



BACTERIAL PATHOGENS AND ANTIBIOTIC SUSCEPTIBILITY PROFILES IN PREGNANT WOMEN WITH UTIS IN OGIDI, NIGERIA

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ABSTRACT: Urinary tract infections (UTI) affect the structures involved in the secretion and elimination of urine. It is a common health concern and contributes about 13-33% of infection in pregnancy. The prevalence of UTI among pregnant women attending antenatal clinic in Iyi-Enu mission hospital and the sensitivity of isolates recovered, to antibiotics was studied. A total of 120 midstream urine samples collected from pregnant women who consented to the study, between January and March, 2023, were cultured on MacConkey agar, Blood agar and Cystine Electrolyte Deficient Agar. Isolates were identified using colony morphology, Gram stain reaction and biochemical tests. Susceptibility of the isolates to antibiotics was determined using KirbyBauer disc diffusion method. Results obtained from the structured questionnaire used for demographic data were statistically analyzed. The prevalence of UTI among pregnant women was 24.2%. The isolates recovered include; *Escherichia coli* (24.1%), *Klebsiella spp.* (18.5%), *Proteus mirabilis* (7.4%), *Pseudomonas spp.* (5.6%) and *Staphylococcus aureus* (44.4%). Gram negative bacteria were most susceptible to Cefuroxime (63%) and resistant to Ceftazidime (80.7%). Gram-positive isolates were susceptible to Levofloxacin (100%) but resistant to Amoxicillin (83.3%). Both Gram negative and Gram-positive organisms were recovered from UTI of pregnant women and were found to be sensitive to Cefuroxime and Levofloxacin respectively. There is need for proper diagnosis of UTI before treatment to avoid resistance of uropathogens to antibiotics.

Keywords: Urinary tract infection, uropathogens, pregnant women, antibiotics, susceptibility test

INTRODUCTION

Urinary tract infection (UTI) is an inflammation of the urinary tract caused by the presence of microorganisms. This may occur either in the lower urinary tract (cystitis or urethritis) and or in the upper urinary tract (pyelonephritis) (Johnson *et al.*, 2021). UTI are a common health concern affecting about 150 million individuals each year (Stamm and Norrby, 2001). Among the various groups susceptible to UTI, pregnant women are particularly vulnerable due to anatomical and hormonal changes that occur during pregnancy (Onyango *et al.*, 2018). UTI contributes 13-33% of all infections encountered during pregnancy in clinical practice (Johnson *et al.*, 2021; Karikari *et al.*, 2020; Mahmoud *et al.*, 2019) with high rate of morbidity and financial burden (Thakur and Nepal, 2020). Infection usually begins at week 6 and peaking at weeks 22-26 (Mahmoud *et al.*, 2019). Common manifestations of UTI in pregnancy are asymptomatic bacteriuria, acute cystitis and acute

pyelonephritis (Loh and Silvslingan, 2007) and the severity is usually influenced by the virulence of the bacteria and the susceptibility of the host (Vicar *et al.*, 2023).

More than 90% of UTI are caused by normal body flora bacteria that can contaminate the genital area and the urinary tract (Nester and Eugene, 1998). The pathogenic bacteria associated with UTI include *Staphylococcus saprophyticus*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Proteus spp.*, *Pseudomonas aeruginosa* and Enterococci (Thakur and Nepal, 2020, Garnizov, 2016) with *E. coli* as the most commonly isolated (Ekwealor *et al.*, 2021; MacLean, 2001).

Women undergo many changes during pregnancy. These changes could be hormonal, anatomical and physiological which increase their chances of acquiring UTI. The increased level of hormones, short urethra in females and pressure caused by the growing uterus can lead to decreased urethral and bladder tone, increased bladder volume and upper urinary tract dilation. These may lead to emptying impairment stasis of urine and ascending of microbiologically contaminated urine to the urethra from the bladder (Thakur and Nepal, 2020; Mahmoud *et al.*, 2019, Taye *et al.*, 2018; Amiri *et al.*, 2015). UTI may present symptoms of dysuria, frequent and urgency in urination, hematuria, suprapubic pain for cystitis, malaise, vomiting, chills, fever, flank pain for pyelonephritis or may be asymptomatic (Johnson *et al.*, 2021, Farazi and Jabbariasl, 2019; Mahmoud *et al.*, 2019).

