ORIGINAL PAPER

Nig. J. Pharm. Res. 2019, 15 (1) pp 45-51
ISSN 0189-8434, e-ISSN 2635-3555 **Available online at http://www.nigjpharmres.com**

Antibiotic Susceptibility Profile of Uropathogens Isolated from Pregnant Women in Selected Hospitals in Ilorin

H. Y. OLUFADI-AHMED[*1A-F], A. ALIYU[1E], M. S. DAVID[1E], A. T. KOLA-MUSTAPHA[3E-F], A. H. DIYAOLU[1, 2A-D]

[1]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria.
[2]Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development (NIPRD), Idu Industrial area, Abuja, F.C.T., Nigeria.
[3]Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria.

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Urinary tract infection (UTI) is one of the most common infections among pregnant women and if untreated could lead to serious complications.
Objective: This work was carried out to determine the prevalence rate of *Enterobacteriaceae* uropathogens and their antibiotics susceptibility profile among pregnant women attending two ante-natal clinics in Ilorin, Nigeria.
Materials and Methods: A total of 111 pregnant women between the ages of 18-50 attending ante-natal clinic at University of Ilorin Teaching Hospital and Civil Service Hospital, Ilorin participated in the study. Mid-stream urine samples were collected and cultured on Cysteine-Lactose-Electrolyte Deficient agar. Presence of significant bacteriuria (> 10^5 cfu/mL) was determined using the plate count method. Antibiotic susceptibility testing was done using Kirby-Bauer disk diffusion technique.
Results: Of the 111 urine samples collected, 27.9% of them were found positive on culture. Among the isolated organisms, *Klebsiella oxytoca* (25.7%) followed by *Klebsiella pneumonia* (22.9%), were the most prevalent. Isolated bacteria were resistant to at least one antibiotic with the highest resistance seen with amoxicillin (94.3%), streptomycin (77.1%) and nitrofurantoin (54. 3%). Amoxicillin-clavulanic acid (51.4%), ceftriaxone (51.4%), ceftazidime (34.3%), cotrimoxazole (51.4%), imipenem (2.9%), ciprofloxacin (14.3%) and gentamicin (25.7%) have the lowest rate of bacteria resistance. Of the isolates, 82.9% showed Multi-Antibiotic Resistance Index (MARI) ≥ 0.3.
Conclusion: High prevalence of bacteriuria in both symptomatic and asymptomatic pregnant women was observed in the study areas. The alarming rate of multi-antibiotic resistance strain is therefore an indicator of a serious clinical problem in the community.
Keywords: Bacteriuria, pregnancy, urine culture, antibiotic susceptibility

INTRODUCTION

Urinary Tract Infection [UTI] is among the most common bacterial infections of Pregnancy. Urinary Tract Infection is defined as the presence of at least 100,000 organisms per milliliter of urine in an asymptomatic patient, or as more than 100 organisms/mL of urine with accompanying pyuria (>7 white blood cells [WBCs]/mL) in a symptomatic patient. (Johnson *et al*., 2016)
Overall, UTIs are 14 times more frequent in women than in men. The physiological make up of a woman is changed in numerous ways during pregnancy. These changes include hormonal and mechanical changes that increase risks of urinary stasis and vesicoureteral reflux. These changes, increased difficulty with

hygiene due to a protruding belly, and a short urethra (approximately 3-4 cm in females) all increase the incidence and frequency of urinary tract infections (UTIs) in pregnant women. (Johnson, 2016)

In pregnancy, asymptomatic or symptomatic bacteriuria if left untreated, is associated with a significant increase in the risk of premature delivery, pre-eclampsia, hypertension, anemia, post- partum endometriosis and a 50% increase in the risk of low birth weight. (Vasudevan, 2014)

A limited and rather predictable spectrum of microorganisms causes UTI in pregnant otherwise healthy females. The primary urinary tract pathogen accounting for 75% to 90% of uncomplicated UTI isolates is reported to be *Escherichia coli*. Other uropathogens include *Staphylococcus saprophyticus*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Proteus mirabilis* and group B *Streptococci*. (Hooton, 2000)

The impact of antibiotic resistance on the morbidity of urinary tract infections in pregnant women is a growing concern in Nigeria. (Okonkwo *et al*., 2009)

Thus, the present study was conducted to determine the prevalence rate of *Enterobacteriaceae* uropathogens and their multi-drug resistant profile among pregnant women attending the two ante-natal clinics in Ilorin, Nigeria.

