

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *KLEBSIELLA PNEUMONIAE* AND *PSEUDOMONAS AERUGINOSA* AMONG WOMEN WITH URINARY TRACT INFECTIONS ATTENDING ANTENATAL CARE IN KADUNA, NIGERIA

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ABSTRACT

This study determines the prevalence and antimicrobial susceptibility pattern of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* among women with urinary tract infections attending antenatal care in Barau Dikko Teaching Hospital Kaduna, Nigeria. A total of 230 mid-stream urine samples were collected and the organisms were identified using cultural (MacConkey agar and Cetrimide agar respectively) and biochemical methods. Antibiotics susceptibility test was performed using commercial disc. The overall UTIs prevalence was 22.17% (51/230) among which 14.78% (34/230) were *Klebsiella pneumoniae* and 7.39% (17/230) were *Pseudomonas aeruginosa*. *Klebsiella pneumoniae* was most sensitive to Augmentin, Ciprofloxacin (100% susceptible to the isolates respectively), and Amoxicillin (88.24%). It was moderately sensitive to chloramphenicol (67.65%), Trimethoprim/Sulfamethoxazole (44.17%), Gentamycin (38.24%) and Pefloxacin (29.24%), while being least sensitive to Sparfloxacin (26.41%), Streptomycin (26.41%) and Ofloxacin (Tarivid) (11.76%). The highest susceptibility of *Pseudomonas aeruginosa* was observed in Amoxicillin (94.12%) followed by Ciprofloxacin (84.35%) and then Augmentin (76.47%), while being least susceptible to Chloramphenicol (41.18%), Sparfloxacin (35.29%), Pefloxacin (23.53%) and Tarivid (14.23%). On the other hand, isolates of *Pseudomonas aeruginosa* obtained from the urine cultures exhibited intermediate susceptibility to Streptomycin (70.59%), Septrin (64.71%), and Gentamycin (58.82%). In conclusion the uropathogens examined were 100% susceptible to Augmentin and Ciprofloxacin for *Klebsiella pneumoniae* and Amoxicillin was more sensitive to *Pseudomonas aeruginosa*. Health education among women, especially pregnant mothers, on the risk, transmission and prevention of infection with uropathogens should be embarked upon by relevant authorities. Furthermore, health care personnel should be proactive in taking steps to prevent nosocomial transmission of these pathogens during antenatal visits of expectant mothers.

Keywords: Prevalence, Susceptibility, Pregnant, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

INTRODUCTION

Urinary tract infections (UTIs) are caused by the presence and growth of micro-organisms in the urinary tract and are the single common bacterial infection of mankind (Onwuezobe and Orok,

2015). In pregnancy, UTIs may involve the lower urinary tract or bladder. The three clinical manifestations of UTIs in pregnancy are asymptomatic bacteriuria, acute cystitis and pyelonephritis (Oluwafami *et al.*, 2018; Odoki *et al.*, 2019). Antibiotic resistance of urinary tract pathogens has been known to be on the increase worldwide especially to commonly used antimicrobials. Bacteria belonging to the genus *Klebsiella* frequently cause human nosocomial infections in particular, the medically important species, *Klebsiella pneumoniae*, accounts for a significant proportion of hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections (Onwuezobe and Orok, 2015). The principal pathogenic reservoirs for transmission of *Klebsiella* are the gastrointestinal tract and the hands of hospital personnel. These bacteria tend to cause nosocomial outbreaks because of their ability to spread rapidly in the hospital environment. These organisms have been isolated from abscesses, blood, catheter tips, lungs, peritoneal fluid, sputum, and throat cultures (Onwuezobe and Orok, 2015). *Klebsiella pneumoniae* is an important nosocomial pathogen that has the potential to cause severe morbidity and mortality. *Pseudomonas aeruginosa* is ubiquitous pathogen prevalent in hospital environments. It can cause severe nosocomial infections, particularly among immunocompromised patients. People with respiratory, gastro-intestinal, urinary tract, and wound infections as well as burn victims, individuals with cancer, and patients hospitalized in intensive care units are affected by *Pseudomonas aeruginosa* mostly due to nosocomial spread and cross contamination (Aernan and Umeh, 2016). *Pseudomonas aeruginosa* accounts for 10% of all hospital acquired infections, a site specific prevalence which may vary from one unit to another. *Pseudomonas aeruginosa* can develop resistance to antibiotics either through the acquisition of resistance genes on mobile genetic elements (i.e., plasmids) or through mutational processes that alter the expression and/or function of chromosomally encoded mechanisms. Both strategies for developing drug resistance can severely limit the therapeutic options for treatment of serious infections. (Ghaima *et al.*, 2018; Rajivghandhi *et al.*, 2018; Behbani *et al.*, 2019; Dabir *et al.*, 2020). This study determine the prevalence and antimicrobial susceptibility pattern of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* among women with urinary tract infections attending antenatal care in Barau Dikko Teaching Hospital Kaduna, Nigeria.

