American Journal of Life Sciences
2014; 2(2): 46-52
Published online March 20, 2014 (http://www.sciencepublishinggroup.com/j/ajls)
doi: 10.11648/j.ajls.20140202.12

Prevalence and antimicrobial susceptibility pattern of Urinary Tract Infection(UTI) among pregnant women in Afikpo, Ebonyi State, Nigeria

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To cite this article:
Stanley Chukwudozie Onuoha, Kayode Fatokun. Prevalence and Antimicrobial Susceptibility Pattern of Urinary Tract Infection(UTI) among Pregnant Women in Afikpo, Ebonyi State, Nigeria. *American Journal of Life Sciences.* Vol. 2, No. 2, 2014, pp. 46-52.
doi: 10.11648/j.ajls.20140202.12

Abstract: Urinary Tract Infections (UTIs) describe the microbial invasion and subsequent multiplication on a part or the entire urinary tract. Urinary Tract Infection (UTI) has become the most common bacterial infections in humans, both at the community and hospital settings. The present study was a hospital-based surveillance conducted in four selected hospitals in Afikpo to assess the prevalence and antimicrobial susceptibility of bacteria from suspected urinary tract infections. A cross-sectional study was conducted on the urine of pregnant women with UTI attending the outpatient clinics between April-August 2013. A total of 200 clean midstream urine samples were collected and a general urine microscopic examination and culture were carried out, information on their age, gestational age, gravidity, parity, level of education and residence were also collected. Susceptibility testing panels of the following antibiotics: Gentamycin, tetracycline amikacin, ampicillin, erythromycin, nalidixic acid, ciprofloxacin, cotrimaxasole and cefaloxine were tested against the isolated organisms using disc diffusion method. The bacteriologic agents of UTI isolated from the patients showed that *Escherichia coli* was the most frequently isolated organism (50.0%) and it is followed by *Staphylococcus aureus* (17.3%), *Proteus mirabilis* (5.5%), *Pseudomonas aeruginosa* (2.7%) and *Enterococcus faecalis* (1.8%). The distribution of UTI among the ages of the antenatal patients showed that age group within the range of 27-32 years recorded the highest incidence of UTI, whereas those of them above 39 years showed the least incidence. Ciprofloxacin, Gentamycin and Erythromycin were the most active antibiotics, while the isolates were highly resistant to cotrimoxasole, cefaloxine and nalidixic acid. Urinary tract infection is a major health problem among pregnant women. Urinalysis with culture and sensitivity test is the major diagnostic measure, while health education with regular antenatal and personal hygiene is recommended as precautionary measures to UTI.

Keywords: Pregnancy, Urinary Tract Infection, Culture, Sensitivity Test, Afikpo

1. Introduction

Urinary Tract Infections (UTIs) describe the microbial invasion and subsequent multiplication on a part or the entire urinary tract. Although, urine is a fluid with a variety of molecules and salts; some of which are waste products, it does not usually have bacteria as a normal component. Therefore, when a bacterium by any means gets into the bladder and subsequently multiplies, it may cause UTI (Patterson and Andriole, 1987).

Urinary tract infection has become the most common bacterial infections in humans, both at the community and hospital settings and has been reported in all age groups in both sexes (Dalela *et al*., 2012). Worldwide, approximately 150 million people are diagonized with UTIs resulting in USD 6 billion health care expenditures (Weichhart *et al*., 2008)

Urinary tract infection has become the most common hospital acquired infection, accounting for as many as 35% of nosocomial infections and is the second most common cause of bacteraemia in hospitalized patients (Kolawole *et al*., 2009). UTI has been reported among 20% of the pregnant women and is the most common cause of admission in obstetrical wards(Bacak *et al*., 2005). Urinary tract infection could be classified into lower urinary tract

infection(cystitis) involving the bladder and urethra and the upper urinary tract infection (pyelonephritis) involving the kidney, pelvis, and ureter.

