



Received: 1st December, 2022 Accepted: 17th December, 2022

Incidence and Antibiogram of Uropathogens Isolated from Pregnant Women Attending Antenatal Clinic in a Public Hospital in Yola, Adamawa State, Nigeria

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Abstract

Urinary tract infection during pregnancy including asymptomatic bacteriuria has been associated with some adverse outcomes for the mother and foetus. Studies have shown that pregnant women with urinary tract infections have a significantly higher rate of intra-uterine growth restriction, pre-eclampsia, caesarean and pre-term deliveries. This study was undertaken to determine the occurrence, distribution and antibacterial susceptibility pattern of uropathogens among pregnant women attending antenatal clinic in a public hospital in Yola. A total of 200 clean catch mid-stream urine samples were collected from pregnant women and inoculated on cysteine lactose electrolyte deficient agar for bacterial count and isolation of uropathogens. The isolates were also screened for ability to form biofilms using Congo Red Agar method and antibiotic susceptibility test was done on the isolates using agar disk diffusion method. The incidence of UTI among the pregnant women was found to be 90%. The predominant uropathogens were *E. coli* (46.0%), *S. aureus* (20.0%) and *Klebsiella pneumoniae* (17.0%) while the least occurring uropathogen was *Proteus sp.* (2.0%). Women in the third and first trimester of pregnancy had the highest occurrence of UTI. A total of 47.0% of the isolates produced biofilm in vitro with the highest biofilm production observed among *Proteus sp.* (75.0%) and *Pseudomonas aeruginosa* (60.0%) isolates. The occurrence of antibiotic resistance among the isolates was high, with greater susceptibility observed to quinolone and third generation cephalosporin. The *E. coli* isolates demonstrated varying levels of resistance to all the antibiotics tested. Because of the gravity of problems of UTI in pregnancy and that 9 in 10 pregnant women from this study have symptomatic or asymptomatic infection, it is concluded that pregnant women should be screened for urinary tract infection during antenatal visits especially during the first and third trimesters of pregnancy.

Keywords: Asymptomatic, Bacteriuria, Uropathogen, Antibiotic susceptibility, Pregnancy

INTRODUCTION

Urinary tract infection (UTI) refers to both symptomatic infection with invasion and asymptomatic microbial colonization of the urinary tract. These infections affect structures such as the kidneys, ureters, urinary bladder and urethra that participate in the secretion and elimination of urine from the body. UTIs are usually named according to the site of infection as Urethritis (urethral infections), cystitis (bladder), pyelonephritis (kidneys) or urosepsis for blood stream infections (Lee *et al.*, 2020). Among pregnant women in Saudi Arabia, the prevalence of UTI was reported to be 20% (12% with symptomatic urinary tract infection and 8% were asymptomatic (Mohamed and Al-Kashif, (2019). Furthermore, Aiyegoro *et al.* (2009) reported that about 20 % of humans suffer from urinary tract infection with 15% adult females suffering from two or more episodes of urinary tract infections annually.

Some of the risk factors associated with recurring urinary tract infection include frequency of intercourse and other sexual behaviours as well as the secretory status of certain blood groups (Johnson *et al.*, 2021; Kodner *et al.*, 2010).

Although UTI is reported to be more common in women than men, reports have also shown that there is an increase in UTI cases among pregnant women because of change in body physiology due to pregnancy. Mohamed and Al-Kashif, (2019) reported that the prevalence of UTI in pregnancy is associated with the socio-economic status of the individual with a frequency of 20% in disadvantaged groups. Some of the other important risk factors of UTI in pregnancy are recurrent UTI, hypertension, anatomical abnormalities, and diabetes mellitus (Johnson *et al.* 2021).

Furthermore, Mohamed and Al-Kashif, (2019) reported that higher parity, sickle cell disease and age less than 15 years at first UTI are also risk factors of UTI in pregnancy.