METHODOLOGY

Study Area and Population

The study was conducted in two major hospitals (a Federal and State hospital) in Ilorin city, The University of Ilorin Teaching Hospital and Civil Service Hospital, Ilorin after ethical clearance from the Ministry of Health, Ilorin.

The study population comprised of 111 pregnant women of age ranges between 18 and 50 years attending antenatal clinics at the two hospitals between the period of April and July 2017.

The research took the form of a descriptive cross-sectional study of pregnant women who were present at the antenatal clinic. The inclusion criteria included pregnant women (symptomatic and asymptomatic) at the study area and who also gave informed consent to participate in the study. Pregnant women who could not give informed consent and those who had taken antibiotics within the last 2 weeks, at the time of collection were excluded.

Preparation of bacterial samples and microbiological analysis

Culture of the urine was carried out by inoculating a portion of the urine samples which had previously been well mixed into Cysteine-Lactose-Electrolyte Deficient (CLED) agar plate using a micropipette to deliver microliter volumes. Inoculated agar plates were incubated at 37°C overnight and read after 24 hours to assess growth of pathogens. Significant bacteriuria is the presence of at least 10^5 single bacteria colonies per mL of urine sample.

The method employed in the identification and characterization of isolated bacteria included examination of morphological features of the colonies on CLED agar and the performance of oxidase test according to Cheesebrough (2006). The isolated *Enterobacteriaceae* organisms were confirmed using a Microbact 12A identification kit in accordance with the manufacturer's directives as well as Microbact System Software. An Octal coding system has been adopted for the Microbact identification kit using the results obtained. The indices of the positive reactions are circled, the sum of these indices in each group of the reaction then form a 9-digit code that is entered into the computer software (Lapage *et al*., 1973). On the software, the percentage figure displayed against the microorganism name is the percentage share of the probability for that organism as a part of the total probabilities for all choices.

The isolated and identified *Enterobacteriaceae* uropathogens were subjected to standard antibiotic sensitivity testing to several classes of antibiotics documented to be used in the treatment of UTI including: Pencillins [Amoxicillin-clavulanic acid (AMC) 30*µ*g, Amoxicillin (AML) 10µg], Sulfonamides [Co-trimoxazole (SXT) 25µg], Nitrofuran derivatives [Nitrofurantoin (F) 300µg], Cephalosporins [Ceftazidime (CAZ) 30µg, Ceftriaxone (CRO) 30µg], Fluoroquinolones [Ciprofloxacin (CIP) 5µg], Aminoglycosides [Gentamicin (CN) 30µg, Streptomycin (S) 5µg] and Carbapenems [Imipenem (IPM) 10 µg]. The Kirby-Bauer disc diffusion technique was employed and results interpreted according to guidelines of Clinical Laboratory Standards Institute ((CLSI, 2016). Resistance pattern of the isolates was done in accordance with the resistance category classification established by the International Expert for Interim Standard Definitions for Acquired resistance by Magiorakose *et al*, (2012).

RESULTS

A total of 111 urine samples were cultured for uropathogens out of which 31 (27.9%) were observed to have significant bacterial growth (>10^5 cfu/mL) among the pregnant women. Table 1 illustrates that the highest prevalence of UTI was seen in pregnant women between 18-24 years (40%). Also, bacteriuria was more frequent in pregnant women within the first three months of gestation (38.9%) as represented in Table 2.

The uropathogens isolated are presented in Figure 1. A total of 35 isolates belonging to eight (8) genera were isolated from the urine samples collected. The most prevalent organism from this study was *Klebsiella oxytoca* (25.7%), followed by *Klebsiella pneumoniae* (22.9%) while *Escherichia coli* showed one of the least prevalence rate (2.9%)

