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ISOLATION, IDENTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF BACTERIA ASSOCIATED WITH URINARY TRACT INFECTIONS (UTIs) IN PREGNANT WOMEN ATTENDING ANTE-NATAL CARE AT REGINA-CAELI HOSPITAL AND MATERNITY AWKA, ANAMBRA STATE, NIGERIA

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ABSTRACT

Urinary tract infection (UTI) is one of the most frequently seen medical conditions in pregnancy with its attendant complications. This study investigated the antibiotic susceptibility pattern of bacteria associated with urinary tract infections among pregnant women attending ante-natal care in Regina-Caeli Hospital and Maternity, and also identified the isolated aetiologic bacteria. A total of 53 clean voided mid-stream urine samples were collected from pregnant women between the ages of 18–49 years and cultured on Cystine Lactose Electrolyte Deficient (CLED) agar using streaking culture techniques. The pure colonies were identified based on colonial morphology, microscopic characteristics, and biochemical tests. The susceptibility pattern of the isolates to common antibiotics was also determined using the Kirby-Bauer disk diffusion techniques. Results showed that among the samples analyzed, 30 bacterial isolates were recovered with Escherichia coli 20 (66.6 %) being the most predominant, followed by Staphylococcus saprophyticus 6 (20.0 %), Klebsiella spp 2 (6.6 %) and Proteus spp 2 (6.6 %). The overall antibiotic susceptibility test revealed that all the Gram negative bacteria isolated were resistant to chloramphenicol, sparflox, ampicillin and tarivid while streptomycin showed a pronounced activity against Proteus sp with an inhibition zone

diameter of 25mm. Escherichia coli was the least susceptible. Staphylococcus aureus was susceptible to ofloxacin and ciprofloxacin but resistant to rocephine, pefloxacin, gentamicin, erythromycin and zinnacef. Routine screening of pregnant women for urinary tract infection during every ante-natal visit should be considered an essential care in the community to avoid complications in early pregnancy.

KEY WORDS: Urinary Tract Infection, antibiotic susceptibility, pregnancy, ante-natal.

INTRODUCTION

Urinary tract infection (UTI) is an infection that occurs along the structures participating in the formation and elimination of urine, particularly the kidneys, ureters, urinary bladder and urethra.[1] UTI is defined as the presence of at least 100,000 organisms per milliliter of urine in an asymptomatic patient,[2] or as more than 100 organisms/ml of urine with accompanying pyuria (> 7 white blood cells [WBCs]/ml) in a symptomatic patient.

UTIs are also described differently depending on the infected part of the tract. When the infection occurs in the bladder, it is called cystitis or a bladder infection, and if one or both of the kidneys are involved, it is known as pyelonephritis. The ureter and urethra may often be infected, and when these occur, they are medically referred to as uretitis and urethritis respectively. UTIs in pregnancy are classified into asymptomatic and symptomatic bacteriuria. While asymptomatic bacteriuria include the true bacteriuria (greater than 100.000 per ml of urine sample) in the absence of specific symptoms of acute UTIs, the symptomatic bacteriuria are grouped into upper tract (pyelonephritis) and lower tract (cystitis) infection.[2] Over the years, UTIs had been regarded as the most common bacterial infection during pregnancy. The hormonal changes during pregnancy, coupled with the fact the growing uterus presses on the bladder, preventing the complete emptying of urine from the bladder are some of the major reasons for more UTIs in pregnant women. Also, during pregnancy about 90% of the women usually develop dilation of the ureter which may persist until delivery thereby leading to increased urinary statsis and ureterovesical reflux as a result of changes in bladder volumes and decreased ureteral tones.[3];[4] These conditions contribute to the development and spread of the infection in pregnant women. If the infection is not well treated, it may lead to kidney infections (pyelonephritis) which are the major causes of complications. This infection occurs most commonly during the second half of pregnancy, but can also occur at any stage in pregnancy.[5]

Women tend to get more infected because their urethera is shorter and closer to the anus than in men. Other factors such as improper cleaning of the perineum, the use of napkins and sanitary towel together with pregnancy and sexual intercourse contribute to the higher incidence of UTIs in various women.[6] They occur most frequently between the ages of 16 and 35 years, with 50- 60% of adult women experiencing a UTI during their lifetime.[7]