MATERIALS AND METHODS

Sample site

The sample site of this study was Barau Dikko Teaching Hospital Kaduna, Kaduna State, Nigeria, which lies between longitude 10° 31' 36.1'' N and latitude 7° 26' 31.5'' E. The samples were collected from Antenatal Units in Barau Dikko Teaching Hospital Kaduna. It is a Hospital that received patients from and within the metropolis and across the state.

Determination of Sample size

The sample size of the study was determined using the Cochran's formula (Cochran 1977) to calculate a representative sample for proportion as follows:

$$N = Z^2 P (1-P)/d^2$$

Where, n_0 is the sample size z is the selected critical value of desired confidence level, p is the estimated proportion of an attribute that is present in the population, $q = 1 - p$ and d is the desired level of precision.

The sample size of these study was calculated using the prevalence rate of 15.4% in Kano state (Egbebi and Famurewa 2011; Tijjani *et al.*, 2012).

$$N = Z^2 P (1-P)/d^2$$

Where N = sample size, P = prevalence rate of previous ESBL (15.4%), Z = confidence interval (1.96) and D = allowable error (5%)

$$N = Z^2 P (1-P)/d^2$$

$$N = 1.96^2 \times 0.154 \times (1-0.154) / (0.05)^2$$

$$N = 200$$

Therefore, the sample size of this study was 230.

Sample Collection

A total of 230 samples of urine were collected from antenatal patients of Barau Dikko Teaching Hospital, Kaduna. All the samples were collected in a sterile urine bottles which were packed in a clean 2 litres closed container and were transported to Medical Microbiology Laboratory of Kaduna State University for analysis. Patients sent to the Microbiology laboratory with suspected cases of Urinary Tract Infection were included in the study. While, patients sent to the Microbiology laboratory with suspected cases other than Urinary Tract Infection were excluded in the study. Structured questionnaire was administered to the patients that fulfil the consent form.

Isolation and Identification of Bacteria

The collected samples were plated on MacConkey agar and cetrinide agar using streak plate procedure and incubated at 37°C for 24 h. Thereafter, the cultures were examined for significant bacterial growth, after which the bacteria were identified based on colony morphology, Gram staining reaction and appropriate biochemical tests including Indole test, Methyl Red - Voges-Proskauer test, citrate utilizations test, urease test, Catalase test, motility test, hydrogen Sulphide Production along with carbohydrate fermentation test (Cowans and Still, 2004; Cheesbrough, 2006).

Antibiotic Susceptibility test

Isolates of *Pseudomonas aeruginos* and *Klebsiella pneumonia* were standardized by inoculating the biochemically identified isolates into a nutrient broth, which was then incubated at 37°C until the visible turbidity was equal to or greater than 0.5 McFarland

standard and then subjected to sensitivity analysis against antibiotics on Mueller Hinton agar by modified Kirby-bauer disc diffusion technique using the following antibiotic discs: Ciprofloxacin (5ug), Cefotaxime (30ug), Ampicillin (30ug), Gentamycin (10ug), Erythromycin (E) 10µg, Pefloxacin (PEF) 30µg, Septrin (SXT) 30µg, Augmentin(AU) 30µg, Ofloxacin (OFX) 5µg and Streptomycin(S)30µg (CLSI 2009).