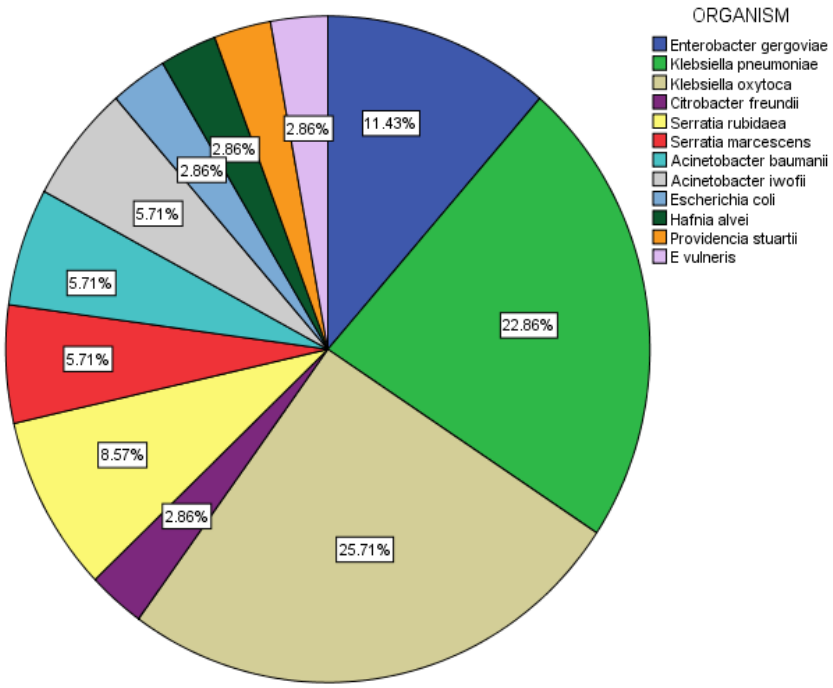

Figure 1. Pie Chart showing uropathogens isolated from pregnant women with urinary tract infection

Antibiotic resistance profile (Figure 2) of the isolates showed that imipenem had the lowest resistance rate (2.9%) with only one (*Serratia marcescens*) organism resistant to its inhibitory effect.

A small percentage of isolates were resistant to Ciprofloxacin (14.3%), Gentamicin (25.7%) and Ceftazidime (34.3%). More of the organisms displayed resistance to Ceftriaxone (51.4%), Amoxicillin/Clavulanic acid (51.4%), Sulphamethoxazole/Trimethprim (51.4%), and Nitrofurantoin (54.3%).

However, a high rate of resistance to Amoxicillin (94.3%) and Streptomycin (77.1%) was seen in the isolates.

The multiple antibiotic resistance index showed that a high percentage of the *Klebsiella pneumonia* was multi-drug resistant. Among the 8 *Klebsiella pneumonia* strains, 87.5% showed MARI $\geq$ 0.3. Overall there was high Multiple Antibiotic Resistance among all the isolates was very high with 82.9% of all the isolates displaying MARI $\geq$ 0.3.

None of the isolates was however resistant to all ten antibiotics while only one organism (*Serratia marcescens)* is resistant to nine antibiotics.

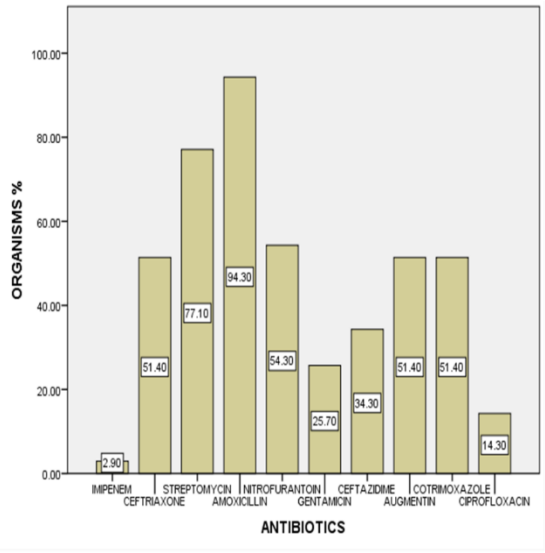

Figure 2. Percentage resistance of organisms to various antibiotics

Table 1: Incidence of Bacteriuria in relation to age distribution of pregnant women

Age group	Frequency	Participants with bacteriuria (%)
<18	1	1 (3.2)
18-24	29	11 (35.5%)
25-30	48	12 (38.7%)
30-39	28	6 (19.4%)
41-50	3	0
NR	2	1 (3.2%)
TOTAL	111	31 (100%)

NR: No response

Table 2: Gestational period distribution of participants with UTI

Gestation period	Frequency	Participants with bacteriuria (%)
0-3 Month	18	7 (38.9%)
4-6 Month	27	9 (33.3%)
6 Months and	58	12 (20.7%)
above	8	3 (37.5%)
NR		
Total	111	31 (27.9%)