Poor personal hygiene and urinary abnormalities, parity, increasing age, previous history of UTI, immunodeficiency, increased condensation of glucose, amino acids in urine during pregnancy and sickle cell anemia have all been implicated as factors that predispose pregnant women to UTI (Kaduma *et al.*, 2019, Li *et al.*, 2019; Kant *et al.*, 2017; Mohamed *et al.*, 2017). Many complications are associated with untreated UTI during pregnancy. These complications may include pyelonephritis, hypertension in pregnancy, severe and septic shock, acute and chronic renal failure, preeclampsia, intrauterine growth restriction, intrauterine fetal death to low birth weight, fetal mortality, preterm labor and premature delivery (Ali *et al.*, 2022; Johnson *et al.*, 2021; Taye *et al.*, 2018). Diagnosis of UTI involves detection of pathogens in routine culture of urine, which allows quantitative estimation of bacteriuria, and antimicrobial susceptibility of uropathogens to available antibiotics. Treatment is usually started empirically while waiting for diagnostic results of the patient (Ali *et al.*, 2022) with antibiotics like Ceftazidime, Norfloxacin, Penicillin and Amoxicillin (Johnson *et al.*, 2021; Vicar *et al.*, 2023). This empirical treatment predisposes the pregnant women to risk of developing drug resistance uropathogens, thereby, increasing the risk of complications and difficulty in eradicating the causative agents (Vicar *et al.*, 2023).

UTI during pregnancy is a major health concern worldwide, especially in developing countries (Baker, *et al.*, 2015). There is need for routine check for uropathogens and treatments during antenatal visits. The purpose of this study, therefore, is to determine the prevalence of UTI among pregnant women attending antenatal clinic at a mission hospital in Southeast, Nigeria.

METHODS

Study area: A cross-sectional study was carried out among pregnant women who attended antenatal clinic at Iyi-Enu Mission hospital Ogidi, Southeast Nigeria between January and March, 2023. Ogidi is the headquarters of Idemili North Local Government Area in Anambra State, Nigeria with estimated population of 70,000 people (NPC, 2016). It shares boundaries with Abatete, Nkpor, Umunnachi, Umuoji, Ogbunike and Umudioka. The mission hospital was recently upgraded to University on The Niger Teaching Hospital, IyiEnu.

Exclusion and inclusion criteria: Pregnant women who received antibiotics two weeks before the sample collection was excluded from the study. All pregnant women who consented orally were included in the study.

Sample size estimation: The sample size was estimated using Kish Leslie (1965) formula.

$$P(1 - P)$$

$$n = (Z_{0.95})^2 \left(\frac{P(1 - P)}{d^2} \right)$$

D^2

Where;

n =Sample size, with 95% confidence interval (i.e. $Z_{0.95} = 1.96$), 31% proportion (P), and absolute precision (D) of 5%.

The minimum number of participants was calculated to give a total of 120 who were selected using systematic random sampling.

Ethical considerations: Ethical approval with Ref. No. IEH/REC/VOL.3/2022/090 was obtained from the ethical Committee of Iyi-Enu Mission Hospital, Ogidi. Oral consent was obtained from pregnant women who participated in the study.

Demographic data: Structured questionnaire was administered to the pregnant women who consented to the study. This was used to obtain information on their age, marital status, occupation, educational level, number of sexual partners, gestational age and parity of the women.

Sample collection: Pregnant women were provided with labeled big mouth sterile bottle and counseled on how to collect urine sample. Each pregnant woman collected about 30ml of clean catch mid-stream urine which was transported to Department of Applied Microbiology and Brewing laboratory, Nnamdi Azikiwe University, Awka, in an ice pack and processed within 3h.

Urine culture: A calibrated wireloop was used to inoculate 0.005ml of urine sample onto MacConkey agar, Blood agar and Cystine Lactose Electrolyte Deficient (CLED) agar plates and incubated at 37°C for 24-48h. Colony forming units were counted and colony counts of 10^3 /ml of urine were regarded as significant for bacteriuria (Johnson *et al.*, 2021). The significant pyuria/ml of urine sample was taken as positive culture. Isolates were purified by sub-culturing on Nutrient agar medium and incubated at 37°C for 24h. Pure cultures of the isolates were inoculated on Nutrient agar slants in tubes and incubated at 37°C for 24h. These were stored at 4°C for further use.