The presence of urinary tract infection in pregnancy has been associated with some adverse outcomes for the mother and baby. Studies have shown that pregnant mothers with urinary tract infections have a significantly higher rate of intra-uterine growth restriction, pre-eclampsia, caesarean deliveries and pre-term deliveries, even after controlling for age and parity. Two studies in Iran by Emmanghorashi *et al.* (2012) and Khalesi *et al.* (2014)] reported a significant ($p=0.001$) increase in the prevalence of UTI among neonates born to mothers who had a UTI during pregnancy (30%) compared to those whose mothers had no UTI in pregnancy (6.8%). Furthermore, asymptomatic bacteriuria is known to increase the risk of developing pyelonephritis in pregnant women compared to healthy non-gravid women. For this reason, it is prescribed that a routine screening for asymptomatic bacteriuria using midstream urine culture early in pregnancy especially during the first antenatal booking or around 10 to 16 weeks gestation (Meads, 2011). Reports have also shown that heavy colonization of the genital tract with Group B Streptococci at the time of delivery or rupture of membranes imposes the risk of vertical transmission, which may manifest as pneumonia and sepsis in the neonate (Allen *et al.*, 2012).

In view of the several deleterious effects of urinary tract infections on mother, foetus and neonates, the study aimed to assess the incidence of urinary tract infection among pregnant women in Yola with a view to providing a baseline data on the occurrence of uropathogens and their antimicrobial susceptibility among pregnant mother in Adamawa State.

MATERIALS AND METHODS

Ethical Approval

The management committee of the hospital used as sample collection sites gave approval for the study. The informed consent of the study subjects was also sought and obtained before enrolling them in the study guaranteeing them of confidentiality.

Study Population

The study population included pregnant women in a public hospital irrespective of age, ethnicity, social status, gravidae and trimester of pregnancy who willingly volunteered to participate in the study.

Sample Collection and Determination of Bacterial Count

A total 200 clean-catch midstream urine samples were collected from the study subjects

in a sterile universal container. Samples were inoculated as described by Imade *et al.* (2010). Briefly, 2 μ L of urine sample was inoculated on dried plates of blood agar and cysteine lactose electrolyte deficient (CLED) agar. The plates were then incubated aerobically at 37°C for 18 hours. Urine samples yielding bacterial growth of 10^5 cfu/mL or more were regarded as significant for Urinary Tract Infection.

Isolation of Uropathogens

A loopful of well-mixed midstream urine sample was inoculated on Cysteine lactose electrolyte deficient (CLED) agar using aseptic procedures. The plates were incubated aerobically at 37 °C for 24 hrs and observed for the presence or absence of bacterial growth. Discrete colonies were sub cultured to obtain pure colonies (Cheesbrough, 2006).

Identification of Isolates

The bacterial isolates were further identified using microscopy and biochemical tests as described by Chessbrough (2006). The biochemical tests are catalase, coagulase, oxidase, Methyl Red, Voges Proskauer, Indole, gas production and sugar fermentation on kligler iron agar.

Screening for Biofilm formation

The qualitative method of screening bacteria for ability to form biofilms using Congo Red Agar method described by Freeman *et al* (1989) was adopted. A wire loop was used to inoculate pure culture of the isolates on the surface of Congo Red Agar after which the plates were incubated at 37 °C for 24 to 48 hours aerobically. After the period of incubation, the plates were observed for the presence of black colonies with dry crystalline consistency which is indicative of biofilm formation.

Antibiotic Susceptibility Testing

Antibacterial susceptibility testing of the isolates was done using the agar disk diffusion method described by CLSI (2012). About 0.1 mL standardized inocula of the test isolates corresponding to 0.5 McFarland were transferred to the surface on Mueller Hinton Agar using sterile pipettes and then spread evenly on the surface using a bent glass spreader. Antibiotic discs of Chloramphenicol (25 μ g), Ampicillin (10 μ g), Amoxicillin (10 μ g), Tetracycline (30 μ g), Cotrimoxazole (25 μ g), Nalidixic acids (30 μ g), ofloxacin (5 μ g) and ciprofloxacin (5 μ g) were placed with the aid of a sterile forceps on the Mueller Hinton agar plates pre-seeded with the test isolates. The plates were then incubated at 37 °C for 24 hrs after which the plates were examined for zone of inhibition which were measured in mm and the zones of inhibition were interpreted using CLSI breakpoints (CLSI, 2012)