RESULTS

Table 1 shows the occurrence of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolated from urine samples of antenatal patients attending Barau Dikko Teaching Hospital Kaduna. The result revealed *Klebsiella pneumoniae* to have higher occurrence with 34 number of isolates (14.78%) compared to *Pseudomonas aeruginosa* with 17 number of isolates (7.39%) among pregnant women with UTI in Kaduna.

Table 1: Occurrence of Bacterial Isolates from Urine Samples of Antenatal Patients Attending Barau Dikko Teaching Hospital Kaduna

Bacteria	Sample size (n)	Number of Positive isolates (%)
<i>Klebsiella pneumoniae</i>		34 (14.78)
<i>Pseudomonas aeruginosa</i>		17 (7.39)
Total	230	51 (22.17)

Table 2 shows that Augmentin and Ciprofloxacin had the highest activity on *Klebsiella pneumonia* while Tarivid shows the lowest activity. Amoxicillin was most active on *Pseudomonas aeruginosa* and Tarivid with lowest activity.

Table 2: Antibiotic Susceptibility Pattern of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* Isolated from Urine Samples of Antenatal Patients Attending Barau Dikko Teaching Hospital

Antibiotics	<i>Klebsiella pneumonia</i>		<i>Pseudomonas aeruginosa</i>	
	Susceptible Isolates (%)	Resistant Isolates (%)	Susceptible Isolates (%)	Resistant Isolates (%)
S (10)	6(14.65)	16(47.06)	8(47.06)	6(35.29)
Am (30)	30(88.24)	14(41.18)	16(94.12)	7(41.18)
Au (10)	34(100)	16(47.06)	13(76.47)	7(41.18)
Cn (30)	13(38.24)	28(82.35)	8(47.06)	2(14.23)
Pef (30)	10(29.24)	11(64.71)	10(58.82)	7(41.18)
Ofx (30)	4(11.76)	13(38.24)	9(52.94)	7(41.18)
Stx (30)	15(44.17)	14(41.18)	8(47.06)	4(23.53)
Ch (30)	23(67.65)	16(47.06)	12(70.59)	7(41.18)
Sp (10)	6(26.47)	12(35.29)	11(64.71)	8(47.06)
Cpx (30)	34(100)	17(50.0)	14(84.35)	7(41.18)

NB: All intermediates are said to be resistant in this research according to CLSI 2018

KEY: S = Streptomycin, Am = Amoxicillin, Au = Augmentin, Cn = Gentamycin, Pef = Pefloxacin, Ofx = Tarivid, Stx = Septrin, Ch = Chloramphenicol, Sp = Sparfloxacin, Cpx = Ciprofloxacin, % = percentage.

The prevalence of bacteriuria due to both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was highest among married women who were between the ages of 21 and 30 years, in their third trimesters, monoporous, and unemployed, with only primary school education (Table 3).

Table 3: Socio-Demographic Characteristics among Antenatal Patients Attending Barau Dikko Teaching Hospital Kaduna

Characteristics	No. Tested (%) n=230	Percentage positive (%)
AGE		
11-20	23 (10.0)	12.8
21-30	126 (54.8)	40.4
31-40	65 (28.3)	24.6
41-50	16 (7.0)	13.9
>50	0 (0.0)	8.3
MARITAL STATUS		
Married	228 (99.1)	82.9
Divorced	2 (0.9)	17.1
EDUCATIONAL BACKGROUND		
Primary	105 (45.7)	52.6
Secondary	72 (31.3)	28.2
Tertiary	34 (14.8)	10.4
Others	19 (8.3)	8.8
GROUPS		
Employed	55 (23.9)	11.4
Unemployed	38 (16.5)	44.6
Student	40 (17.4)	24.3
Others	97 (42.2)	19.7
GESTATION PERIOD		
First trimester	25 (10.9)	5.8
Second trimester	133 (57.8)	26.9
Third trimester	72 (31.3)	67.3
PARITY		
None	23 (10.0)	21.2
Once	35 (15.2)	37.3
Twice	41 (17.8)	26.1
Trice	68 (29.6)	10.2
>4	63 (27.4)	5.2