Bacteria identification: Isolates were identified based on colony morphology, Gram's reaction, biochemical reactions and sugar fermentation tests (Cheesbrough, 2016).

Susceptibility test: Antibiotic sensitivity test was carried out using Kirby-Bauer disc diffusion method (Cheesbrough, 2016). Bacteria suspension was standardized to 0.5 Macfarlan turbidity and inoculated on Mueller Hinton agar plates using sterile swab stick. This was allowed to stand for 5min. to dry.

Commercially available antibiotic discs containing Ceftriaxone (30µg), Gentamicin (10µg), Cefuroxime (30µg), Ciprofloxacin (10µg), Ceftazidime (30µg), Ofloxacin (10µg), Augmentin (30µg), Pefloxacin (10µg), Streptomycin (30µg),

Ceporex (10µg) for Gram negative isolates and Amoxicilline (20µg), Ciprofloxacin (10µg), Erythromycin (30µg), Gentamicin (10µg), Cefuroxime (30µg), Levofloxacin (20µg), Azithromycin (10µg), Streptomycin (30µg), Ceflaxidime (20µg), Rifampic (20µg) for Gram positive isolates were aseptically placed on the inoculated plate using sterile forceps. This was incubated at 37°C for 24h.

Data analysis: Data obtained were statistically described in terms of frequency and percentages. The Pearson Chi-Square test was applied to analyze association of urinary tract infection with age, marital status, occupation, educational level, number of sexual partners, gestation period and parity of the participants. P value less than 0.05 ($p < 0.05$) was considered statistically significant at 95% confidence level.

RESULTS

From the colony morphology, biochemical characteristics and sugar fermentation tests, five bacterial isolates were recovered (Table 1), namely, *Escherichia coli*, *Klebsiella spp.*, *Pseudomonas spp.*, *Proteus mirabilis* and

Staphylococcus aureus. Gram negative bacteria were mostly isolated. Out of the 120 urine samples collected, 24.2% (29) were positive for UTI. The frequency of UTI causing bacterial isolates is shown in Table 2. Gram negative bacteria accounted for 55.6% (30) of the isolates and 44.4% (24) were Gram positive. *Escherichia coli* was the most frequently isolated Gram-negative bacteria while *Pseudomonas spp.* was the least. *Staphylococcus aureus* was observed to be the only Gram-positive bacteria isolated and had the highest frequency of UTI. Prevalence of UTI bacteria distributed according to the demographic data and obstetric factors is as presented in Table 3. Result shows that UTI was more prevalent among age group 26-35 years, pregnant women that are single, are subsistence farmers, and that have attained secondary education, those that keep multiple sexual partners, women in their 3rd trimester and the multigravida. Although a significant relationship was observed between UTI and marital status, occupation and multiple partners, there was no significant association recorded between age, educational level, parity, gestation period and UTI.

Table 4a shows the antimicrobial susceptibility pattern of Gram-negative bacteria isolated from UTI in pregnant women. Antibacterial susceptibility of Gram-negative isolates show that Cefuroxime 19 (63.3%) was the most sensitive antibiotics while Ceftazidime 24 (80%) was the most resisted (Table 4a). It can be observed from Table 4a, that *Pseudomonas spp.*, seem to be the most resistant organism to the antibiotics tested, while *Klebsiella spp.* were found to be more sensitive to the antibiotics.

Table 3:

Gram positive bacterium isolated was 100% susceptible to Levofloxacin and 75% sensitive to Gentamicin. Resistance was recorded for Amoxicillin (83.3%), Streptomycin (70.8%) and Rimfampic (62.5%).