Most UTIs are caused by Gram-negative bacteria Like *Escherichia coli* , *Klebsiella spp, Proteus mirabilis, Pseudomonas aeruginosa, Acinetobacter spp* and *Serratia* spp and Gram postive bacteria such as *Enteroccocus* spp and *Staphlococcus* spp (Kashef *et al*., 2010; Theodros, 2010; Mulugeta and Bayeh, 2014)

The most common clinical manifestation of UTIs in pregnancy are: asymptomatic bacteriuria, acute cystitis and acute pylonephritis(Loh *et al*., 2007). Also, UTI contributes significantly to maternal and perinatal morbidity (Akerele, 2002). Abortion, small birth size, maternal anemia, hypertension, preterm labour, phlebitis, thrombosis and chronic pyelonephritis are related to UTIs during pregnancy.

Pregnancy increases the risk of UTIs. At around the 6^{th} week pregnancy numerous changes in the physiology of a woman's body occur. Hormonal and physical changes increase urine stasis and the climbing up of urine from the bladder into the ureters. These changes along side with an already short urethra (approximately 3-4cm in females), increases the frequency of urinary tract infection in pregnant women (Johnson and Kim, 2012). Additionally, Pregnant women are considered immune-compromised UTI host because of the physiological changes associated with pregnancy. These changes increase the risk of infections that could be either symptomatic or asymptomatic.

Drug resistance among bacteria causing UTI has increased since the introduction of UTI chemotherapy (Nerurkar *et al.,* 2012; Sood and Gupta, 2012; Bahadin *et al*., 2011; Haider *et al.,* 2010). The aetiological agent and their susceptibilty patterns of UTIs vary in regiona and geographical location (Mulugeta and Bayeh, 2014). Besides, the aetiology and drug resistance change through time (de Francesco *et al.,* 2007). Knowledge of the local bacterial etiology and susceptibility patterns is required to trace any change that might have occurred in time so that updated recommendation for optimal empirical therapy of UTI can be made(Leegaard *et al.,* 2000). In Nigeria, a number of studies have been done on the prevalence and antimicrobial resistance patterns of UTIs (Onuh *et al*., 2006 ; Mbata, 2007, Okonko *et al.,* 2009).

However, no data have been reported from the present study area, hence the study was designed to isolate and identify the bacteria associated with urinary tract infection among antenatal women in Afikpo, to determine the distribution of UTI among the women and to investigate the sensitivity of isolated bacteria to various antibiotics for effective treatment.

2. Materials and Methods

2.1. Study Area

The study was conducted in Afikpo. Afikpo comprises of the following urban communities: Ozizza, Ohaisu, Nkpoghoro, Ugwuegu and Itim. Afikpo city is the headquarters of Afikpo North Local government and is the second most popular and largest city in Ebonyi State. It lies on latitude 5.89°N and longitude 7.94°E and it has a population of 71,866. There are many government and some private health centres in Afikpo. Laboratory services for antenatal screening are available in these facilities.

2.2. Study Design

This is a descriptive cross sectional study done at antenatal screening for pregnant women attending selected Hospitals in Afikpo, Ebonyi State, Nigeria.

2.3. Study Population and Sampling Techniques

A total of 200 antenatal patients from the Four Hospitals were randomly selected for the study. The essence of using random sampling techniques in this evaluation is to ensure that each of the antenatal patients who were willing and available at the time of study, satisfied the inclusion and exclusion criteria to be recruited in the evaluation for UTIs.

2.4. Ethical Considerations

Ethical clearance was sought for and granted by Ethic committee of the Hospitals involved. Permission was also obtained from Administrators of the Hospitals before the commencement of the study. Also, the entire antenatal patients selected for the study agreed to a written consent after thorough explanation of the rationale for the study.