The clinical manifestations of UTIs vary but ranges from pyrexia, dysuria (painful urination), lower abdomen cramp, frequent passage of small volume of foul smelling urine.[8] Complications of UTI in pregnancy which include but not limited to pyelonephritis, anaemia, chronic renal failure, pre-mature delivery, low birth weight and foetal death have been documented.[9,10] Pyelonephritis is the second most common medical complications of pregnancy after anaemia.[11]

A limited number of organisms cause UTI and these include Escherichia coli, which accounts for the majority of uncomplicated UTI cases.[12] Others are Staphylococcus saprophyticus, Klebsiella species, Proteus species, Enterococcus species and Enterobacter species.[13] [14]listed group B Streptococcus and Staphylococcus saprophyticus, Enterococci, Gardnerella vaginalis and Ureaplasma ureolyticum as other less implicated aetiological agents of UTIs.

Scientifically, several researches had been carried out on the prevalence of UTIs in pregnant women. Findings from these report suggested that the prevalence of asymptomatic UTI is relatively on the increase especially among pregnant women.[2] Hence, there is the need for unrelenting surveillance and awareness pattern on the predisposing factors vis-à-vis the prompt and efficacious treatment of symptomatic UTIs in pregnant women. Nevertheless, there is still some controversy regarding the screening and treatment of asymptomatic forms during gestation.[15]

Given the prevalence and the potential impacts of UTIs on the health, of the mother and her child, this work is aimed at isolating, identifying bacteria associated with urinary tract infections in pregnant women attending ante-natal care at Regina Caeli Hospital and Maternity in Awka, Anambra State, and testing the antimicrobial susceptibility of the isolates against some commercial antibacterial drugs.

MATERIALS AND METHOD

Study Area and Population

A total of 53 samples were collected from pregnant women attending out-patient ante-natal care at Regina Caeli Hospital and Maternity Awka, Anambra State, Nigeria. The women who were on antibiotic treatment prior to the sampling period were excluded from the study. A questionnaire was designed containing demographic variables like age, trimester, occupation etc.

Ethical Consideration

Informed consent for this study was obtained from each of the subjects after the aim of the study has been explained to them. Also, the anonymity of each of the subjects was treated with confidentiality and for the purpose of this research.

Sample Collection and Handling

Mid-stream urine (MSU) samples were employed to minimize contamination with normal flora of genitourinary tract. A total of fifty-three (53) freshly voided MSU samples (about 10-20ml) were collected into sterile containers after properly cleaning the genitals. The urine samples were then carried to the laboratory immediately and processed within one hour.

Culture of Sample

The freshly collected mid-stream urine was mixed by shaking the container. With the aid of a flamed wire loop 0.002ml of the urine sample was inoculated on CLED agar using the spread plate method. The plates were incubated at 35-37^{0}C for 24hrs.The plates were observed for growth after the incubation period .The presence of colonies indicated positive urine culture and this was defined by their characteristic appearance on the media and confirmed by the detailed panel of biochemical reactions.

Isolation of colonies

Pure colonies were obtained by sub-culturing them in fresh media plates. After purification, the isolates were maintained using nutrient agar slant in Bijou bottles and were kept in the refrigerator after appreciable growth has been noticed to prevent overgrowth.

Identification of the various isolates obtained

The various isolates were identified using the colony descriptions of the isolates, morphological characteristics and biochemical reactions of the isolates.[16] The following

microbiological and biochemical tests were carried out for the characterization and identification of the organisms viz: Gram staining, catalase test, citrate utilization test, indole test, sugar fermentation test, motility test, methyl red test, oxidase test, urease test, coagulase test and growth on eosin methylene blue (EMB) agar.

Standardization of Inoculum

One (1) ml of $H_2SO_4$was added to 99ml of distilled H_20 in a conical flask and mixed well. 0.5g of dehydrated $BaCl_2$ salt was dissolve in 50ml of distilled water. To make the turbidity standard 0.6ml of $Bacl_2$ solution was added to 99.4ml of H_2SO_4 solution to make up to 100ml and mixed well .This gives 0.5 MacFarland turbidity standard which is equivalent to $1x10^6$ cells/ml.[17] The solution was transferred into capped tubes and stored at room temperature. Using a sterile wire loop, a pure colony of the isolate was transferred to a tube containing 5ml of normal saline and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was then adjusted to the density of the 0.5 McFarland turbidity standard.