Table 3: Multi-Antibiotic Resistance Indices of Isolates

MARI	*Klebsiella oxytoca* (n=9)	*Enterobacter* spp (n=4)	*Serratia* spp (n=5)	Frequency *Escherichia* spp (n=2)	*Acinetobacter* spp (n=4)	*Klebsiella pneumonia* (n=8)	TOTAL (n=35)
0.0	0	0	0	0	0	0	0
0.1	2	0	0	0	0	0	2
0.2	0	1	1	1	0	1	4
0.3	2	1	0	1	0	1	5
0.4	1	0	2	0	2	0	5
0.5	3	0	1	0	2	3	9
0.6	0	0	0	0	0	1	1
0.7	0	1	0	0	0	0	1
0.8	1	0	1	0	0	2	4
0.9	0	1	0	0	0	0	1
1.0	0	0	0	0	0	0	0

DISCUSSION

Age group of participants is displayed on Table 1. A higher percentage of pregnant women (38.7%) with UTI was found within the age bracket of 25-30 years. Meanwhile, the age groups with the least percentage (3.2%) are the participants less than 18 years. Only 19.4% of the participants within the age bracket of 30-39 years displayed bacteriuria. The older pregnant women (41-50 years) did not present with bacteriuria at all. The aforementioned age group having the highest infection is in conformity with previous studies (Turpin *et al*., 2007; Amadi *et al*., 2007). Although a reports states that advanced maternal age (≥35 years) is a risk factor for asymptomatic bacteriuria in pregnancy (Akinloye *et al*., 2006), this study does not conform with that research as older participants had fewer instances of bacteriuria, even more so in pregnant women 41 years and older.

The prevalence rate of asymptomatic bacteriuria in this study was found to be 27.9%, which differs from 40% rate reported in a previous study in Ilorin (Ajayi *et al.*, 2012). However, the value is similar to 24.7% reported in Abakaliki (Onuh *et al*., 2013), 26% in Kaduna (Muhammed, 2014), 23.9% in Zaria (Oko *et al*., 2017) but very high compared to 10.6% in Enugu. (Ojide *et al.*, 2014)

The percentage distribution of organisms isolated is represented on Figure 1. The results of the study in the two hospitals revealed a high prevalence of *Klebsiella* spp including *K. pneumonia* and *K. oxytoca* both of which collectively account for 48.6% of all the isolates. Other organisms isolated from the study include *Serratia* spp (14.3%), *Enterobacter gergoviae* (11.4%) and *Acinetobacter* spp (11.4%).

The higher prevalence of *Klebsiella* spp. in this study contradicts, popular belief that *Escherichia coli* is responsible for 80-90% of urinary tract infections. (Patterson *et al.*, 1987; Barr *et al*., 1985; McDowall *et al*., 1981)

This research is in agreement with a previous study carried out in 2007 at the University of Ilorin Teaching Hospital (UITH) which reported lower incidence of *Escherichia coli* (4%). (Ajayi *et al.*,2012)

Also, a previous study carried out in 2014 revealed that *Klebsiella* spp accounts for 87% of all respiratory tract infections in UITH (Kalgo *et al.*, 2014) this might be a pointer to the relative abundance of pathogenic *Klebsiella* spp in this clime since respiratory tract infections are usually airborne.

According to this study, there is a very high rate of resistance to amoxicillin (94.3%) and also to streptomycin (77.1%), in fact, of all thirteen organisms isolated, only *Serratia* spp was susceptible to the inhibitory effect of amoxicillin.

Enterobacteriaceae resistance to Ciprofloxacin in the study area was greater than 10% unlike 1% reported for fluoroquinolones (Christiaen *et al.* in 1998). This might not be unconnected to the indiscriminate use of fluoroquinolones which has increased in many developing countries leading to the emergence of resistance amongst bacterial isolates. (Pickering, 2004)

Also, self-medication with antibiotics is common in many parts of the world. Antibiotics are illegally sold in many countries without prescription (Morgan *et al*., 2011). This practice is particularly common in several countries in Asia, Africa, South and Central America and even in South European countries such as Italy, Spain, Greece and Malta (Borg and Sciclunca, 2002; Vaananen *et al*., 2006; Carrasco-Garrido *et al*., 2008; Plachouras *et al*., 2010).

