Original Research Article

Antimicrobial profile and Asymptomatic Urinary Tract Infections among Pregnant Women Attending Antenatal Clinic in Bolgatanga Regional Hospital, Ghana

ABSTRACT

Background: Urinary tract infection (UTI) in pregnancy is associated with significant morbidity for both the mother and the baby. Proper investigation and prompt treatment are needed to prevent the serious life-threatening condition and morbidity associated with UTI in pregnant women. **Aim**: This study was designed to detect common uropathogens and their antibiotic susceptibility pattern among asymptomatic pregnant women attending antenatal care in the Bolgatanga Regional Hospital. **Methodology**: Mid-stream urine samples were collected from 200 individuals and inoculated onto cysteine lactose electrolyte deficient (CLED) agar media. Colony counts yielding bacterial growth of $\geq 10^5$ CFU /ml was regarded as significant bacteriuria. Pure isolates of bacterial pathogens were characterized by colony morphology, Gram-stain and standard biochemical procedures. Kirby Bauer disc diffusion method was used for antimicrobial susceptibility testing of all identified isolates. **Results:** The overall prevalence of bacteria-associated asymptomatic UTI was 17.5%. *Escherichia coli* (42.9%) was the most isolated organism followed by *Staphylococcus aureus* (34.3%), *Klebsiella pneumoniae* (11.4%), *Staphylococcus saprophyticoccus* (5.7%) and *Proteus mirabilis* 2 (5.7%). Yeast cells and *Schistosoma haematobium* were also recorded in 2% of the women. Isolates showed significant sensitivity to commercially prepared antibiotic discs. However, higher level of resistance was recorded with tetracycline, nitrofurantoin and nalidixic acid. **Conclusion**: Early screening for UTI should be done for all pregnant women and those found to be infected need to be treated with appropriate antimicrobial agents to avoid complications.

Keywords: Urinary tract infection, Bacteriuria, Pregnant women, Antimicrobial resistance.

1 INTRODUCTION

Urinary tract infection (UTI) is an infection caused by the presence and growth of microorganisms anywhere in the urinary tract. The proximity of the urethra to the anus more frequently predisposes females to UTI's due to easy contamination of the urinary tract with fecal flora [1]. More so, the presence of short urethra, absence of prostatic secretion, and pregnancies are few factors deemed to foster rapid progression of UTI's in females [2].

Pregnancy is a special condition presented with marked changes in the anatomical and physiological make-up of the urinary tract. During pregnancy, there is a profound alteration in hormonal levels and physiological and anatomical changes in the female urinary tract. Changes during pregnancy are usually marked by glucosuria, gradual dilatation of renal pelvis and ureters in the eighth week, increased levels of estrogen and progesterone, and also the displacement of the bladder superiorly and anteriorly [3, 4]. The hormonal changes cause mechanical alteration in the urinary tract by promoting urinary stasis and vesicourethra reflux thereby increasing the risk of urinary tract infection in pregnant women. The combination of anatomical, hormonal and physiological changes during pregnancy contribute to significant changes in the urinary tract, which has a profound impact on the acquisition of bacteriuria during pregnancy [5].

UTI infections usually present as asymptomatic or symptomatic. Nonetheless, both asymptomatic or symptomatic UTI could potentially cause considerable morbidity or mortality if not detected early and treated. In symptomatic pregnant women, urinary tract infection may manifest as preterm delivery, low birth weight, pre-eclampsia toxaemia, pyelonephritis, anaemia, chronic renal failure and foetal mortality [6]. Diagnosis and treatment of UTI's should ideally be prompt and accurate, especially in this era where there are increasing reports of antimicrobial resistance worldwide. Unfortunately, in many developing countries management of UTIs is

usually empirical, and exhaustive screening in pregnancy is not considered as an essential part of antenatal care. This study was designed to detect common uropathogens and their antibiotic susceptibility pattern in pregnant women.

2 METHODS

2.1 Study population and sample collection

This study included urine samples from 200 pregnant women attending antenatal clinic at the Bolgatanga Regional Hospital in the Upper East region of Ghana. The age of the participants ranged from 15 to 46 years. Information on parity and gestational age were extracted from the antenatal records of pregnant women. Other information was obtained using a well-structured questionnaire. Clean-catch midstream urine specimens were collected. The participants were instructed on how to collect the urine sample by using a leak-proof, wide-mouth sterile plastic containers. The urine specimens were then delivered to the laboratory and processed within one hour. Pregnant women who were on antibiotic treatment two weeks prior to their initial visit, those who exhibited clinical signs and symptoms of UTI and those at 38 weeks of gestation or more were excluded from the study.