Antibiotic Susceptibility Testing

The antibiotic susceptibility profile of all isolates was done using the standard disk diffusion method. The commercial antibiotic disks used for the Gram positive bacteria were Amoxil, Ofloxacin, Rocephine, Ciprofloxacin, Gentamycin, Streptomycin, Zinnacef, Erythromycin, Perfloxacin and Septrin while those for the Gram negative bacteria included Amoxil, Augmentin, Rocephine, Chloramphenicol, Sparflox, Ofloxacin, Septrin, Perfloxacin, Streptomycin and Tarivid. A sterile swab stick was dipped into the standardized inoculum suspension and the excess was removed. The surface of already solidified Mueller-Hinton agar was then seeded by gently distributing the inoculum evenly over the entire surface of agar. The inoculated plate was left to dry for 3-5minutes. With the aid of sterile forceps the antibiotic sensitivity disk was placed on the surface of the agar. The plates were incubated at 37^0C for 24 hrs. After the incubation period, the plates were examined for zones of inhibition and the zones of inhibition were measured in millimeters with the aid of a pair of dividers and meter rule.

RESULTS AND DISCUSSION

In this study, the prevalence, identity and antibiotic sensitivity of microorganisms associated with urinary tract infections (UTIs) in pregnant women attending ante-natal care at Regina-Caeli Hospital and Maternity Awka, Anambra State, Nigeria was investigated. Results obtained showed that 30 (56.6%) out of the 53 pregnant women included in the study had

urinary tract infections. Similar prevalence rates have been reported by some other researchers.[18];[19] However, this result does not agree with the prevalence rates of 32.85% and 10.4% respectively reported by [20] and [21] .The relatively high prevalence rate of UTIs in this study could be attributed to low economic status and low level of personal hygiene among the pregnant women. Also, the relative variation in the prevalence rate from one geographical location to another could be attributed to differences in UTI perception, mode of screening, and compounding risk factors such as age, parity, pregnancy and host behavioural factors.[10]

Table 1: Distribution of UTI in the pregnant women according to age groups

Age Group (yrs)	Sample Tested	Positive Sample	Negative sample
18 -25	14 (26.4 %)	7 (23.3 %)	7 (30.4 %)
26-33	20 (37.7 %)	9 (30.0 %)	11 (47.8 %)
34-41	15 (28.3 %)	11 (36.6 %)	4 (17.3 %)
42-49	4 (7.5 %)	3 (10.0 %)	1 (4.3 %)
> 50	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)
Total	53(100%)	30(56.6%)	23(43.4 %)

Relating the findings to the age group mostly affected, it was observed that pregnant women within the age group 34-41 years were most infected, with a prevalence rate of 36.6% though a high percentage of the bacterial isolates were obtained mainly from pregnant women in age group 26 – 33 years. This confirms the usual report that the risk of UTIs increases with age bracket of high sexual activity of women and men.[10]

It was also observed from this study that pregnant women in their third trimester were most infected 16 (72.2%) than those in the first and second trimesters (Table 2). A possible suggestion is the general anatomical and physiological changes that occur at these stages of their pregnancy. Also, observed low bacteria count in the women in their first trimester could be that these microbes are at their infection and incubation stages before manifestation in the subsequent trimesters. A similar observation was reported by[22] and.[10] Thus, pregnant women should be routinely screened for UTI by urine culture at 12 to 16 weeks of gestation.

Table 2: Distribution of UTI in the pregnant women according to trimester

Trimester	Sample Tested	No. Positive (%)
First	2	Nil
Second trimester	29	14 (48.2 %)
Third trimester	22	16 (72.7 %)
Total	53	30 (56.6 %)

Table 3: Distribution of UTI according to Occupation

Occupational group	Sample Tested	Positive sample
Student	20	7 (35.0 %)
Teachers	10	7 (70.0 %)
Civil Servants	7	4(57.1%)
Business women	7	4 (57.1%)
Traders	5	4 (80.0 %)
House wives	4	1 (25.0 %)
Total	53	27 (54.0 %)

On the incidence of UTI by occupational group, the infection was more prevalent among petty-traders (80.00 %) followed by teachers (70.00 %) and civil servants/business women (57.1%). Housewives and students occupied the least with prevalence of 25.0% and 35% respectively (Table 3). The low incidence rates of urinary tract infection reported among students in this work may be attributed to the extensive health care talk given regularly in universities and public awareness programmes among the students. UTIs were common among business women, civil servants, and traders because of their busy schedule and may probably not have time for health talk and public awareness programmes.