KEY: n=total number

DISCUSSION

Urinary tract infections (UTIs), which occur as a result of colonization of urine and the invasion of any structure of the urinary tract by microorganisms such as bacteria, viruses, yeasts and protozoa, have been recognized as a common infection of human (Chijioko *et al.*, 2016; Almedhawi *et al.*, 2017). Among individuals presenting clinical symptoms of UTIs, females have been reported to show increased susceptibility, especially during pregnancy with significant maternal and fetal risks (Almedhawi *et al.*, 2017; Vaishali and Aarti, 2017). The results of the present study indicated an overall UTIs prevalence of 22.17% among pregnant women

attending antenatal care facility in Barau Dikko Teaching Hospital Kaduna. Different authors have reported widely varying bacteriuria among pregnant women. Muhammad and Muhammad (2019) reported an incidence rate of 15.4% UTIs among pregnant women in Kano state, Northern Nigeria. In a 2016 study conducted among pregnant women in Owerri, South-eastern Nigeria, a 25% bacteriuria was recorded by Onuorah *et al.* (2016). According to a study conducted by Oladipo (2013), prevalence of 56.7% UTIs among pregnant women in South-western Nigeria was recorded. In another study, 61.5% prevalence of UTI among pregnant women in Aba, South-eastern Nigeria was recorded by Ezeigbo *et al.*, (2016). Immunological, physiological, anatomical and hormonal factors have been identified as responsible for incidences of urinary tract infections among pregnant women (Samuel *et al.*, 2016). Almedhawi *et al.*, (2017) indicated that shortened urethra, absence of prostatic secretion, contamination of urinary tract with fecal flora increase the risk of UTIs among women, conditions which are further exacerbated in pregnancy due to concomitant hemodilution, urinary stasis, decreased urine concentration and glycosuria, all of which favour bacterial colonization and growth within the urinary tracts. The weakened immune mechanisms associated with pregnancy also make pregnant women more susceptible to infection with bacterial pathogens (Almukhtar, 2018). Urinary tract and vaginal trauma during sexual intercourse have also been put forward as probable causes of increased UTIs among women (Tamalli *et al.*, 2013). The seeming disparity in prevalence of UTIs recorded in this study and those of Oladipo (2013) and Ezeigbo *et al.* (2016) which recorded higher prevalence may be due to the number of bacterial pathogens identified and the culture media used. In this study, only two organisms were of experimental interest, namely *Klebsiella* spp and *Pseudomonas aeruginosa* while in the studies by the aforementioned authors a wider range of bacterial pathogens were isolated and identified. *Klebsiella* spp and *Pseudomonas aeruginosa* have been variously identified in urine cultures of patients presenting with clinical symptoms of urinary tract infections (Oluwafemi *et al.*, 2018; Odoki *et al.*, 2019). In the present study, a prevalence of 14.78% infection with *K. pneumoniae* was recorded among urine samples collected from the pregnant women. This closely agrees with the findings of Almedhawi *et al.* (2017), Chijioko *et al.* (2016), Oladipo (2013) Tamalli *et al.* (2013), and Umar *et al.* (2018) who reported prevalence of 17.3%, 17.2%, 16.8%, 13.3% and 10%, respectively, for *K. pneumoniae* in urine cultures of pregnant women.

In a study by Mohammad and Mohammed (2019), *Pseudomonas aeruginosa* accounted for 14.8% of identified bacterial pathogen in the urine cultures of pregnant women in Kano, Northern Nigeria. Prevalence of 13.0% *P. aeruginosa* in urine samples obtained from pregnant women attending antenatal clinics in Aba, South-eastern Nigeria as recorded by Ezeigbo *et al.* (2016). According to a (2013) study, a prevalence of 2.6% in urine samples collected from pregnant women in Ile-Ife, Southwestern Nigeria was recorded by Oladipo. In another study, Farouk *et al.* (2019) reported 15.8% prevalence for *P. aeruginosa* among pregnant women in Kano, Northern Nigeria. The results from the present study showed that the prevalence of *Pseudomonas aeruginosa* among the pregnant women was 7.39%, a value marginally lower than most of the aforementioned authors. The differences in prevalence of both *Klebsiella* spp and *Pseudomonas aeruginosa* recorded in this and other studies may be due to differences factors including but not limited to socio-economic status, environmental condition, social