2.2 Bacteria culture and identification

Using sterile calibrated wire inoculating loop, approximately 0.01 ml urine samples were inoculated onto cysteine lactose electrolyte deficient (CLED) (Oxoids, England) agar plate. Cultures were incubated under aerobic condition at 37ºC for 24 hrs. Colonies were counted to check the presence of significant bacteriuria. Colony count yielding bacterial growth of $\geq 10^5$ CFU/ml of urine was regarded as significant bacteriuria [7] hence positive for UTI. On the other hand, colony count $<10^5$ CFU/ml was taken as negative. All positive cultures with significant

bacteriuria were then identified by their colony characteristics, gram-staining reaction and standard biochemical protocol [8].

2.3 Urine Chemistry, Microscopic and Macroscopic Examination

A volume of about 6 ml of well-mixed urine sample was centrifuged at 3000 rpm for 10 minutes and the supernatant was subsequently dispensed into a clean sterile tube. The supernatant was tested against combi 10 urine reagent strips (URIT Medical electronics, China). The sediment obtained after centrifugation was mixed and a drop was placed on a microscopic slide for examination using a compound light microscope for the presence of parasites, pus cells, epithelial cells, cast and crystals.

2.4 Antimicrobial susceptibility testing

Kirby Bauer disc diffusion method was used for antimicrobial susceptibility testing of all identified isolates following the Clinical and Laboratory Standards Institute protocol [9] using Muller Hinton agar (Oxoids, England). Commercially available antibiotic discs including Cefoperazone (CPZ), Ciprofloxacin (CIP), Ceftriaxone (CTR), Piperacillin (PIT), Cefotaxime (CTX), Tetracycline (TET), Levofloxacin (LEV), Nitrofurantoin (NIT), Gentamicin (GEN), Norfloxacin (NX), Amikacin (AMK) and Nalidixic Acid (NA) were used for the antimicrobial susceptibility testing. *Escherichia coli* ATCC 25299 was used as a quality control strain. The diameter of the zone of inhibition was measured as the index for isolate susceptibility.

2.5 Ethical Consideration

All experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Additionally, informed written consent was obtained from the study participants and confidentiality was kept.

2.6 Data analysis

Socio-demographic, clinical and laboratory data were entered and analyzed using IBM SPSS version 20. Chi-square analysis was used to compare all categorical data. Fisher's exact analysis was used for cells with an expected count less than 5. An alpha level of 0.05 was used for all statistical tests.

3 RESULTS

A total of 200 pregnant women were included in the study. The age of the study participants ranged from 19 to 45 years. Notably, most of the participants (57.5%) had only primary education. Anaemia was more prevalent 31(15.5%) among the study participants. Details on the baseline characteristics of the study participants are provided in Table 1.

Table 1 Baseline characteristics of study participants in the Bolgatanga Regional Hospital

VARIABLE	NUMBER(n=200)	PERCENTAGE (%)
Parity		
Primigrividae	59	29.5
Pausigravidae	26	13
Multigravidae	115	57.5
Marital Status		
Single	4	2.5
Married	196	97.5
Religion		
Muslim	25	12.5
Christian	175	87.5
UTI In Previous Pregnancy		
Yes	37	18.5
No	163	81.5
Place of Treatment		
Hospital	21	56.8
Others	16	43.2
Awareness of UTI		
Yes	84	42
No	116	58
Knowledge of Mode of Infection		
Sexual Contact	40	20

Uncleanness	10	5
No Idea	150	75
Education		
Primary	115	57.5
Secondary	9	4.5
Tertiary	49	24.5
Vocational Skills	5	2.5
Illiterate	21	10.5
Complications in Previous Pregnancy		
Anaemia	31	15.5
Eclampsia	1	0.5
Hypertension	6	3
Preterm Delivery	2	1
BMI(Kg/M2)		
Lean (<20)	23	11.5
Normal (20-24.9)	107	53.5
Overweight (25-29.9)	43	21.5
Obese (≥30)	27	13.5