2.5. Sample Collection and Preparation

A total of 200 antenatal patients were sampled from Four Hospitals namely: Mater Misericordiae Hospital, Enohia-Itim General Hospital, His Grace Hospital and Maternity and Afikpo Medical Centre between Aprils to August 2013. Fifty samples were collected at each of the four hospitals. Ten urine samples of non-pregnant women were also collected as control. Freshly voided mid-stream urine was collected into sterile universal bottle. The samples were conveyed to the Microbiology Laboratory of Akanu Ibiam Federal Polytechnic Unwana, were urine samples were subjected to a semi-qualitative test using combi-9 urine test Strip (SGL) to characterize the presence and levels of chemical entities such as Protein, Glucose, Blood, Urobilinogen, Bilirubin, Ketones, Nitrite and pH. Pus cells were examined microscopically. The microorganisms were isolated by pour plate method. The bacteria were identified on the basis of their morphological, biochemical and fermentation tests as mentioned by Cheesbrough (2006). The protozoa were identified according to the microscopic appearance as mentioned by(Cheesbrough, 2006). The susceptibility of the isolates to selected antimicrobial agents was determined by the disc diffusion method and the Kirby-Bauer method (Bauer *et al*., 1966) using antibiotic impregnated paper disc (s).

3. Results

A total of two hundred (200) urine samples were collected from antenatal patients attending four of the hospitals selected for the study, out of which 110 (55%) of the antenatal patients showed significant bacteriuria, while 90 (45%) of the patients were negative. After chemical and microscopic analysis, followed by urine culture using CLED agar as described by Cheesbrough (2006). The results showed that 105 samples (52.5%) tested positive for nitrate; traces of protein were recorded in 40(20%) samples, whereas 25(12.5%) samples tested positive for glucose. About 15 (7.5%) samples tested positive for ketone (Table 1). Microscopic examination of the samples showed a high percentage of pus cells (35.5%), followed by bacteria (28%), yeast (20%) and a low percentage of red blood cells (1%) and *Schistosoma haematobium ova* (1.5%) (Table 2). The distribution of UTI among the ages of the antenatal patients showed that age group within the range of 27-32 years recorded the highest incidence of UTI, whereas those of them above 39 years showed the least incidence as shown in (Table 3). The distributions of UTI among the infected women according to their socio-demographic and obstetric characteristics are as shown in Table 4. The highest proportion (74.5%) of UTI was seen among antenatal patients of age 21-38 years. Housewives constituted 77.3% of the studied population with 63.6% living in rural areas. Also, patients' with 6-12 years level of education made up 45.5% and only 29.1% attend antenatal care regularly, with about 61.8% showing no concern for antenatal services. Going by their obstetric history, 72.7% of the infected women were multigravidia, those who had 1-4 children constituted 47.3%, while the grandmultiparous (≥5children) women made up about 22.7%. Short child spacing (<2 years birth space) was recorded among 36.4% of the antenatal patients evaluated. During microbiological studies a total of six (6) bacteria species were isolated and identified using standard methods.

Table 1. *Urine analysis recorded from the 200 urine samples obtained from the antenatal patients.*

Chemical entity	Number of positive test (%)
Protein	40 (20.0)
Glucose	25 (12.5)
Urobilinogen	5 (2.5)
Bilirubin	10(5.0)
Nitrate	105 (52.5)
Ketone	15 (7.5)
Blood	0
Total	200 (100)

Table 2. *Microscopic examination of urine samples.*

Deposits	Number positive (%)
Yeast cells	40 (20)
Trichomonas vaginalis	19 (9.5)
Schistosoma haematobium	3 (1.5)
Calcium oxalate crystal	9 (4.5)
Red blood cells	2(1)
Pus cells	71 (35.5)
Blood	56 (28)
Bacteria	200 (100)

Table 3. *Incidence of UTI in relation to age among the antenatal patients.*

Age group (years)	Number tested (%)	Number positive (%)
15-20	55 (27.5)	26 (23.6)
21-26	57 (28.5)	30 (27.3)
27-32	58 (29)	42 (38.1)
33-38	22 (11)	10 (9.1)
39-above	8 (4)	2 (1.8)
Total	200	110

Table 4. *Socio-demographic and Obstetric Characteristics among the infected antenatal patients.*

