Original Research Article

Prevalence of Asymptomatic Bacteriuria among Pregnant Women Attending Antenatal Care at the Korle-Bu Teaching Hospital Accra, Ghana: A cross-sectional study

ABSTRACT

Background: The significance of asymptomatic bacteriuria (ASB) relates to its potential to cause acute pyelonephritis, preterm labor and preterm rupture of the membranes. Additionally, it has been associated with clinical conditions such as anemia, preterm birth, low birth weight and perinatal mortality**.**

Aim: This study therefore determined the prevalence of ASB among pregnant women attending antenatal clinic.

Materials and Methods: A cross-sectional study using convenient sampling method was used to recruit 200 pregnant women attending antenatal care at the Korle-Bu Teaching Hospital (KBTH) from January – April, 2014. Bacteriological, urine chemistry and routine urine analyses as well as antibiotic susceptibility profile of bacterial isolates from mid-stream urine samples of pregnant women were carried out using appropriate standard methods. Variables were reported in mean, standard deviation, percentages and bar graph. Chi square test was used to establish statistical difference and association between variables where p-value <0.05 was considered statistically significant.

Results: Out of the total participants, 23 had asymptomatic bacteriuria but no prevalence of ASB was found among Muslims. The association between marital status and prevalence of ASB was significant (Pearson chi2 = 4.88, p-value = 0.027). Five bacterial isolates were obtained with *Escherichia coli* (43.6%) being the most prevalent organism. There were more negative reactions than positive reactions with regards to nitrite and leukocyte esterase determination. Both gram positive and negative isolates recorded high susceptibility to Gentamicin and Norfloxacin. However, Ciprofloxacin showed a high rate of resistance to only gram negative isolates whiles Chloramphenicol, Ampicillin and Amoxillin showed high resistance to gram positive isolates.

Conclusions: The overall prevalence of asymptomatic bacteriuria was 11.5% [95%CI: 7.4% - 16.8%] among the referral pregnant women attending KBTH with *Escherichia coli* being the most prevalent organism whiles the most susceptible antibiotics were Gentamicin and Norfloxacin.

Keywords: Urinary tract infections, antibiotic susceptibility, asymptomatic bacteriuria (ASB), bacterial profile.

1. INTRODUCTION

Urinary tract infections (UTI's) are diseases that affect any part of the urinary system being it the kidney, ureters, bladder or the urethra (Kriplain, Bukshee , & Rafan, 1993). Most UTI's affect the lower urinary tract (bladder and urethra) before they complicate to the upper tract level (Mezue, Ofong, Nmezi, & Ugochukwu-Obi, 2006). Urinary Tract Infections are a common problem encountered in pregnancy due to the morphological and physiological changes that take place in the genitourinary tract (Sharma, 1990). It is of two types, symptomatic and asymptomatic (Akinloye, Ogbolu, Akinloye, & Alli, 2006). Asymptomatic bacteriuria refers to a condition in which urine culture reveals a significant growth of pathogens greater than 10^5 bacteria per milliliter in the absence of urinary symptoms (Lavanya & Jogalakshmi, 2008).

Urinary Tract Infections affect all age group, but women particularly pregnant women are more susceptible than men due to their pregnancy condition and anatomically short urethra which is easily contaminated by fecal flora (Gupta, Sahm, Mayfield, & Stamm, 2001). A bacterium during pregnancy has a greater propensity of progressing to pyelonephritis due to the following; reduction of glycogen secretion to the intracellular cavity of the vagina (Patterson & Andriole, 1997), relaxation of muscles of the ureters (Aboderin, Ako-Nai, Zailani, Ajayi, & Adedosu, 2004) which causes backward flow of urine and the cortisol production from progesterone secretion (Maclean, 1997).

The significance of ASB relates to its potential to cause acute pyelonephritis, preterm labour and preterm rupture of the membranes, other clinical condition includes; anemia, preterm birth, low birth weight and perinatal mortality (J. Akerele, Abhulimen, & Okonofua, 2001; Patterson & Andriole, 1997). A study carried out on ASB showed a prevalence between 2-7% (Okpere, 2003) but may be as high as 10% in high risk population (Kacmaz, Cakir, Aksoy, & Biri 2006). Furthermore, prevalence of ASB conditions may be as twice as common in pregnant women with sickle cell trait and three times as common in pregnant women with diabetic mellitus status (Kiningham, 1993). Studies done so far show that the prevalence rate of ASB in developing countries is higher than that in the developed states (Hooton , Winter , Tiu , & Stamm 1995).