3.1 Urine Chemistry, Microscopic and Macroscopic Examination

The appearances of the urine samples examined during this study were as follows; clear 171(85.5), cloudy 7(3.5%), hazy 22(11%) amber 14(7%) and 186(93%) straw in colour. The majority of the participants with urine pH less than 7 were 169(80.5%) and those ≥7 were 59(29.5%). The study showed that 169 (84.5%) of the subjects' urine had specific gravity < 1.025. Further, the urine of 7(3.5%) participants was positive for haematuria. There were 19(9.5%) positive cases of leucocytes esterase and 3 (1.5%) leucocyte esterase trace. In addition, positives for protein were 2 (1%), 9 (4.5%) for trace and 189(94.5%) were negative for proteins. Moreover, glucosuria was found in 5 (2.5%) of the participants, whereas 3 (1.5%) of the participants were positives for ketones**.** Microscopic examination showed 4 (2%) were infected with *Schistosoma haematobium* and varying proportions of epithelial cells, crystals and cast (Table 2). Notably, of the "cast and crystal" identified, the proportion of calcium oxalate was relatively high.

Table 2. Microscopic examination of urine samples recorded

URINE DEPOSITES	COUNT (%)
Pus Cells <10	189(94.5)
Pus Cells≥10	11(5.5)
Epithelial Cells<10	150(75)
Epithelial Cells≥10	50(25)
Crystals & Casts	
Calcium Oxalate	10(5)
Calcium Phosphate	1(0.5)
Hyaline Cast	1(0.5)
Triple Phosphate	1(0.5)
Parasites	
Yeast cells	4(2)
Schistosoma haematobium	4(2)

3.2 Prevalence of bacteria-associated urinary tract infection

The results showed that of the 200 samples cultured, 165 (82.5%) had insignificant bacterial growth, while 35 (17.5%) showed significant bacterial growth. Of the total recovered isolates were 15 (42.9%) *Escherichia coli*, 4 (11.4%) *Klebsiella pneumoniae*, 12 (34.3%) *Staphylococcus aureus*, 2(5.7%) *Staphylococcus saprophyticus* and 2 (5.7%) *Proteus mirabilis*. This study reports an overall prevalence of bacterial urinary tract infection of 17.5% (35/200) among pregnant women attending a clinic in Bolgatanga regional hospital.

3.3 The distribution of uropathogens among pregnant women

There was evidence of an association between *E. coli* and *S. aureus* infection and parity ($p<0.05$). Overall, there was evidence of an association between uropathogen infection and parity among

pregnant women. Table 3 depicts the distribution of uropathogens among pregnant women stratified into age, parity, and gestational age.

Table 3. The distribution of uropathogens among pregnant women stratified into age group, parity and gestational age in the Bolgatanga Regional Hospital

ISOLATE	Age			P-value	Parity			P-value	Gestational age			P-value
	(≤19) N=19	(20-30) N=122	(>30) N=59		Primigravidae N=59	Paucigravidae N=26	Multigravidae N=115		1ST Trimester N=35	2ND Trimester N=0	3RD Trimester N=0	
E. Coli	3(15.8)	9(7.4)	3(10.0)	0.304	5(8.5)	7(26.9)	3(2.6)	**<0.05**	15(42.9)	0(0.0)	0(0.0)	-
Klebsiella pneumoniae	0(0.0)	4(3.3)	0(0.0)	0.535	2(3.4)	1(3.8)	1(0.9)	0.240	4(11.4)	0(0.0)	0(0.0)	-
Proteus mirabilis	0(0.0)	2(1.6)	0(0.0)	1.000	0(0.0)	1(3.8)	1(0.9)	0.130	2(5.7)	0(0.0)	0(0.0)	-
S. aureus	2(10.5)	5(4.1)	5(8.5)	0.259	3(5.1)	9(34.6)	0(0.0)	**<0.05**	12(34.3)	0(0.0)	0(0.0)	-
S. saproprophyticus	0(0.0)	2(1.6)	0(0.0)	1.000	0(0.0)	1(3.8)	1(0.9)	0.330	2(5.7)	0(0.0)	0(0.0)	-
TOTAL INFECTION	5(26.3)	22(18.0)	8(13.6)	0.435	10(16.9)	19(73.1)	6(5.2)	**<0.05**	35(100)	0(0.0)	0(0.0)	-