Characteristics	Antenatal patients number infected	Percentage
Age (years)		
≤ 20	26	23.6
21-38	82	74.5
> 38	2	1.8
Employment		
House wives	85	77.3
Employed	25	22.7
Residence		
Rural	70	63.6
Urban	40	36.4
Level of education		
No formal	40	36.4
6-12	50	45.5
>12	20	8.2
Antenatal care visit		
Regular	32	29.1
No need	68	61.8
Not present	10	9.1
Gravidity		
Primigravidia	30	27.3
Multigravidia	80	72.7
Parity		
Nullipara	33	30.0
1-4	52	47.3
≥5	25	22.7
Child spacing		
< 2 years	40	36.4
2 years	70	63.6

Table 5. *Frequency occurrence of bacterial isolates.*

Bacteria	Number isolated	Percentage (%)
Pseudomonas aeruginosa	3	2.7
Klebsiella pneumonia	25	22.7
Proteus mirabilis	6	5.5
Enterococcus faecalis	2	1.8
Escherichia coli	55	50.0
Staphylococcus aureus	19	17.3
Total	110	100.0

Five (5) of the bacteria isolates were Gram-negative and one was Gram-positive. The results shows that the most common uropathogen isolated from urine of infected subject is *Escherichia coli* which constituted 50.0% and followed by *Staphylococcus aureus* (17.3%), *Proteus mirabilis* (5.5%), *Pseudomonas aeruginosa* (2.7%) and *Enterococcus faecalis* (1.8%) shared to a lesser extent, in causing UTIs as shown in Table 6. The distribution of the bacteria species from Gram-negative to Gram-positive organism ranges in the order *Escherichia coli* > *Klebsiella pneumoniae*>*Proteus mirabilis*>*Pseudomonas aeruginosa*>*Enterococcus faecalis* and then *Staphylococcus aureus*.

The bacteria species showed varying susceptibility patterns to nine of the antimicrobial drugs as shown in Table 6, While *E.coli* showed high susceptibility to the aminoglycosides-ciprofloxacin (45.5%) and gentamicin (36.4%), it showed low susceptibility to erythromycin (18.2%) and was resitant to the others. *K. faecalis* also showed high susceptibility to gentamycin and ciprofloxacin at (50.0%) respectively. All the isolates were resistant to Nalidixic acid, cotrimoxasole and cefaloxine.

Table 6. *Distribution of bacteria susceptibility to antibiotics.*

Isolates	Total no of isolates	Antimicrobial drugs								
		GEN	TET	AMK	AMP	ERY	NAL	CIP	COT	CEF
E. coli	55	20(36.4)	0(0.0)	0(0.0)	0(0.0)	10(18.2)	0(0.0)	25(45.5)	0(0.0)	0(0.0)
Proteus mirabilis	6	2(33.3)	1(16.7)	0(0.0)	0(0.0)	1(16.7)	0(0.0)	2(33.3)	0(0.0)	0(0.0)
Staph. aureus	19	8(42.1)	3(15.8)	0(0.0)	0(0.0)	1(5.3)	0(0.0)	7(36.8)	0(0.0)	0(0.0)
K. pneumonia.	25	6(24.0)	2(8.0)	6(24.0)	3(12.0)	0(0.0)	0(0.0)	8(32.0)	0(0.0)	0(0.0)
E. faecalis	2	1(50.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(50.0)	0(0.0)	0(0.0)
P. aeruginosa	3	13(3.3)	0(0.0)	0(0.0)	0(0.0)	1(33.3)	0(0.0)	1(33.3)	0(0.0)	0(0.0)

Key: GEN = gentamycin, TET = tetracycline, AMK = amikacin, AMP = ampicillin, ERY = erythromycin, NAL = nalidixic acid, CIP = ciprofloxacin, COT= cotrimoxasole, CEF = cefaloxine, Numbers in bracket are percentage values

4. Discussion

Urinary tract infections are the widely spread infections seen in hospital settings and the second commonest infections seen in the general population (Valiquette, 2001).

UTI occurs in every age and in every gender. It is however more frequent in women, and pregnancy increases the risk of UTI in women. The high incidence of UTI in pregnant women is as result of some physiological and hormonal changes observed during pregnancy. At 6^{th} week of pregnancy the ureter begins to dilate and it continues until delivery. Increased progesterone and estrogens levels normally leads to decreased ureteral and bladder tone. Increased plasma volume during pregnancy leads to decrease urine concentration and increase bladder volume. The combination of these factors leads to urinary stasis and uretero-vesical refux (Delzell and Lefevre, 2000).