habit, personal hygiene and educational level of the women (Almukhtar, 2018; Farouk *et al.*, 2019; Gebremariam *et al.*, 2019). Urinary tract infections predispose both pregnant women and neonates to adverse outcomes. The expectant mother, with UTIs, has increased risks of pyelonephritis, premature labour, preterm rupture of the membranes, pre-eclampsia and anaemia of pregnancy. Neonates may develop fatal sepsis, experience intrauterine growth retardation and low birth weight (Ballen *et al.*, 2014; Valentina and Srirangaraj, 2016).

The results of the antibiotic susceptibility indicated that the isolates of *Klebsiella pneumoniae* obtained from the urine cultures of the pregnant women exhibited varying degrees of susceptibility to the drugs. *Klebsiella pneumoniae* was most sensitive to the effects of Augmentin, Ciprofloxacin (100% susceptibility of the isolates), and Amoxicillin (88.24%). It was moderately sensitive to the effects of chloramphenicol (67.65%), Trimethoprim/Sulfamethoxazole (44.17%), Gentamycin (38.24%) and Pefloxacin (29.24%), while being least sensitive to the effects of Sparfloxacin (26.41%), Streptomycin (14.65%) and Ofloxacin (Tarivid) (11.76%). In a 2018 study, Balkhi *et al.* showed that *Klebsiella pneumoniae* was highly sensitive to the effects of Ciprofloxacin and another Quinolone, Levofloxacin, as well as to the Aminoglycoside Gentamycin, and the β -lactams, Ampicillin and Augmentin, with Seprin showing only moderate activity.

The isolates of *Pseudomonas aeruginosa* identified in the urine cultures of the pregnant women also exhibited varying degrees of susceptibilities to the antibiotics tested. The highest susceptibility of isolates of *Pseudomonas aeruginosa* was observed in Amoxicillin (94.12%) followed by Ciprofloxacin (84.35%) and then Augmentin (76.47%), while being least susceptible to Chloramphenicol (41.18%), Sparfloxacin (35.29%), Pefloxacin (23.53%) and Tarivid (14.23%). On the other hand, isolates of *Pseudomonas aeruginosa* obtained from the urine cultures exhibited intermediate susceptibility to Streptomycin (70.59%), Trimethoprim (64.71%), and Gentamycin (58.82%). These results closely agree with those of Aernan and Umeh, (2016) who showed that *Pseudomonas aeruginosa* was very sensitive to the effects of both Augmentin (92.9%) and Ciprofloxacin (100%). However, their results showed that *Pseudomonas aeruginosa* was also highly sensitive to the effects of other quinolones (Ofloxacin, Pefloxacin and Sparfloxacin) as well as to the Aminoglycosides, Gentamycin and Streptomycin.

Similarly, the susceptibility pattern of the isolates of *Pseudomonas aeruginosa* from the urine cultures exhibited multiple drug resistance, a finding which agrees with that of Aernan and Umeh, (2016) who also reported that isolates of *Pseudomonas aeruginosa* obtained from urine samples of pregnant women were multidrug resistant. The occurrence of multidrug-resistant strains is increasing worldwide and limiting therapeutic options for clinicians (Anil *et al.*, 2016). Akpan *et al.* (2019) noted that MDR is usually high against commonly used antibiotic, citing self-medication, unnecessary use of antibiotics and noncompliance to antibiotic regimen as some of the factors promoting the rise in multidrug resistance. The multidrug resistant phenotypes could be mediated by several mechanisms including multidrug efflux systems, enzyme production, outer membrane protein (porin) loss and target mutations (Aernan and Umeh, 2016).