Data is presented in count (percentage)

3.4 Antimicrobial susceptibility testing

Evaluation of the susceptibility pattern of the organisms to the antibiotics showed Amikacin was the most effective, recording 94.3% susceptibility. Ceftriaxone and Ciprofloxacin were also active against the organisms, recording 91.4% and 85.7% susceptibility respectively. On the other hand, Tetracycline and Nalidixic Acid were the least effective antimicrobial agents recording 20% susceptibility each. Details of the antimicrobial susceptibility pattern are provided in Figure 1.

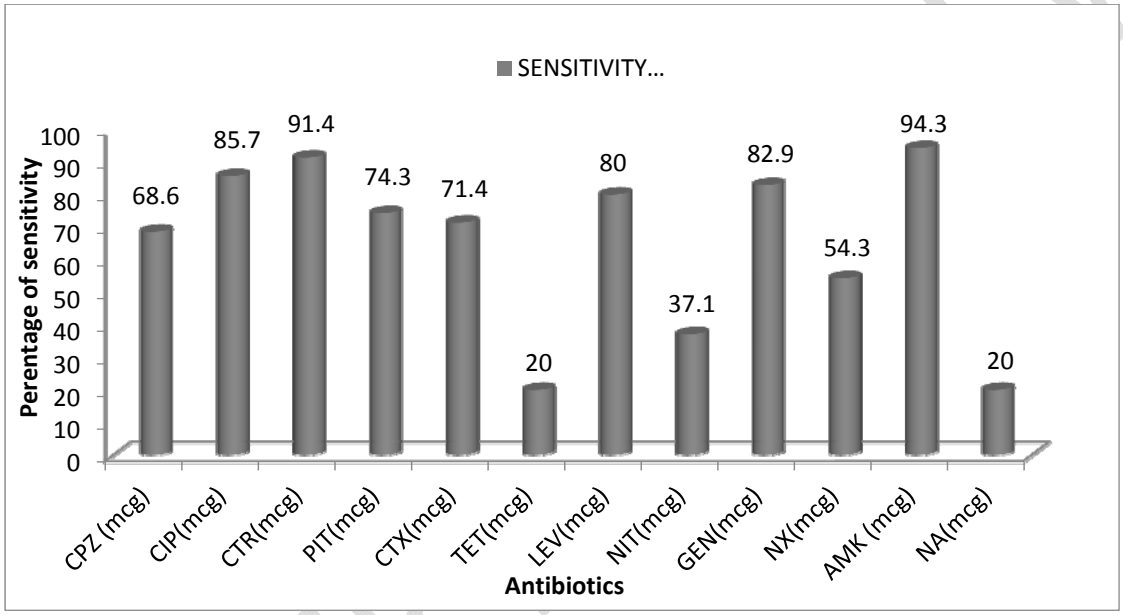

Figure 1. Susceptibility of the uropathogens to the antimicrobial agents used.
Cefoperazone (CPZ), Ciprofloxacin (CIP), Ceftriaxone (CTR), Piperacillin (PIT), Cefotaxime (CTX), Tetracycline (TET), Levofloxacin (LEV), Nitrofurantoin (NIT), Gentamicin (GEN), Norfloxacin (NX), Amikacin (AMK) and Nalidixic Acid (NA)

4 DISCUSSION

It is of great importance to detect and effectively treat urinary tract infections earlier especially, among the risk group of pregnant women. The signs and symptoms of UTI's in asymptomatic patients are not always obvious, hence the need for routine screening to aid in early detection. The

study sought to investigate the incidence of asymptomatic uropathogens infections among pregnant women aged between 19-35 years using microscopic and microbiological methods.

The results of this present study showed that 17.7% of pregnant women attending a clinic at the Bolgatanga Regional hospital were infected with bacteria-associated asymptomatic UTI. The prevalence of asymptomatic UTI recorded by this present study discords with earlier findings obtained previously in Ghana. This is significantly higher than the findings obtained previously in Ghana [10, 11], [12], and Ethiopia [13] but comparable to that conducted in Northern Tanzania [14]. The discrepancies in prevalence could reflect the difference in sociocultural practice, the economic status between the study areas, sexual contact, personal hygiene [15], the year difference and the variations in diagnostic protocols employed.