The main findings of this study were: the prevalence of UTI among pregnant women was 55%; *E. coli* was the commonest isolated pathogen; there was resistance towards some of the most common antibiotics in use.

The result of the urinalysis showed that, forty(40) urine samples, representing 20% of the samples contained proteins, twenty (20) which represents 12.5% of the samples had glucose in the urine, while high number of 105 pregnant women had nitrate in their urine. The presence of protein in the urine in could be due to pathological disorders. Proteinuria in some of the samples could be a non-specific biomarker for UTI. Nitrites were always detected in urine samples for which Gram-negative bacteria were isolated. Gram-negative organism like *Escherichia coli, Klebsiella pneumonia and Proteus mirabilis* are able to reduce nitrate present in urine to nitrite, hence could account for the nitrate present. This agrees with Cheesbrough (2006) who had linked bacteria invasion to the presence of protein and nitrite in urine.

Microscopic examination of urine samples showed that 71 urine samples which represent 35.5% of the samples had pus cells, while 40 samples representing 20% of the samples had yeast cells. The high percentage of pus cells in the urine samples is an indication of the presence of urinary tract infection. Also, the presence of high percentage of glucose in this study might be responsible for high number of yeast cells in the urine. Sugar is known to be a good culture medium for the growth of fungi and usually diabetic patients suffer more from fungi infection.

In this study, one hundred and one(110) urine samples gave significant growth representing 55% prevalence. This study is similar to figures reported in previous studies. The report is comparable to 58% incidence rate reported by Onifade *et al.*, 2005, 47.5% by Okonko *et al* (2009) but lower than the prevalence rate of 71.6% reported in a similar study by Mbata (2007) who recorded 77.9% and among prison inmates. Furthermore, the result of the study represents high incidence when compared to earlier reports such as Onuh *et al* (2006) who obtained 32.7%, 3.3% (Moghadas and Irajian, 2009), 3.7% (Mobasheri *et al*., 2002), 28% (Hamdan *et al*., 2011), 22.7% (Mulugeta and Bayeh, 2014). Similar studies conducted all over the world have reported the different variations in UTI incidence from one geographical location to another, the differences could be attributed to the differences in UTI perception, mode of screening and compounding risk factors, such as

age, parity and variation in the environment, socio-economic status of the pregnant women and the standard of personal hygiene and education.

The most common uropathogen isolated from the urine of infected subjects in this study was *Escherichia coli* which constituted 50%. It was followed by *Klebsiella pneumonia* (22.7%), *Staphylococcus aureus* (17.3%), *Proteus mirabilis* (5.5%), *Pseudomonas aeruginosa* (2.7%), *and Enterococcus faecalis* (1.8 %.) *E. coli* and other pathogens in this study were comparable to the rates documented previously (Demile *et al*., 2012, de Francesco *et al*., 2007). Onifade *et al*., (2005) and Aiyegoro *et al*., (2007) also reported in their study high incidence of *E. coli* in UTIs. The high incidence of *Klebsiella species* (22.7%) goes to buttress the fact that the organism is achieving prominence as aetiological agents of UTIs than previously reported (Okonko *et al.*, 2009). *Staphylococcus aureus* was known for years as rare urinary isolate (Arpi and Rennberg, 1984), recently it has been reported to be the most frequent pathogen among pregnant women in Nigeria (Akinloye *et al*., 2006). In this study, with prevalence of 17.3% it was also found to constitute recognizable percentage. The organism is known to causes cystitis in young active females and it has been found to be responsible for majority of UTIs in pregnant women. The other organisms isolated were *P. aeruginosa, E. faecalis and P. mirabilis* accounting for 2.7, 1.8 and 5.5% of the isolates respectively. Both have been reported as agent of UTIs and their presence in the sample population was not unusual.