RESULTS

Incidence of Uropathogens

Culture results of urine samples collected from study subjects enrolled in the study revealed that 180 (90%) yielded growth uropathogens implying an incidence of 90%. Of this number, only 130 representing 65% of the samples are positive for significant asymptomatic bacteriuria (Figure 1). Mixed isolates were

observed in 32 samples. Biochemical, morphological and cultural characterisation of the isolates revealed that *E. coli* was the most abundant isolates with an occurrence of 46% followed by *S. aureus* with an occurrence of 20%. The least occurring isolate was *Proteus* (2%) and *Pseudomonas aeruginosa* with an occurrence of 5% (Figure 2).

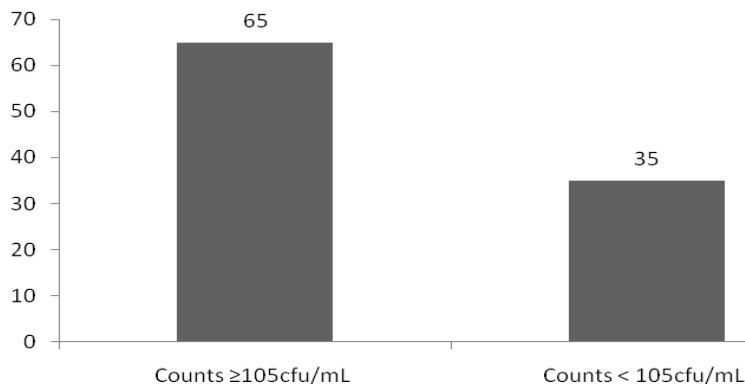


Figure 1 Distribution of samples in relation to percentage of significant bacteriuria

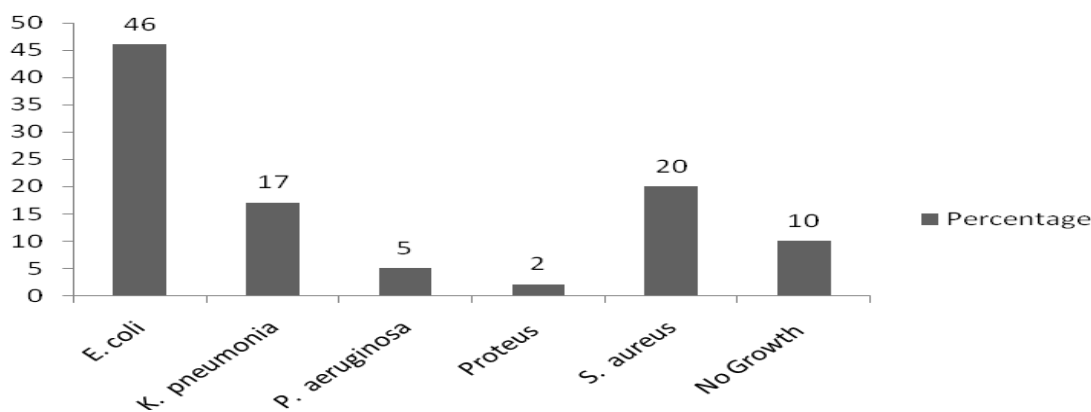


Figure 2: Percentage Occurrence of Uropathogens Isolated in the study population

Distribution of Uropathogens by Trimester of Pregnancy

The result of the study with respect to trimester of pregnancy show that women in the third trimester of pregnancy has the highest

uropathogens occurrence (95.6%) followed by those in the first trimester (88.9%). Although these differences were however not statistically significant (p=0.05) (Table 1).