Findings from this study indicate that *E. coli* and *Staphylococcus aureus* were the most prevalent bacteria implicated in urinary tract infection among the pregnant women and this harmonizes with these earlier studies in Ghana [16] and other African countries [17, 18]. In this study, the second dominated isolate was coagulase-negative *Staphylococcus* 12(34.3%), which is slightly higher than other studies [10, 12] but comparable to reports elsewhere [5, 19]. Urogenital infection with *Schistosoma haematobium* is associated with severe morbidity and mortality and it is characterized by granuloma formation in uterus, fallopian tube, and ovaries [20]. This usually occurs when *Schistosoma haematobium* successfully penetrates the urinary tract into the genital region. This can result in infertility. The study reported *Schistosoma haematobium* infections among four participants, highlighting the need to look at the source of drinking water of the catchment areas surrounding Bolgatanga.

Age, parity, and gestation are purported to be a risk factor for the acquisition of UTI among pregnant women [21, 22]. In agreement with this post, Chi-square analysis revealed a significant association ($p<0.05$) between parity and urinary tract infection. However, no evidence of association was found between gestational age and UTI and this is in contrast to the findings by Obeng-Forson et al. [16]. The incidence of bacteria-associated urinary tract infection was the highest among pregnant women age <20 years followed by 20-30 years and >30years. This finding discords with earlier studies were UTI was found to be more prevalent among women age >30 years [4, 16, 23]. Further, all UTI cases were recorded among pregnant women in their 1st trimester and none among pregnant women in their and 2nd and 3rd trimesters. This disagrees with a study in Ghana by Obeng-Forson et al. [16] where UTI was most prevalent among women in their 2nd and 3rd trimesters.

Substantially high sensitivity rates were recorded for the following antibiotics in descending order, AMK 94.3%, CTR 91.4%, CIP 85.7%, GEN 82.9%, LEV 80% and PIT 74.3%. The activeness of AMK against the isolates could be attributed to the fact that AMK is reserved for the treatment of serious infections and are not available over the counter. This may result in low drug-pressure and consequently the development of low-level resistance against AMK. A similar finding was reported against bacteria isolates in a different study carried out in Ghana [24]. On the other hand, low susceptibility rates were observed for NX, NIT, TET and NA. The findings of this study are similar for some antibiotics reported in other studies [5, 10, 25].

5 CONCLUSION

The present study estimated the overall prevalence of urinary tract infection among pregnant women attending a clinic at Bolgatanga regional hospital at 17.5 %. Of the isolated, *Escherichia coli* and *Staphylococcus aureus* were the most prevalent implicated in the UTI infection. The study

also found evidence of the association between parity and UTI infection. Though the study reported low-level schistosomiasis infection, there is the need to assess the water source in these areas. Amikacin seems to be the most effective antibiotic against all the recovered bacteria isolates.

6 CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

[1] Manjula N, Math GC, Patil A, Gaddad SM, Shivannavar CT. Incidence of urinary tract infections and its aetiological agents among pregnant women in Karnataka region. Advances in Microbiology 2013;2013.

[2] Haider G, Zehra N, Munir AA, Haider A. Risk factors of urinary tract infection in pregnancy. J Pak Med Assoc 2010;60:213-6.

[3] Ciliberto CF, Marx GF. Physiological changes associated with pregnancy. Physiology 1998;9:1-3.

[4] Amiri M, Lavasani Z, Norouzirad R, Najibpour R, Mohamadpour M, Nikpoor AR, et al. 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. Iranian Red Crescent medical journal 2015;17:e26946-e.

[5] Ephrem T. Bacterial Profile and Drug Susceptibility Pattern of Urinary Tract Infection in Pregnant Women Attending Antenatal Care at Mekelle Hospital, Mekelle, Northern Ethiopia. AAU, 2015.

[6] Moyo SJ, Aboud S, Kasubi M, Maselle SY. Bacterial isolates and drug susceptibility patterns of urinary tract infection among pregnant women at Muhimbili National Hospital in Tanzania. Tanzania journal of health research 2010;12:233-6.

[7] Demilie T, Beyene G, Melaku S, Tsegaye W. Urinary bacterial profile and antibiotic susceptibility pattern among pregnant women in North West Ethiopia. Ethiopian journal of health sciences 2012;22.

