NPS Non-pregnant subjects

Factors associated with asymptomatic bacteriuria in pregnant subjects were evaluated and presented in Table IV. The analysis showed that pregnant women with tertiary education (72.6%, OR = 1.45, p = 0.014), at third trimester (77.6%, OR = 1.39, p = 0.042), with multi-gravidity (79.0%, OR = 1.41, p = 0.0017) and multiparity (75.9%, OR = 1.49, p = 0.03) were associated with asymptomatic bacteriuria. Table V presents the results of

antibacterial susceptibility of the 118 bacterial isolates. The results showed that all the bacterial isolates were susceptible to quinolones (ciprofloxacin, pefloxacin and ofloxacin). In addition to this, *S. aureus* was also susceptible to erythromycin. However, all bacterial isolates were resistant to readily available antibacterial drugs including augmentin®, cotrimoxazole, penicillin and cephalexin

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**Table IV. Factors associated with asymptomatic urinary tract infections in pregnant women**

<b>Variables</b>	<b>Total enrolled (N = 150)</b>	<b>Positive urine (N = 95)</b>	<b>Odd ratios</b>	<b>P value</b>
<b>Educational background</b>				
Primary	2	1 (50.0)	1.00	
Secondary	30	17 (56.7)	1.13	
Tertiary	62	45 (72.6)	1.45	
Quranic	13	7 (53.8)	1.08	
None	43	25 (58.1)	1.16	<b>0.014</b>
<b>Gestational age</b>				
First trimester	53	30 (56.6)	1.00	
Second trimester	48	27 (56.3)	1.00	
Third trimester	49	38 (77.6)	1.39	<b>0.042</b>
<b>Gravidity</b>				
Primigravid	58	33 (56.9)	1.00	
Secondigravid	30	13 (43.3)	0.76	
Multigravid	62	49 (79.0)	1.41	<b>0.0017</b>
<b>Parity</b>				
None	66	35 (53.0)	1.00	
Single	26	16 (61.5)	1.17	
Multiple	58	44 (75.9)	1.49	<b>0.03</b>

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**Table V. Antibacterial susceptibility pattern of the bacterial isolates**

<b>Drugs</b>	<b>Klebsiella</b>	<b><i>E. coli</i></b>	<b><i>S. aureus</i></b>	<b><i>P. mirabilis</i></b>	<b><i>P. aeruginosa</i></b>
<b>Ofloxacin</b>	<b>91.3</b>	<b>95.5</b>	<b>80.0</b>	<b>83.3</b>	<b>88.9</b>
<b>Pefloxacin</b>	<b>75.6</b>	<b>60.9</b>	<b>80.0</b>	<b>75.0</b>	<b>88.9</b>
<b>Ciprofloxacin</b>	<b>87.0</b>	<b>87.0</b>	<b>92.6</b>	<b>91.7</b>	<b>66.7</b>
<b>Gentamycin</b>	<b>52.2</b>	<b>52.2</b>	<b>44.4</b>	<b>50.0</b>	<b>55.6</b>
<b>Streptomycin</b>	<b>51.1</b>	<b>21.7</b>	<b>37.0</b>	<b>41.7</b>	<b>44.4</b>
<b>Erythromycin</b>	<b>50.0</b>	<b>39.1</b>	<b>77.8</b>	<b>45.5</b>	<b>0.0</b>
<b>Lincomycin</b>	<b>28.3</b>	<b>5.0</b>	<b>42.3</b>	<b>0.0</b>	<b>0.0</b>
<b>Cephalexin</b>	<b>27.4</b>	<b>17.4</b>	<b>0</b>	<b>25.0</b>	<b>0.0</b>
<b>Augmentin</b>	<b>19.6</b>	<b>26.1</b>	<b>12.5</b>	<b>0.0</b>	<b>11.1</b>
<b>Cotrimoxazole</b>	<b>17.4</b>	<b>8.7</b>	<b>14.8</b>	<b>8.3</b>	<b>0.0</b>
<b>Nalidixic acid</b>	<b>15.6</b>	<b>8.7</b>	<b>4.2</b>	<b>8.3</b>	<b>11.1</b>
<b>Flucloxacillin</b>	<b>14.4</b>	<b>34.8</b>	<b>37.0</b>	<b>25.0</b>	<b>22.2</b>
<b>Penicillin</b>	<b>12.8</b>	<b>26.1</b>	<b>4.0</b>	<b>0.0</b>	<b>0.0</b>

**Discussion**

Urinary tract infection (UTI) is a common clinical episode seen at both hospital and community settings. In women, it is a major problem as up to a third of women experienced UTI at some point in their life (Valiquette. 2001). However, clinical presentations are always asymptomatic especially among pregnant women. The asymptomatic nature makes the condition progresses undetected and untreated in most patients. This could lead to more serious health conditions such as cystitis and kidney disorders. In our study, we investigated the prevalence and factors associated with asymptomatic bacteriuria in pregnant women.