The findings on the distribution of UTIs in relation to clinical symptoms are detailed in Table 4. The results show that symptom of burning sensation while passing urine was found in 66.6 % of the infected women, while 35.0 % and 41.6% of them had vagina itching and discharges respectively. Burning with urination has been recorded as one of the most common symptoms of UTIs.[23] The clinical symptoms of painful urination and intercourse had zero prevalence among them. The clinical symptoms are reflections of the common pathogens encountered in this study.

Table 4: Distribution of UTI by clinical symptoms

Clinical symptoms	Sample tested	Positive sample	Negative sample
Burning	15	10(66.6 %)	5(33.4 %)
Itching	20	7(35.0 %)	13(65.0 %)
Painful urination	-	-	-
Painful intercourse	-	-	-
Discharge	12	5 (41.6 %)	7 (58.4 %)

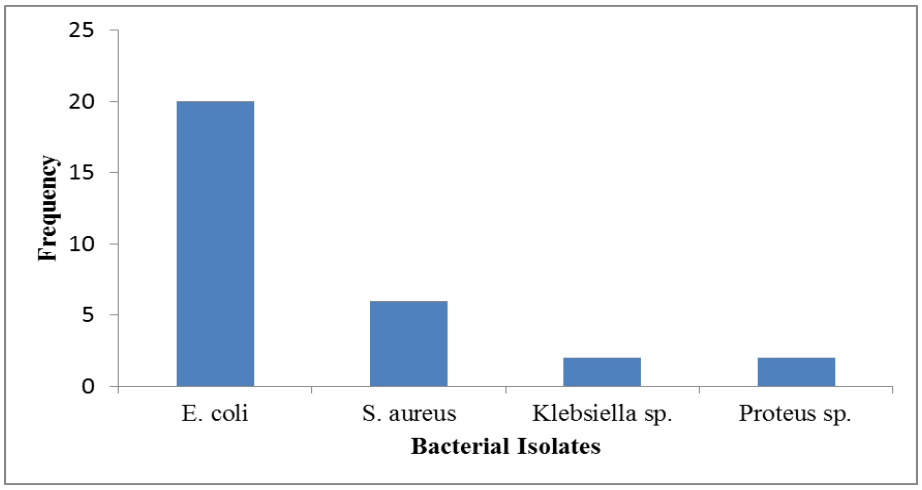

Fig. 1: Frequency of bacteria isolated from the urine samples

Figure 1 shows the frequency of isolation of the bacteria from the urine samples of the pregnant women. The bacteria encountered were Escherichia coli, Klebsiella sp. Proteus sp and Staphylococcus saprophyticus. Of the 30 bacterial isolates obtained, Gram-negative bacteria occur more frequently than Gram-positive bacteria constituting 24 (80.00%) of the total isolates (Fig 1). Similar results have been documented.[24,21] In this sturdy, Escherichia coli (66.6%) was the most common bacteria isolated. This is in agreement with the findings of [25], who found that E. coli represents 80.0% of bacterial isolates in bacteriuria in pregnant women. Similar results have been reported by some other researchers.[21,20,26] The high prevalence of E. coli could be attributed to the fact that the bacterium is a common commensal of the bowel and is more likely to cause infection by fecal contamination due to poor hygiene, the anatomic proximity to the genito-urinary area in females and the urinary stasis during pregnancy. The second most common pathogen in this work is S. saprophyticus which agrees with the work of [26] .This, however, contradicts the results of [24] and [20] who found out that S. aureus was the second most common bacterium isolated. This variation shows that etiologic pattern of urinary tract infection with respect to bacteria pathogen is not similar worldwide.

Result of the susceptibility of the isolates against the antibiotics is presented in table 5. From this study, it can be seen that while all the Gram negative bacteria isolated were resistant to chloramphenicol, sparflox, ampicillin and tarivid, streptomycin showed a pronounced activity against Proteus sp with an inhibition zone diameter of 25mm. Escherichia coli was the least susceptible to the Gram negative antibiotic drugs. Ofloxacin and ciprofloxacin showed the greatest activity against the Gram positive bacterium (Staphylococcus aureus) with inhibition

zone diameters of 25mm and 23mm respectively. This microorganism was resistant to rocephine, pefloxacin, gentamicin, erythromycin and zinnacef.