A hospital based study carried out in some developed states including Canada showed a prevalence rate of 4-7% as compared to that of developing countries like Nigeria (i.e. Benin City and Sagamu) and Ethiopia. In Ghana, a study conducted in Komfo Anokye Teaching Hospital (KATH) and University of Cape Coast (UCC) hospital showed prevalence rates of 7.3% and 56.5% respectively (P. Akerele, Abhuliren, & Okonofua, 2001.; A. Alemu *et al.*, 2013; A. Boye *et al.*, 2012; Turpin, Minkah, Danso, & Frimpong, 2007). Another report also showed that ASB occurs between 5-9% for both non-pregnant and pregnant women and if left untreated, can progress to symptomatic UTI's including acute cystitis and pyelonephritis; this progress to symptomatic UTI's is likely to be more in pregnant women than non-pregnant women. However, other conditions that increase the incidence include low socio-economic status, grand multi-parity and advance in age. A recommendation was made by the Infectious Diseases Society of America that there is a need to screen and treat all positive cultures during pregnancy. The study therefore determined the prevalence of ASB (Asymptomatic bacteriuria) in pregnant women attending antenatal clinic at the Korle-Bu Teaching Hospital. The research work also went ahead to ascertain the antibiotic susceptibility patterns of these significant bacteriuria.

2. MATERIAL AND METHODS

2.1 Study design / Study site

A cross-sectional study design was carried out to recruit pregnant women from January to April 2014 at the Obstetrics and Gynecology Department of Korle-Bu Teaching Hospital (KBTH). Korle-Bu Teaching Hospital is a premier health care facility in Ghana, it's one of the tertiary hospitals in the country and is located in the Accra metropolis in the Greater Accra region of Ghana.

2.2 Study participants/ Eligibility criteria

A total of 200 pregnant women attending antenatal clinic at KBTH within the study period were recruited using convenient sampling method. Pregnant women within the ages of 15-45years were included in this study, however, participants with signs and symptoms of Urinary tract infections (UTI's), kidney diseases, diabetes, urological surgery, carcinoma and spinal cord injuries were excluded.

2.3 Data Collection and Processing

2.3.1 Urine Sample Collection

An interview was conducted to obtain information on age, marital status, level of education, area of residence, parity, gestational age, gravidity, body mass index, blood pressure, hemoglobin level, religion and many other socio-demographic characteristics.

Study participants after completing the interview were given labelled sterile universal containers by the interviewer and counselled on how to collect midstream urine. Approximately 15mls of urine were collected from each participant. The urine samples were transported immediately on ice to the Central laboratory, Department of Microbiology, KBTH for processing within four hours of collection to ensure that the pathogenic organisms present in the urine were isolated and also to avoid the possible proliferation of the pathogenic organisms.

2.3.2 Urine Sample Processing

The samples were subjected to routine microscopy and culture. A dip stick test strip was first inserted into the urine samples to check for the presence of nitrites and leukocytes. The urine samples were further centrifuged and their sediments were observed under a microscope for the presence of pus cells, red blood cells, casts and epithelial cells. The Urine samples were also cultured on Cysteine Lactose electrolyte deficiency (CLED) agar media using the streak plate method by use of a calibrated inoculation loop of size 0.001CFU/L, the plates were then incubated at 37˚C and read within 18-24 hours for significant bacteriuria. Significant bacteriuria was defined as a single colony count of $\geq 10^5$ organisms. The organisms were isolated and identification of pathogens was done using standard biochemical and sugar fermentation tests. Antibiotic susceptibility tests were also conducted on them by plating on Muller Hinton agar employing the Kirby Bauer disc diffusion method.

2.4 Statistical Analysis

Data collected was entered to Microsoft Excel and further analyzed using SPSS version 22.0. Most variables were reported in frequencies and percentages whiles hemoglobin and bacteria isolates were reported in mean and bar graph respectively. Chi square and fisher's exact test were also used to establish statistical difference and association between variables where *p*-value <0.05 was considered as statistically significant.