Antibiotic sensitivity pattern of *Staph. aureus* to various antibiotics is as presented in Table 4b. All the *S. aureus* isolates were sensitive (100%) to Levofloxacin while 83.3% of the isolates were resistant to Amoxicilline

Prevalence of UTI bacteria based on demographic data and obstetric factors

Variables		Frequency (%) (n= 120)	UTI positive (%)	p-value
Age group (years)	15-25	42 (35)	3 (7.1)	0.051
	26-35	68 (56.7)	24 (35.3)	
	36-45	10 (8.3)	2 (20)	
Marital Status	Married	102 (85)	22(21.6)	0.024
	Single	14 (11.7)	6 (42.9)	
	Divorced	4 (3.3)	1 (25)	
Occupation	Students	6 (5)	2 (33.3)	0.048
	Civil servants	28 (23.3)	8 (28.6)	
	Traders	57 (47.5)	12 (21.1)	
	Artisans	21 (17.5)	3 (14.3)	
	Subsistence farmers	8 (6.7)	4 (50)	
Educational level attained	Primary	22 (18.3)	4 (18.2)	0.062
	Secondary	60 (50)	19 (31.7)	
	Tertiary	38 (31.7)	6 (15.8)	
Number of sexual partners	Single	92 (76.7)	11 (12)	0.008
	Multiple	28 (23.3)	18 (64.3)	
Gestation period	1 st trimester	26 (21.7)	4 (15.4)	0.052
	2 nd trimester	40 (33.3)	7 (17.5)	
	3 rd trimester	54 (45)	18 (33.3)	

Parity	Primigravida	43 (35.8)	7 (16.3)	0.087
	Multigravida	77 (64.2)	22 (28.6)	

Table 4a: pattern of Gram negative bacteria isolated

Antimicrobial susceptibility		Antibiotics									
Bacteria/ Sensitivity		TRX	CN	CEF	CPX	CTZ	OFX	AU	PEF	S	CIP
No. of Pattern isolates											
<i>E. coli</i> (13)	S	2(15.4)	2(15.4)	12(92.3)	9(69.2)	1(7.7)	5(38.5)	1(7.7)	5(38.5)	6(46.2)	8(61.5)
	I	1(7.7)	0(0)	0(0)	1(7.7)	0(0)	1(7.7)	0(0)	1(7.7)	0(0)	2(15.4)
	R	10(77)	11(84.6)	1(7.7)	3(23.1)	12(92.3)	7(53.8)	12(92.3)	7(53.8)	7(53.8)	3(23.1)
<i>Klebsiella</i> spp. (10)	S	6(60)	7(70)	3(30)	8(80)	4(40)	7(70)	9(90)	4(40)	4(40)	0(0)
	I	3(30)	2(20)	1(10)	2(20)	1(10)	3(30)	1(10)	1(10)	1(10)	2(20)
	R	1(10)	1(10)	6(60)	0(0)	5(50)	0(0)	0(0)	5(50)	5(50)	8(80)
<i>Pseudomonas</i> spp (3)	S	0(0)	0(0)	0(0)	0(0)	0(0)	2(66.7)	1(33.3)	1(33.3)	1(33.3)	0(0)
	I	0(0)	0(0)	3(100)	0(0)	0(0)	0(0)	0(0)	0(0)	1(33.3)	0(0)
	R	3(100)	3(100)	0(0)	3(100)	3(100)	1(33.3)	2(66.7)	2(66.7)	1(33.3)	3(100)
<i>Proteus</i> spp (4)	S	0(0)	0(0)	4(100)	0(0)	0(0)	0(0)	1(25)	1(25)	2(50)	0(0)
	I	4(100)	0(0)	0(0)	4(100)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
	R	0(0)	4(100)	0(0)	0(0)	4(100)	4(100)	3(75)	3(75)	2(50)	4(100)
Total	S	8(26.7)	9(30)	19(63.3)	17(56.7)	5(16.7)	14(46.7)	12(40)	11(36.7)	13(43.3)	8(26.7)
	I	8(26.7)	2(6.7)	4(13.3)	3(10)	1(3.3)	4(13.3)	1(3.3)	2(6.7)	2(6.7)	4(13.3)
	R	14(46.7)	19(63.3)	7(23.3)	10(30)	24(80.7)	12(40)	17(56.7)	17(56.7)	15(50)	18(60)

Key: S- Sensitive (mm), I-Intermediate (mm), R-Resistant (mm), TRX-Ceftriaxone (30 µg), CN-Gentamicin (10 µg), CEF-Cefuroxime (30 µg), CPX-Ciprofloxacin (10 µg), CTZ-Ceflaxidime (20 µg), OFX-Ofloxacin (10 µg), AU-Augumentin (30 µg), PEF-Pefloxacin (10µg), S-Streptomycin (30 µg), CIP-Ceporex (10 µg).