The present study showed that the prevalence of UTI was high among the reproductive age group than others. The highest pevalence was recorded among age group between 21-38 years. While, the least prevalence was among age group above 39 years. This finding was in agreement with the result of studies done in Nigeria and other countries(Onuh *et al*., 2006; Desai *et al.*, 2012; Adeleke *et al.*, 2013). The reason for high incidence of UTI among this age range is because women in this age group are more sexually active and this may have predisposed them to UTI . The result of this study is however in sharp contrast to a similar study conducted by Okonko *et al* (2009) who confirmed high prevalence among age group between 36-40 years, he contended that the risk of UTIs increases with age.

The distributions of UTI among the infected women according to their socio-demographic and obstetric characteristics is shown in Table 4. The highest proportion (74.5%) of UTI was seen among antenatal patients of age 21-38 years. Housewives constituted 77.3% of the studied population with 63.6% living in rural areas. Also, patients' with 6-12 years level of education made up 45.5% and only 29.1% attend antenatal care regularly, with about 61.8% showing no concern for antenatal services. Going by their obstetric history, 72.7% of the infected women were multigravidia, those who had 1-4 children constituted 47.3%, while the grandmultiparous (≥5children) women made up about 22.7%. Short child spacing (<2 years birth space) was recorded among 36.4% of the antenatal patients evaluated. Based on the data obtained from this study, it was observed that both socio-economic status and obstetric characteristic of the pregnant women were risk factors for UTI among these women. However, to corroborate my findings previous reseachers have shown that maternal age, parity, morbid obesity and socio-economic status are risk factors for UTI among pregnant women (Dimetry *et al.*, 2007; Haider *et al*., 2010; Basu *et al*., 2010).

The result of the antibiotic susceptibility tests showed that all the isolated bacteria were highly susceptible to gentamicin, ciprofloxacin, tetracycline and erythromycin to certain degrees with the exception of *E. coli, E. faecalis and P. aeruginosa* that were resistant to tetracycline, while *K. pneumonia* and *E. faecalis* were resistant to erythromycin.This observation compares favourably with the observations made by (Adedeji and Abdulkadir, 2009) that Gram-negative bacteria have the highest sensitivity to Gentamycin and Ciprofloxacin. Gentamycin is an aminoglycoside antibiotic that works by binding to the subunit of the bacteria ribosome, interrupting protein synthesis thereby preventing bacteria from performing vital functions needed for survival. Gentamicin are used for serious UTIs but it could have serious side effects such as damage to hearing, sense of balance and kidneys.The highest efficacy of gentamicin in the treatment of UTIs has been reported by (Al-sweih *et al*., 2005). The ciprofloxacin, a member of the fluoroquinolones is effective against a wide range of organisms(Cheesbrough, 2006).

All the isolates however were resistant to nalidixic acid, cotrimaxasole, cefalixine and ampicillin, except for *K. pneumonia* which showed low susceptibility towards ampicilin. The high level of resistance to these medications could probably be because they have been in the market for a long time, thus allowing microorganisms time to develop resistance mechanisms towards the antibiotics. Inaddition, this high level of resistance noticed could equally be attributed to easy access of antibiotics across the counter in developing countries like Nigeria and the running of pharmacies by unlicenced personnel.The need for the development and enforcement of antibiotic policies and proper antibiotic stewardship in developing countries cannot be overemphasized

5. Conclusion

The study reveals that UTI is a major health problem among pregnant women within Afikpo area. *Escherichia coli, Klebsiella pneumonia* and *Staphylococcus aureus* were the predominant uropathogen that causes UTI. All the isolates were sensitive to Gentamycin and Ciprofloxacin. There is also association of women's age, gestational age, parity, and other socio-economic status with the rate of urinary tract infection during pregnancy. These call for frequent and consistent evaluation of the prevalence, aetiologic agents and predisposing factors of urinary tract infections during pregnancy in developing countries in

order to reduce the devastation effects of urinary tract infections in pregnancy on both maternal and foetal health.

Acknowledgement

Researcher showed appreciation to ethical review committe of the selected health facilities and the pregnant women who consented to taking part in the study. Funding for this study was from personal contributions from the authors, no external funding.

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