The prevalence of asymptomatic bacteriuria (ASB), 63.3%, observed among the pregnant women in our study is considered high; particularly when compared to other similar studies within and outside Nigeria. Prevalence of 23.3% was reported in Sagamu, Nigeria (Olusanya et al., 1993) and 58.0% in Ondo, Nigeria (Onifade et al., 2005). In addition, 7.0 and 7.3% were reported in Ethiopia (Gabre-Selassie, 1998) and Kumasi, Ghana (Turpin et al., 2007), respectively. Outside Africa, significantly lower rates have also been reported - 14.2% in Saudi Arabia (Al-Sibai et al., 1989), 38.0% in Iraq (Al-Dujiaily, 2000), 30% in Yemen (Al-Haddad 2005), 4-7% in

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Canada (Nicolle, 1994), 10.6% in Turkey (Kutlay et al., 2003) and 28.5% in Pakistan (Sheik, 2000). However, significantly higher rate (86.6%) was reported in Benin City, Nigeria (Akerlele et al., 2001). This variation could be attributed to the fact that prevalence of ASB varies with geographical locations, age of the subjects, studied population and diagnostic methods. The observed difference in the prevalence levels could also be linked to environment, social habits of the community, personal hygiene and level of educational status.

In most documented studies on bacterial pathogens implicated in UTI among pregnant women, members of the family Enterobacteriaceae predominate, with *E.coli* and enterobacter species as the most common. In this study, *Klebsiella* species accounted for highest proportion of 39.8% (47/118) contrary to previous studies that reported *E. coli* as most prevalent isolates (Olusanya et al., 1993, Akerlele et al., 2001, Onifade et al., 2005.). This finding may not be surprising as incidence of bacterial pathogens varies in hospitals and geographical locations (Fatima and Ishrat, 2006). In addition to Gram negative organisms, we also isolated Gram positive bacteria like *S. aureus*. This is similar to previous studies that have also reported *S. aureus* (Al-Sibai et al., 1989, Gabre-Selassie 1998, Olusanya et al., 1993, Al-Dujaily 2000, Akerlele et al., 2001, Onifade et al., 2005, Al-Haddad 2005, Turpin et al., 2007). The high prevalence rate of the urinary pathogens in female population, especially pregnant women might not be a surprising issue considering

the anatomical structure and the proximity of the genital tract to the bowel that allows for easy contamination.

In our study, tertiary education, trimester, multi-gravidity and multi-parity were factors associated with ASB in pregnant women. These factors were similar to previously reported study (Fatima and Ishrat, 2006). This observation might not be too surprising as these individual falls within age-group that experiences increased sexual activities, which invariably leads to high probability of experiencing UTI episode early in life, also recent history of UTI have been identified as a contributory factor (Blumberg and Abrutyn, 1997). Educational status has not been known as predisposing risk factor of ASB. Studies have shown that increased sexual activities, particularly within the sexually active age could serve as a risk factor for UTI, as most of the bacterial pathogens isolated formed the normal flora of vagina region. Also, massaging process or manipulation of urethra are also contributory factors (Blumberg and Abrutyn, 1997, Kromery et al., 2001). It is well recognized that urinary stasis increases with advancing pregnancy, and thus the incidence of UTI could increase in the last trimester (Nicolle, 1994). In addition, hormonal changes which occur with pregnancy as it advances, certain chemicals such as glucose is released in to the urine which changes the pH of the urine and favor the growth of most pathogens present in the bladder (Kromery et al., 2001). The obstetric history evaluation of the pregnant women studied

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showed that high proportion of the bacterial pathogens was isolated from pregnant women who previously had miscarriage which accounted for 20 (21.1%) cases. Similarly it was also observed that pregnant women who had 3 or more pregnancies and those with multiparity accounted for high occurrence with 49(51.6%) and 44(46.3%) respectively. The high frequency of occurrence of such pathogens among such groups is in accordance with reported study by Turpin et al. (Turpin et al., 2007). The risk of acquiring bacteriuria among those groups is common due to frequent exposure to hospital equipment and environment, as the infections may be endogenous but there is possibility of being introduced through exogenous means (Kromery et al., 2001) especially during surgical manipulations and evacuations.

The antibiotic susceptibility pattern of the bacterial isolates in this study showed high susceptibility to the quinolones family, followed by aminoglycosides and high resistant pattern was observed with nalidixic acid, sulphonamide and penicillin. With such high prevalence of ASB in this study, and its clinical

implication, routine urine culture may be necessary for early detection and need for initiation of antibiotic therapy. However, antibiotic prescription in pregnancy depends on proper assessment of the pregnant women by the physician, based on the pharmacokinetic property of the drugs, thereby evaluating the drugs side-effect, level of toxicity particularly on the fetus. Early identification of ASB and treatment could help stemmed down progression of clinical condition to cases like renal dysfunction.

In conclusion, the prevalence of ASB observed in this study is considered to be high, which could pose serious clinical implication on both pregnant women and fetus. Also, considering the clinical effect of untreated UTI, that may progress leading to ascending infections. Therefore, it would be of great importance if urine culture determination is included as part of routine antenatal care.

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