Table 5: Susceptibility of the Isolates Against the Antibiotics

Isolates	Diameter of zone of inhibition of Antibacterial Drugs (mm)									
Gram Negative	**AMX**	**AUG**	**ROC**	**CHL**	**SPA**	**OFL**	**AMP**	**TAR**	**PEF**	**STR**
E. coli	16	-	16	-	-	17	-	-	-	20
Klesiella sp.	-	-	20	-	-	20	-	-	-	-
Proteus sp.	-	20	-	-	-	-	-	-	20	25
Gram Positive	AMX	ROC	SEP	OFL	PEF	STR	GEN	ERY	CIP	ZIN
S. saprophyticus	20	-	18	25	-	22	-	-	23	-

Key

AMX= Amoxil, AUG= Augmentin, ROC = Rocephine, CHL = Chloramphenicol, SPA = Sparflox, OFL = Ofloxacin, AMP = Ampiclox, TAR = Tarivid, PEF = Pefloxacin, STR = Streptomycin; SEP = Septrin, GEN = Gentamicin, ERY = Erythromycin, CIP = Ciprofloxacin, ZIN = Zinnacef; - = No zone of inhibition.

CONCLUSION

This study has shown that urinary tract infection is still one of the commonest problems during pregnancy as 56.6% of the 53 pregnant women included in the study had urinary tract infections and this was most observed among women within the age group 34-41 years. Thus, urine culture must be done at the first antenatal visit, and repeated cultures should be obtained at different trimesters, because the urine of treated patients may not remain sterile for the entire pregnancy. Burning sensation while passing urine and vagina itching was respectively found in 66.6% and 35.0% of the infected women. It is therefore suggested that routine screening of patients during ante-natal visits be practiced, and if need be antibiotics treatment commenced and guided by antibiotics sensitivity results to forestall the untoward complications that might ensue.

In this study, Escherichia coli was the most common bacterium isolated and the least susceptible to the Gram negative antibiotic drugs. Ofloxacin and ciprofloxacin showed the greatest activity against the Gram positive bacterium, Staphylococcus aureus. It is thus recommended that pregnant women be treated when infection is identified with appropriate antibiotic therapy based on sensitivity test so as to avoid maternal-fetal complications.

REFERENCES

1. Johansen TEB, Botto H, Cek M, Grabe M, Tenke P, Wagenlehner FME, Naber KG. Critical review of current definitions of urinary tract infections and proposal of an EAU/ESIU classification system. Int J Antimicrob Agents, 2011; 64-70.
2. Fasalu ROM, Balasubramanian T, Shejina M, Musambil, M. A. Review on Urinary Tract Infection in Pregnancy. Int J Pharma Res Rev., 2015; 4(2): 26-33.
3. Patterson TF, Andriole VT. Bacteriuria in pregnancy. Infect Dis Clin North Am., 1987; 1:807-8022.
4. Turay AA, Eke SO, Oleghe PO, Ozekhome MC, The prevalence of urinary tract infections among pregnant women attending antenatal clinic at ujoelen primary health care centre, Ekpoma, Edo State, Nigeria. Int J Basic Appl Innov Res., 2014; 3(1): 86 – 94.
5. Schnarr J, Smaill F. Asymptomatic bacteriuria and symptomatic urinary tract infections in pregnancy. Eur J Clin Invest., 2008; 38(2): 50-57.
6. Obiogbolu CH, Okonko IO, Anyamere CO, Adedeji AO, Akanbi AO, Ogun AA, Ejembi J, Faleye TOC. Incidence of urinary tract infections (UTIs) among pregnant women in Awka metropolis, Southeastern Nigeria. Sci Res Essay, 2009; 4: 820-824.
7. Czaja CA, Hooton TM. Update on acute uncomplicated urinary tract infection in women. Postgrad Med., 2006; 119: 39-45.
8. Davidson S, Edwards CRW, Bouchier IAO. Principles and practice of medicine. UpCambridge, 1989; 654-661.
9. Foxman B. Epidemiology of urinary infections: incidence, morbidity, and economic costs. Am J Med., 2002; 113: 5–113.
10. Boyle A, Siakwa PM, Boampong JN, Koffuor GA, Ephraim RKD, Amoateng P, Obodai G, Penu D. Asymptomatic urinary tract infections in pregnant women attending antenatal clinic in Cape Coast, Ghana. E3 J Med Res., 2012; 1(6): 074-083.
11. Amiri M, Lavasani Z, Norouzirad R, Najibpour R, Mohamadpour M, Nikpoor AR, Raeisi M, Hadi Zare Marzouni HZ. Prevalence of urinary tract infection among pregnant women and its complications in their newborns during the birth in the hospitals of Dezful City, Iran, 2012 – 2013. Iran Red Crescent Med J., 2015; 17(8): 1-8.
12. Gupta K, Hooten TM, Stamm WE. Increasing antimicrobial resistance and the management of uncomplicated community acquired urinary tract infections. Annals of Int Med., 2001; 135: 41-50.