Table 1: Distribution of Urinary tract infections according to Trimester of pregnancy

Trimesters	No. positive (%)	No. Negative (%)	Total No. (%)	P value
First Trimester	40(88.9)	5 (11.1)	45 (22.5)	P=0.4545
Second Trimester	52 (86.7)	8 (13.3)	60 (20)	
Third Trimester	88(95.6)	7 (3.4)	95(47.5)	
Total	180 (90)	20 (10)	200	

Biofilm Formation among Uropathogens

Result on biofilm production by the test isolate revealed that 47% of the isolates produce biofilm *in vitro*. The highest occurrence of biofilm producing isolates was *Proteus* spp (75%) followed by *Pseudomonasa eruginosa*

(60%). Among the *Klebsiella pneumoniae*, 41.2% of the isolates produced biofilms. The difference however among the isolates in terms of the ability to produce biofilm was not statistically significant (p=0.05) (Table 2).

Table 2: Occurrence of Uropathogens with Biofilm Forming Abilities

Isolates	Biofilm producing isolates No.(%)	Non biofilm producing isolates No.(%)	Total	P value
<i>Escherichia coli</i>	40 (43.5)	52 (46.5)	92 (51)	P=0.3335
<i>Pseudomonas aeruginosa</i>	6 (60.0)	4 (40.0)	10 (5.6)	
<i>Klebsiella pneumoniae</i>	14 (41.2)	20 (59.8)	34 (18.9)	
<i>Proteus spp</i>	3 (75.0)	1 (25.0)	4 (2.2)	
<i>Staphylococcus aureus</i>	23 (57.5)	17 (42.5)	40 (22.2)	
	86 (47.8)	94 (52.2)	180 (100)	

Antimicrobial susceptibility test

Results of antibacterial susceptibility test for uropathogens recovered in this study as revealed in Tables 3 and 4. Table 4 shows that *Proteus spp* isolates demonstrated absolute resistance to ampicillin, levofloxacin, streptomycin and nalidixic acid but were all susceptible to gentamycin. Among the *P. aeruginosa* isolates, greater susceptibility to ciprofloxacin (60%), gentamycin (60%), and ofloxacin (60%) were observed as well as greater resistance to streptomycin (80%) and nalidixic acid (80%). For the *E coli* isolates, varying levels of resistance to all the antibiotics

tested was observed but of particular note is high level of resistance to streptomycin (64.1%), cephalixin (56%) and cotrimoxazole (43.5%) (Table 3). Although susceptibility to ciprofloxacin was high among all the Gram-negative isolates, some isolates demonstrated reduced susceptibility and resistance to the drug *in vitro*.

The antibiotic susceptibility profile of *S. aureus* revealed that 75% of the isolates were resistant to ceftriaxone, followed by amoxicillin 60%. The highest susceptibility of *S. aureus* isolates was to ampiclox, 75% followed by pefloxacin (65%) and ciprofloxacin 65% (Table 5).

Table 3: Antibiogram of *E. coli* and *P. aeruginosa* isolated from the study subjects

Antibiotics	<i>E. coli</i> (n=92)			<i>P. aeruginosa</i> (n=10)		
	R	I	S	R	I	S
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Ciprofloxacin	12 (13.0)	18 (19.6)	62 (67.4)	2 (20.0)	2 (20.0)	6 (60.0)
Gentamicin	18 (19.6)	20 (21.7)	54(58.7)	0 (0.0)	4 (40.0)	6 (60.0)
Ofloxacin	12 (13.0)	39 (42.4)	41(44.6)	4 (40.0)	0 (0.0)	6 (60.0)
Ampicillin	38 (41.3)	30 (32.6)	24(26.0)	4 (40.0)	4 (40.0)	2 (20.0)
Levofloxacin	48 (52.2)	4 (4.3)	40 (43.5)	6 (60.0)	0 (0.0)	4 (40.0)
Cephalixin	52 (56.5)	6 (6.5)	34 (37)	2 (20.0)	6 (60.0)	2 (20.0)
Streptomycin	59 (64.1)	5 (5.4)	28 (30.4)	8 (80.0)	1 (10.0)	1 (10.0)
Cotrimoxazole	40 (43.5)	6 (6.5)	46 (50.0)	4 (40.0)	2 (20.0)	4 (40.0)
Nalidixic Acid	24 (26.0)	16 (17.4)	52 (56.5)	8 (80.0)	0 (0.0)	2 (20.0)
Augmentin	24 (26.0)	24 (26.0)	44 (47.8)	6 (60.0)	2 (20.0)	2 (20.0)