[8] Tille P. Bailey & Scott's diagnostic microbiology-E-Book. Elsevier Health Sciences, 2015.

[9] CSLI. Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standards Institute, Wayne, PA., 2017.

[10] Turpin C, Minkah B, Danso K, Frimpong E. Asymptomatic bacteriuria in pregnant women attending antenatal clinic at komfo anokye teaching hospital, kumasi, ghana. Ghana Med J 2007;41:26-9.

[11] Boye A, Siakwa MA, J. BB, G. KA, Ephraim DKR, P. A, et al. Asymptomatic urinary tract infections in pregnant women attending antenatal clinic in Cape Coast, Ghana. E3 Journal of Medical Research 2012;1:074-83.

[12] Obirikorang C, Quaye L, Bio F, Amidu N, Acheampong I, Addo K. Asymptomatic Bacteriuria among Pregnant Women Attending Antenatal Clinic at the Uni-versity Hospital, Kumasi, Ghana. Journal of Medical and Biomedical Sciences 2012;1:38-44.

[13] Assefa A, Asrat D, Woldeamanuel Y, Abdella A, Melesse T. Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at Tikur Anbessa Specialized Hospital Addis Ababa, Ethiopia. Ethiopian medical journal 2008;46:227-35.

[14] Olsen BE, Hinderaker SG, Lie RT, Gasheka P, Baerheim A, Bergsjø P, et al. The diagnosis of urinary tract infections among pregnant women in rural Tanzania; prevalences and correspondence between different diagnostic methods. Acta obstetricia et gynecologica Scandinavica 2000;79:729-36.

[15] Abdel-Aziz Elzayat M, Barnett-Vanes A, Dabour MFE, Cheng F. Prevalence of undiagnosed asymptomatic bacteriuria and associated risk factors during pregnancy: a cross-sectional study at two tertiary centres in Cairo, Egypt. BMJ Open 2017;7:e013198.

[16] Forson AO, Tsidi WB, Nana-Adjei D, Quarchie MN, Obeng-Nkrumah N. Escherichia coli bacteriuria in pregnant women in Ghana: antibiotic resistance patterns and virulence factors. BMC research notes 2018;11:901-.

[17] Hamdan HZ, Ziad AH, Ali SK, Adam I. Epidemiology of urinary tract infections and antibiotics sensitivity among pregnant women at Khartoum North Hospital. Ann Clin Microbiol Antimicrob 2011;10:2.

[18] Akobi O, Inyinbor H, Akobi E, Emumwen E, Ogedengbe S, Uzoigwe E, et al. Incidence of urinary tract infection among pregnant women attending antenatal clinic at Federal Medical Centre, Bida, Niger-State, North Central Nigeria. Am J Infect Dis Microbiol 2014;2:34-8.

[19] Mobbasheri E, Ghaemi E, Moujloo M, Vakili M. Prevalence of bacteriuria during pregnancy in Gorgan, Iran. Gorgan Medical Journal 2001;9:42-7.

[20] Nour NM. Schistosomiasis: health effects on women. Reviews in Obstetrics and Gynecology 2010;3:28.

[21] Haider G, Zehra N, Munir AA, Haider A. Risk factors of urinary tract infection in pregnancy. JPMA. The Journal of the Pakistan Medical Association 2010;60:213.

[22] Kline KA, Schwartz DJ, Gilbert NM, Lewis AL. Impact of host age and parity on susceptibility to severe urinary tract infection in a murine model. PloS one 2014;9:e97798-e.

[23] Sibi G, Kumari P, Kabungulundabungi N. Antibiotic sensitivity pattern from pregnant women with urinary tract infection in Bangalore, India. Asian Pacific journal of tropical medicine 2014;7s1:S116-20.

[24] Newman MJ, Frimpong E, Donkor ES, Opintan JA, Asamoah-Adu A. Resistance to antimicrobial drugs in Ghana. Infection and drug resistance 2011;4:215-20.

[25] Boye A, Siakwa PM, Boampong JN, Koffuor GA, Ephraim RKD, Amoateng P, et al. Asymptomatic urinary tract infections in pregnant women attending antenatal clinic in Cape Coast, Ghana. E3 J Med Res 2012;1:74-83.