13. Masinde A, Gumodoka B, Kilonzo A, Mshana SE. Prevalence of urinary tract infection among pregnant women at Bugando Medical Centre, Mwanza, Tanzania. Tanzania J Health Res., 2009; 11: 154-159.
14. Delzell JE, Lefevre ML. Urinary tract infection during pregnancy. Am Fa. Physician, 2000; 61(3): 713-720.
15. Schmiemann G, Kniehl E, Gebhardt K, Matejczyk MM, Hummers-Pradier E. The Diagnosis of Urinary Tract Infection: A Systematic Review. Deutsches Ärzteblatt Int., 2010; 107(21): 361-367.
16. Obirikwurang C, Quaye L, Bio FY, Amidu N. Asymptomatic bacteriuria among pregnant women attending antenatal clinic at the University Hospital, Kumasi, Ghana. J Med and Biomed Sc., 2012; 1(1): 38-44.
17. Cheesbrough M. District Laboratory Practice in Tropical Countries, Part 2, Cambridge University Press, Canbridge, UK, 2010; 40-197.
18. Onifade AK, Omoya FO, Adegunloye DV. Incidence and control of urinary tract infections among pregnant women attending antennal clinics in government hospitals in Ondo State, Nigeria. J Food Agric Environ., 2005; 3(1): 37-38.
19. Okonko IO, Ijandipe LA, Ilusanya OA, Donbraye-Emmanuel OB, Ejembi J, Udeze AO, Egun OC, Fowotade A, Nkang AO. Incidence of urinary tract infection (UTI) among pregnant women in Ibadan, South-Western Nigeria. Afr J Biotechnol, 2009; 8(23): 6649-6657.
20. Poonam US, and Ulka B. Isolation and identification of bacteria causing urinary tract infections in pregnant women in Vidarbha and their drug susceptibility pattern in them. Int J Curr Microbiol Appl Sc., 2013; 2(4): 97-103.
21. Agersew A, Feleke M, Yittayal S, Ketema T, Afework K, Belay A, Abebe A. Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at university of Gondar Teaching Hospital, Northwest Ethiopia. BMC Research Notes, 2012; 5: 197-103.
22. Vazquez JC, Villar J. Treatment for Symptomatic Urinary Tract Infections during Pregnancy (Cochrane Review). In: Cochrane Library. Issue 3. John Wiley and Sons. Ltd., Chichester, U.K, 2000.
23. Nicolle LE. Uncomplicated urinary tract infection in adults including uncomplicated pyelonephritis. Urol Clin North Am., 2008; 35(1): 1–12.

24. Lamido TZ, Ibrahim AR, Halima SM. Isolation and antibiotic sensitivity of E. coli from pregnant and non-pregnant women attending the University of Maiduguri Teaching Hospital (UMTH), Maiduguri, Nigeria. Int J Biomed and Health Sc., 2010; 6(3): 159-164.
25. Sheiner E, Mazor E and Levey A. Asymtomatic Bacteriuria during Pregnancy. J Maternity Fetal Neonatal Med., 2009; 22: 423-427.
26. Ezeadila JO, Echetabu IE, Ogu GI, Aneke FA. Isolation, identification and antibiotic sensitivity pattern of bacteria from urine samples of female students living in the hostels of Chukwuemeka Odumegwu Ojukwu University, Uli campus, Anambra State, Nigeria. Int J Curr Microbiol and Appl Sc., 2015; 4(12): 255-262.