Furthermore, the susceptibility/resistance pattern of *Klebsiella pneumoniae* to the antibiotics as observed from the present results also revealed the likelihood of ESBL production among *Klebsiella pneumoniae* isolates. This can be seen in its enhanced in the presence of a beta-lactamase inhibitor in Augmentin (Amoxicillin and Clavulanate) compared to when only a β -lactam drug (Amoxicillin) was used.

The highest prevalence of bacteriuria was recorded in pregnant women between ages 21-30 years followed by those between 31-40 years. These agree with the findings of Tamalli *et al.* (2013), Samuel *et al.* (2016); Laily *et al.* (2018); Nwachukwu *et al.* (2018) and Thapa *et al.* (2018); who reported high incidence of bacteriuria and/or urinary tract infections in pregnant women between ages 21 and 30 years. Odoki *et al.* (2019) had remarked that there is a significant association between age and prevalence of UTIs in pregnant women. Reasons that have been alluded to for these high incidences of UTIs among women within these age categories include associated increased sexual activity and multiple parity (Laily *et al.*, 2018; Nwachukwu *et al.*, 2018; and Oluwafumi *et al.*, 2020).

Women in their third trimester had the highest prevalence of bacteriuria due to both *K. pneumoniae* and *P. aeruginosa*. Previous studies have reported varying prevalence of UTIs among women in their second and third trimesters of pregnancy, with the least prevalence recorded in the first trimester. In the study of Tamalli *et al.* (2013) and Akpan *et al.* (2019), peak prevalence of UTIs in pregnant women was recorded among those in their 3rd trimester of pregnancy, while Laily *et al.* (2018) recorded peak prevalence of UTIs during 2nd trimester. The increase risk of UTIs during the 2nd and 3rd trimesters may be due to increased urethral dilation, decreased bladder tone, decreased urine concentration, increased stasis and hormonal changes (Tamalli *et al.*, 2013).

UTIs was most prevalent in primigravid women followed by those with 2 to 3 births with least prevalence recorded among those with four or more births as observed by Nwachukwu *et al.* (2018), Thapa *et al.* (2018) and Kaduma *et al.* (2019). This present result agrees with the findings of the aforementioned authors. While there are no tentative reasons proffered for this correlation between parity and prevalence of UTIs in pregnant women, it may not be unconnected with the lifestyle of women within this category. It is likely that women with their first pregnancy are newly or recently weds, and are more prone to increased frequency of coitus which may predispose them to UTIs due to vaginal trauma Laily *et al.*, (2018). According to Tamalli *et al.* (2013), He noted that UTIs is more prevalent among uneducated than educated pregnant women. However, Akpan *et al.* (2019) in sampling the occurrence of bacteriuria among women visiting some primary health care in South-South Nigeria observed that UTIs is most prevalent among educated group of women, and that within those with some form of education, it is most prevalent in women with secondary school education and least among those with tertiary education. A study conducted by Oladeinde *et al.* (2015), also made similar observation in their study of UTIs among pregnant women in a traditional birth home in Benin City, Nigeria. It is expected that education, or literacy or some sort, should help reduce incidences of ill health and infections among a given population. This suggests a faulty education system that does not cater for sexual education, or poor learning outcome for the people so taught.

Conclusion

Klebsiella pneumoniae and *Pseudomonas aeruginosa* are pathogens which colonize the urinary tracts and/or urine of pregnant women attending Barau Dikko Teaching Hospital, Kaduna with prevalence of 14.78 and 7.39 %, respectively. Isolates of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were highly sensitive to Augmentin, Ciprofloxacin, and Amoxicillin with *Klebsiella pneumoniae* showing higher susceptibility to the antibiotics. Furthermore, both uropathogens exhibited antibiotic susceptibility patterns that were indicative of multidrug resistance with some isolates presenting as extended beta-lactamase producers (*Klebsiella pneumoniae*: 11.76%; *Pseudomonas aeruginosa*: 14.29%). The result of the study shows that the prevalence of bacteriuria due to both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was highest among married women who were between the ages of 21 and 30 years, in their third trimesters, mono-parous, and unemployed, with only primary school education.

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