Table 4b:

Antimicrobial susceptibility pattern of Gram-positive bacteria isolated.

Isolates Pattern		Antibiotics (%)									
		AMX	CIPRO	ERY	CN	CEF	LEV	AZM	S	CTZ	RD
<i>S. aureus</i> (24)	S	3(12.5)			18(75)						
	I	1(4.2)	13(54.2)	9(37.5)	2(8.3)	9(37.5)	24(100)	5(20.8)	6(25)	10(41.7)	8(33.3)
	R	20(83.3)	7(29.2)	14(58.3)	4(16.7)	14(58.3)	0(0)	18(75)	17(70.8)	8(33.3)	15(62.5)

Key: S- Sensitive (mm), I-Intermediate (mm), R-Resistant (mm), AMX-Amoxicilline (20 µg), CIPRO-Ciprofloxacin (10 µg), ERY-Erythromycin (30 µg), CN-Gentamicin (10 µg), CEF-Cefuroxime (30 µg), LEV-Levofloxacin (20 µg), AZM-Azithromycin (10 µg), S-Streptomycin (30 µg), CTZ-Ceflaxidime (20 µg), RD-Rifampic (20 µg).

DISCUSSION

Urinary tract infection is among the most frequent bacterial infections in pregnancy, accounting for about 25% of all infections (Johnson *et al.*, 2021). Previous reports have it that untreated UTI during pregnancy cause several complications and adverse effects to both mother and fetus (Johnson *et al.*, 2021; Vicar *et al.*, 2023; Taye *et al.*, 2018)

Out of the 120 urine samples analyzed, it was observed that the prevalence of urinary tract infection among pregnant women attending antenatal clinic in Iyi-Enu Mission Hospital Ogidi was 24.2%. This is in line with the global prevalence rate of 13%-33% also observed by Tibyangye *et al.* (2015), 22.33% and Odongo *et al.* (2013), 24.2%. Lower prevalence rates have been reported by Agersew *et al.* (2012), 10.4%, Onyango *et al.* (2018),

(15.8%) in Kenya and Ali and Abdallah (2019), (15.8%) in Kano, Northern Nigeria. However, prevalence rate as low as 4% has been reported by Sekikubo *et al.* (2017) in Uganda and as high as 35% by Johnson *et al.* (2021) in Uganda, 37.8% by Thakur and Nepal (2020) in Nepal, 49.3% by Younis *et al.* (2019) in Libya and 53.5% by El-Kashif (2019) in Saudi Arabia. The variations in the results reported from different geographic areas may be attributed to different factors that may include geographic location, mode of screening, personal and environmental hygiene practices, differences in UTI perception, sexual behaviors and risk factors like age, marital status, parity and gestation period (Vicar *et al.*, 2023; El-Kashif, 2019).

In this research, Gram negative organisms accounted for 30 (55.6%) of the isolates with *E. coli* 13 (24.1%) as the most prevalent while *Pseudomonas spp.* 3 (5.6%) was the least. The high prevalence of Gram-negative organisms among the pregnant women was also reported by Ali *et al.* (2022) and Johnson *et al.* (2021) who recorded 71%- and 76.45%-Gram negative bacteria respectively. Onyango *et al.* (2018) and Vicar *et al.* 2023 also reported *E. coli* as the most common Gram-negative bacteria involved in UTI. The presence of *E. coli* in the vaginal and rectal area may have influence in its prevalence in UTI. *E. coli* has also been reported by as responsible for 55%-72% of cystitis in younger women and over 50% in women above 50 years by Egwari (1997). According to Onyango *et al.* (2018) and Plos *et al.* (1991), about 80% of uropathogenic *E. coli* express P fimbriae, which is a virulent factor that helps it to anchor to the glycolipid of the outer membranes of urothelial cells in the kidney.