The Multi-drug resistance indices of the isolates is represented on Table 3. High percentages of *Klebsiella pneumonia* and *Klebsiella oxytoca*, which were the most prevalent organisms isolated, were discovered to be multi-drug resistant. Among the 8 *Klebsiella pneumonia* strains, 87.5% showed MARI ≥ 0.3 and among the 9 Klebsiella oxtoca, 78% showed MARI ≥ 0.3. Overall, the multiple antibiotic resistance observed among all the isolates was very high with 82.9% of all the isolates displaying MARI ≥ 0.3. None of the isolates was however resistant to all ten antibiotics while only one organism (*Serratia marcescens)* is resistant to nine antibiotics. The Multiple Antibiotic Resistance Index (MARI), which is a measure of exposure of organism to antibiotics shows that most of the isolated organisms exhibited resistance to multiple antibiotics belonging to different classes of antibiotics. This therefore indicates that a large percentage of the uropathogenic isolates have been exposed to a cocktail of antibiotics.

CONCLUSION

In conclusion, age of pregnant woman and gestational age might not be such a big risk factor for urinary tract infection in the study area. The most predominant *Enterobacteriaceae* uropathogens in the study area are *Klebsiella oxytoca* and *Klebsiella pneumonia* and imipenem has the widest coverage against isolated *Enterobacteriaceae* uropathogens and is therefore recommended that imipenem be used for empirical treatment of Urinary tract infections while urine culture and sensitivity tests are being carried out.

The high incidence of bacteriuria in the study as well as the high rate of Multi- Antibiotic Resistance strains among the isolates is a serious cause of concern especially in pregnant women whose treatment options are already limited given their physiological condition.

DECLARATIONS

Ethical approval

Ethical approval for this research was given by University of Ilorin Teaching Hospital Ethics Review Committee (ERC) with approval number ERC PAN/2017/04/1669 as well as Kwara State Ministry of Health with ref no. MOH/KS/EU/777/180. All experiments were carried out according to the ethical standards of the microbiology laboratory operation**.**

REFERENCES

Ajayi, A.B., Nwabuisi, C., Aboyeji, P.A., Ajayi, N.S., Fowotade, A., Fakeye, O.O. (2012). Asymptomatic bacteriuria in Antenatal patients in Ilorin, Nigeria. *Oman Med J*., 27(1), 31–35.

Akinloye O., Ogbolu D.O., Akinloye O.M, Alli D.A. Terry. (2006). Asymptomatic bacteriuria of pregnancy in Ibadan, Nigeria: a reassessment. *Br J Biomed Sci.* 63: 109-112

Amadi E.S., Enemuo O.B, Uneke C.J, Nwosu O.K, Onyeagba R.A, Ugbogu O.C (2007) Asymptomatic bacteriuria among pregnant women in Abakaliki, Ebonyi State, Nigeria. *J Med Sci.* 7(4):698-700

Amin, M., Mehdinejad, M., Pourdangchi, Z. (2009). Study of bacteria isolated from urinary tract infections and determination of their susceptibility to antibiotics. *Jundishapur J Microbiol*., 2(3, 118-123.

Bauer, A.W., Kirby, W.M., Sherris, J.C. (1966). Antibiotic susceptibility testing by a standard single disk method. *Am J Clin Pathol*., 45, 493–506

Borg M., Sciclunca E. (2002) Over-the-counter acquisition of antibiotics in the Maltese general population. *Int J Antimicrob Agents* 20: 253-257

Carrasco-Garrido P. Jimenez-Garcia R., Barrera V., Gil de Miguel A. (2008) Predictive factors of self-medicated drug use among the Spanish adult population. *Pharmacoepidemiol Drug Saf* 17: 193-199

Christiaens, T. H., Heytens, S., Verichraegen, G., DeMeyers, D. J. (1998). Which bacteria are found among Belgian women with uncomplicated urinary tract infections in primary health care and what is their sensitivity pattern? Acta *Clinica Belgica* 53(3), 184-188.

Clinical and Laboratory Standards Institute. (2016). Performance standards for antimicrobial susceptibility testing; Twenty-Sixth Informational Supplement. 950 West Valley Road, Suite 2500 Wayne, PA 19087 USA.

Hooton, T.M. (2000). Pathogenesis of urinary tract infections: an update. *J Antimicrob Chemother*. 46 (Suppl A), 1–7.

Johnson, E.K. (2016). Urinary Tract Infections in Pregnancy Treatment & Management, *Medscape*.Accessed: January 23, 2017. <http://emedicine.medscape.com/article/452604-overview>

Kalgo, M.Z., Nwabuisi, C., Manga, S.S. (2016). Bacteria Pathogens of Lower Respiratory Tract in University of Ilorin Teaching Hospital, Ilorin, Nigeria. *IJISSET*, 2(3). 1-6.