Table 4: Antibiogram of Gram negative *K. pneumoniae* and *Proteus sp* isolated from the study subjects

	<i>K. pneumoniae</i> (n=34)			<i>Proteus spp.</i> (n=4)		
	R	I	S	R	I	S
	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Ciprofloxacin	4 (11.8)	4 (11.8)	26 (76.5)	2 (50.0)	0 (0.0)	2 (50.0)
Gentamicin	8 (23.5)	10 (29.4)	16 (47.1)	0 (0.0)	0 (0.0)	4 (100.0)
Ofloxacin	12 (35.3)	12 (35.3)	10 (29.4)	2 (50.0)	2 (50.0)	0 (0.0)
Ampicillin	24 (70.6)	4 (11.8)	6 (17.6)	4 (100.0)	0 (0.0)	0 (0.0)
Levofloxacin	20(58.8)	4 (11.8)	10 (29.4)	4 (100.0)	0 (0.0)	0 (0.0)
Cephalixin	14 (41.1)	10 (29.4)	10 (29.4)	0 (0.0)	4 (100.0)	0 (0.0)
Streptomycin	10 (29.4)	4 (11.8)	20 (58.8)	4 (100.0)	0 (0.0)	0 (0.0)
Cotrimoxazole	16 (47.1)	6 (17.6)	12 (35.3)	2 (50.0)	2 (50.0)	0 (0.0)
Nalidixic Acid	4 (11.8)	10 (29.4)	20 (58.8)	4 (100.0)	0 (0.0)	0 (0.0)
Augmentin	26 (76.5)	4 (11.8)	4 (11.8)	0 (0.0)	2 (50.0)	2 (50.0)

KEY: n= Number of isolates, R= Resistance, I = Intermediate, S = Susceptible

Table 5: Antibiogram of *S. aureus* (n=40) isolated from study subjects

Antibiotics	Resistant No (%)	Intermediate No (%)	Susceptible No (%)
Pefloxacin	10 (25)	6 (15)	26 (65)
Gentamycin	19 (47.5)	16 (40)	5 (12.5)
Ampiclox	4 (10)	6 (15)	30 (75)
Cefuroxime	30 (75)	6 (15)	4 (10)
Amoxicillin	24 (60)	8 (20)	8 (20)
Ceftriaxone	20 (50)	14 (35)	6 (15)
Ciprofloxacin	10 (25)	4 (10)	26 (65)
Streptomycin	16 (40)	8 (20)	16 (40)
Cotrimoxazole	20 (50)	0 (0)	20 (50)
Erythromycin	24 (60)	4 (10)	12 (30)

DISCUSSION

Both gram positive and gram-negative uropathogenic bacteria were recovered from urine of pregnant women. The overall occurrence of uropathogens from pregnant women in this study is 90.0%, however only 65.0% of the samples reflected significant bacteriuria. This value is quite high when compared to the 46.5% in Ebonyi reported by Onuh *et al.* (2014), 35.5% in Ilorin reported by Çelen *et al.*, (2011). However, the high prevalence is not surprising as pregnancy has been reported to increase the vulnerability of women to UTIs (Johnson *et al.*, 2021; Mohamed and Al-Kashif, (2019). Furthermore, the hormonal and physiological changes and distending abdomen promote urinary stasis and vesicoureteral reflux which along with an already short urethra and difficulty with hygiene due to a distended pregnant belly, make UTIs the most common bacterial infections during pregnancy (Imade *et al.*, 2010). These factors might have contribution to the high prevalence in this study.