Contrary to our observation, Johnson *et al.* (2021) isolated *Klebsiella pneumonia* (37.41%) as the most frequent Gram-negative organism. *Klebsiella spp.* is now known to emerge as a dominant community acquired uropathogen (Kaduma *et al.*, 2019; Taye *et al.*, 2018), although observed as the second most prevalent bacteria 10 (18.5%) isolated in this study. Its emergence has also been linked to the presence of inherent factors like siderophore, types 1 and 3 fimbriae, biofilm formation and capsules (Johnson *et al.*, 2021). Other Gram-negative bacteria isolated were *Proteus mirabilis* 4 (7.4%) and *Pseudomonas spp.* 3 (5.6%). Their involvement in UTI among pregnant women had also been reported (Ali *et al.*, 2022; Johnson *et al.*, 2021).

Staphylococcus aureus was the only Gram-positive bacteria 24 (44.4%) isolated. This finding is supported by the reports of Johnson *et al.*, (2021) and Muhammed (2015), who also recorded *S. aureus* as the only Gram-positive bacteria isolated in their work. As suggested by Kaambo and Charlene (2017) and Top *et al.* (2012), the high incidence of *S. aureus* may be attributed to the rate of vaginal carriage of *S. aureus*, which has been reported to be 4% - 22% of the vaginal microbiota in pregnant women (Muhammed, 2015)

Various factors like maternal age, parity, gestation period of pregnancy, marital status, multiple sex partners and level of income, have been associated with higher prevalence of urinary tract infection in pregnant women (Baba *et al.*, 2023; Lee *et al.*, 2020). From the demographic and clinical factors of the pregnant women studied, prevalence was highest among the following: 26-35years (35.3%), single mothers (42.9%), those with farming as their occupation (50%), pregnant women with secondary educational level 19 (31.7%). Others include women with multiple sex partners 18 (64.3%), in their third trimester 18 (33.3%) and among the multigravida 22 (28.6%). There was no significant association between age group ($p=0.051$), educational level attained ($p=0.062$), gestation period ($p=0.052$) and parity ($p=0.087$) with UTI among pregnant women. However, significant relationship between marital status ($p=0.024$), occupation ($p=0.048$) and number of sexual partners ($p=0.008$) were observed. The results obtained indicted sexual behaviors of the pregnant women as a predisposing factor for urinary tract infection. It has been reported that treatment of urinary tract infection is usually started empirically (Ali *et al.*, 2022) with antibiotics like Ceftazidime, Norfloxacin, Penicillin and Amoxicillin (Vicar *et al.*, 2023; Johnson *et al.*, 2021). In this work, Cefuroxime (63%) was the most sensitive antibiotics against the Gram-negative bacteria isolated, followed by Ciprofloxacin (56.7%). High resistance was recorded to Gentamicin (63.3%), Ceftazidime

(80.7%), and Ceporex (60%). The Gram-positive isolates were all susceptible to Levofloxacin (100%). This is in line with the results of Ali *et al.* (2022) who recorded highest resistance to Gentamicin (75%) among Gram negative bacteria. Gram positive bacteria were susceptible to Gentamicin (75%), Rifampic (61.5%) and Ciprofloxacin (54.2%). High resistance was recorded by Amoxicillin 20 (83.3%), Azithromycin 18 (75%) and Streptomycin 17 (70.8%). This agrees with Ali *et al.* (2022) who reported 66.7% - 88.9% susceptibility to gentamicin, Ciprofloxacin and Ceftriaxone in Gram positive organisms.

Akpan *et al.* (2019) also recorded 53% - 100% sensitivity to Gentamicin and 57% - 75% to Ciprofloxacin for Gram positive organisms. It is important to note that Ceftazidime and Amoxicillin which were drugs of choice for empirical treatment (Vicar *et al.*, 2023) were highly resisted in this study. These antibiotics are affordable and easily accessible in Nigeria, either as prescription or over the counter drugs and may have contributed to the high resistance of uropathogens to antibiotics amongst pregnant women. In conclusion, Prevalence of UTI among pregnant women in this study showed that *Staph. aureus* was the most prevalent bacteria. Levofloxacin was the antibiotic of choice for Gram positive bacteria while Gram negative bacteria were sensitive to Cefuroxime. There is need for proper diagnosis of UTI before treatment, to avoid resistance of uropathogens to antibiotics.

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