Krieg, N. R., and Holt, J. G., eds. (1984). Bergey's Manual of Systematic Bacteriology 1 (First Ed.). Baltimore: *The Williams & Wilkins Co*. 408–420. ISBN 0- 683- 04108-8.

Krumperman, P. H. (1983). Multiple antibiotic indexing Escherichia coli to identifying risk sources of faecal contamination of foods. *Applied and Environmental Microbiology*, 46: 165-170.

Lapage SP, Bascomb S., Willcox W.P. Curtis M.A (1973). Identification of bacteria by computer – general aspects and perspectives. *J. Gen Microbiol* Aug; 77 (2): 273-90

Magiorakose, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., … Harbarth, S. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbial Infection*, 18, 268–281

Morgan D., Okeke I., Laxminarayan R., Perencevich E., Weisenberg S. (2011) Non-prescription antimicrobial use worldwide: a systemic review. *Lancet infect Dis* 11:692-701

Muhammed, M. (2014) Urinary Tract Infections amongst pregnant women attending a medical Centre in Kaduna, Nigeria. *Afr. J. Cln. Exper. Microbiol*. 16(1): 7-11 http://dx.doi.org/10.4314/ajcem.v16i1.2

Nubia, L.R., Musa, S., Dang Thi, N.D., Corinna, K., Fred, K., Mirembe, F., Braune, A. (2012). Uropathogenic *Escherichia coli* Isolates from Pregnant Women in Different Countries. *J. Clin Microbiol*. 50 (11), 3569–3574.

Ojide, C.K., Wagbatsoma, V.A., Kalu, E.I., Nwadike, V.U. (2014). Asymptomatic bacteriuria among antenatal care women in a tertiary hospital in Benin, Nigeria. *Niger J Exp Clin Biosci*. 2, 79-85

Oko, J.O., Abriba, C., Umar, M., Asitok, A.D., Audu, J.A., Jakheng, S.P.E., Ojeleye, F.S., (2017). Antibiotics Susceptibility Study of Uropathogens Isolated from Asymptomatic Pregnant Women Attending a Tertiary Maternity Clinic in Northern Nigeria. *JOCAMR*, 2(3), 1-9, Article no. JOCAMR.32159

Okonko, I.O., Ijandipe, L.A., Ilusanya, A.O., Donbraye-Emmanuel, O.B., Ejembi, J., Udeze, A.O., Egun, O.C., Fowotade, A., Nkang, A.O. (2009). Incidence of Urinary Tract Infection (UTI) among Pregnant Women in Ibadan, South-Western Nigeria. *African Journal of Biotechnology*. 8(23), 6649–6657

Onuh, S.O., Umeora, O.U.J., Igberase, G. O., Azikem, M.E. and Okpere, E.E. (2006). Microbiological issues and sensitivity pattern of urinary tract infection in pregnancy in Benin City, Nigeria. *Ebonyi Medical Journal*, 5(2), 48-52.

Pickering, L. K. (2004). Antimicrobial resistance among enteric pathogens. *Seminars in Pediatric Infectious Disease*. 15, 71– 77

Plachouras D., Kavatha D., Antoniadou A. Giannitsioti E., Poulakou G., Kanellakopoulou K., (2010) Dispensing of antibiotics without prescription in Greece, 2008: another link in the antibiotic resistance chain. *Eurosurveillance* 15 19488

Turpin C.A, Minkali B. Danso K.A, Frimpong E.H. (2007). Asymptomatic bacteriuria in pregnant women attending antenatal clinic in Komfo Anokye Teaching Hospital, Kumasi, Ghana. *Ghana Med J*. 41(1):26-29

Vaananen M., Pietila K., Airaksinen M. (2006) Self-medication with antibiotics – does it really happen in Europe? Health Policy 77: 166-171

Vasudevan, R. (2014). Urinary Tract Infection: An Overview of the Infection and the associated risk factors *J. Microbiol. Exp*.1 (2), 00008.DOI:10.15406/jmen.2014.01.00008.

*Address for correspondence: Olufadi-Ahmed H. Y.

Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Kwara state, Nigeria.

Telephone: +2348141539861

E-mails: ah.olufadi@unilorin.edu.ng

Conflict of Interest: None declared

Received: 21 September, 2018

Accepted: 1 March, 2019