The most prevalent uropathogens isolated from this study were *E. coli* (46.0%) followed by *Staphylococcus aureus* (20.0%). These two bacteria vary in their recovery from cases of urinary tract infections. From some studies, *E. coli* dominates as the most common etiologic agents of UTI (references of such studies) while in other studies *S. aureus* dominated (references of such studies). Findings from this study agree with the report of Mohammed (2015) which states that *E. coli* accounts for the majority (80.0%) of urinary tract infections. From this study it was also observed that the second highest uropathogens recovered from the study was *S. aureus* (20.0%). This prevalence is lower than the 24.4% prevalence reported by Imade *et al.*, (2010) among pregnant women in Benin, Nigeria but higher than the 18.0% reported by Mohammed (2015) in Kaduna. This incidence indicates that *S. aureus* is emerging as an important uropathogens which may be attributed to the fact that *S. aureus* is reported to colonize the vagina of 4% - 22% of pregnant

women (Nester, 1998; Akerele and Okonofua, 2001).

Meads (2011) reported that the frequency of asymptomatic bacteriuria in pregnancy ranges between 2%-18.5%. He further reported that 24.8% of pregnant women whose asymptomatic bacteriuria was untreated developed pyelonephritis compared to 3.2% of those who were treated. In another study, 30% of pregnant women with bacteriuria were reported to develop pyelonephritis compared to 1.8% of those without pregnancy (Meads, 2011; Imade, 2010). These reports indicate that the high prevalence of uropathogens from this study implies that the pregnant women are at risk of developing serious health complication (Johnson *et al.*, 2021) It has been reported that when asymptomatic bacteriuria is not treated in pregnancies, there are risks of kidney infection, intrauterine growth retardation and pre term birth.

The 88.9% occurrence of Uropathogens in the first trimester of pregnancy is very significant. This is because it has been reported that asymptomatic bacteriuria increases the risk of pyelonephritis in pregnant women unlike in healthy non gravid women. As an important precautionary observation, it is expected that midstream urine culture be done for pregnant women during their booking visit for ante natal irrespective of whether they have signs and symptoms of UTI or not (Nicolle *et al.*, 2019; Meads, 2011).

Results from the study showed that 47.8% of the isolates demonstrated the ability to produce biofilms *in vitro*. Biofilms production is an environmental adaptation mechanism by pathogens and it plays an important role in pathogen survival in the natural environment. This ability to form biofilms is a dangerous trend because they can become a persistent source of contamination as well as making the organisms to show high level of tolerance to prolonged antibiotic therapy. The ability of the uropathogens to form biofilms in conjunction with resistance to antibiotics could probably be responsible for some treatment failures,

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frequent relapses, recurrent infection and persistent transmission of the disease among pregnant women (Imade *et al.* 2010; Okonofua, 2001).

Results from antibiotic susceptibility screening revealed varying levels of antibiotic resistance among the isolate. From absolute resistance of *Proteus* species isolates to ampicillin, levofloxacin, streptomycin and nalidixic acid to varying resistance to cephalexin, cotrimoxazole, ceftriaxone and amoxicillin by the other isolate. Hitherto, it has been reported that the efficacious drug for the treatment of UTI in pregnancy are varying combinations of ceftriaxone, amoxicillin, cephalexin and gentamycin among others (Amadi *et al.*, 2007). This implication of this finding is that effective treatment options are narrowing due to resistance and laboratory based confirmation of treatment option instead of empirical diagnosis needs to be adhered to avoid treatment failure and relapse of urinary tract infection in pregnant women.

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E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

CONCLUSION

Findings from this study revealed that the occurrence of asymptomatic bacteriuria is high (65.0%) while the occurrence of uropathogens from the study population is equally high (90.0%). The study further revealed that the isolates have the ability to form biofilms and demonstrated varying degree of resistance to tested antibiotics.

RECOMMENDATION

Because of the high incidence of asymptomatic bacteriuria from this study and its consequences on the health of the mother and foetus, it is important that pregnant women be promptly screened for asymptomatic bacteriuria instead of just the proteinuria done during the focal antenatal visits. This will go a long way towards mitigating the problems of UTI in pregnancy and consequently improve the quality of life of the mother and foetus.

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