3. RESULTS AND DISCUSSION

Table 1 depicts demographical data of 200 pregnant women enrolled in the study. Out of this, majority of participants (30.5%) were found to be in both 26-30 and 31-35 year groups and (90.5%) of participants being Christians. The proportions of married participants were also higher (88.0%). Additionally, (44.0%) and (49.0%) of participants had secondary education and in their second trimester respectively. The mean hemoglobin level was 11.45±0.08 whiles greater portions (42.0) and (41.5) of these pregnant women were overweight and obese correspondingly.

The overall prevalence of ASB was 23 (11.5%). Among the age ranges, 41-45 age group had the highest prevalence (28.6%) of ASB whiles the least (3.4%) was found among 36-40 age group. Prevalence of ASB was also found higher, (12.7%) and (25.0%) among pregnant women who were not married and Christians respectively. However, no prevalence of ASB was found among Muslims. Also, ASB prevalence was highest (18.8%) and (14.3%) among women who had no formal education and in their first trimester duration of pregnancy correspondingly. Pregnant women who were overweight had the highest (14.3%) prevalence of ASB. **(Table 1)**

The association between marital status and prevalence of ASB was significant (Pearson chi2 = 4.88, p-value = 0.027). Also, the relationship between haemoglobin level and prevalence of ASB was significant (p = 0.018). All the other demographic factors were not significant and had no association with prevalence of ASB. **(Table 1)**

Table 1: Prevalence of ASB and demographic characteristics of study population

Characteristic	Non-Bacteriuria N (%)	Bacteriuria N (%)	Total N (%)	X^2	*P*-value
Age (years)					
15-20	11 (78.6)	3 (21.4)	14 (7.0)	6.513	0.259
21-25	25 (89.3)	3 (10.7)	28 (14.0)		
26-30	52 (85.2)	9 (14.8)	61 (30.5)		
31-35	56 (91.8)	5 (8.2)	61 (30.5)		
36-40	28 (96.6)	1 (3.4)	29 (14.5)		
41-45	5 (71.4)	2 (28.6)	7 (3.5)		
Marital Status				4.884	0.027*[a]
Married	159 (90.3)	17 (9.7)	176 (88.0)		
Single	18 (75.0)	6 (25.0)	24 (12.0)		
Education				2.434	0.487
None	13 (81.2)	3 (18.8)	16 (8.0)		
Basic	55 (87.3)	8 (12.7)	63 (31.5)		

Secondary	81 (92.0)	7 (8.0)	88 (44.0)		
Tertiary	28 (84.8)	5 (15.2)	33 (16.5)		
Trimester				1.015	0.602
1st	6 (85.7)	1 (14.3)	7 (3.5)		
2nd	89 (90.8)	9 (9.2)	98 (49.0)		
3rd	82 (86.3)	13 (13.7)	95 (47.5)		
Religion				2.728	0.099
Christian	158 (87.3)	23 (12.7)	181 (90.5)		
Muslim	19 (100.0)	0 (0.0)	19 (9.5)		
BMI				1.997	0.850
Underweight	1 (100.0)	0 (0.0)	1 (0.5)		
Normal	28 (87.5)	4 (12.5)	32 (16.0)		
Overweight	72 (85.7)	12 (14.3)	84 (42.0)		
Obese	76 (91.6)	7 (8.4)	83 (41.5)		
ASB Status	177 (88.5)	23 (11.5)	200 (100.0)	-	-
Hb g/dl	11.45±0.08	10.76±1.35	10.83±1.32		0.018*[a]

N: Number; * Significant at p< 0.05; ASB: Asymptomatic Bacteriuria; all p-values calculated from fisher's exact test except those denoted by a.

Table 2 shows the dip stick examination for urine chemistry which revealed majority of the participants testing negative for both nitrite and leukocyte esterase test. However, 24(12.0%) and 38(19.0%) tested positive for nitrite and leukocytes esterase respectively.

Table 2: Urinary parameters among pregnant women

Urine Chemistry Parameters	N (%)
Leukocytes	
Positive	38 (19.0)
Negative	162 (81.0)
Nitrite	
Positive	24 (12.0)
Negative	176 (88.0)

N: Number

Figure 1 represents frequency of the bacteria isolates, the predominant isolate was *E coli* (43.6%), followed by *CoNS*, *Citrobacter spp, Enterococcus spp* and *Klebsiella spp* as (26.1%), (21.7%), (4.3%) and (4.3%) respectively.

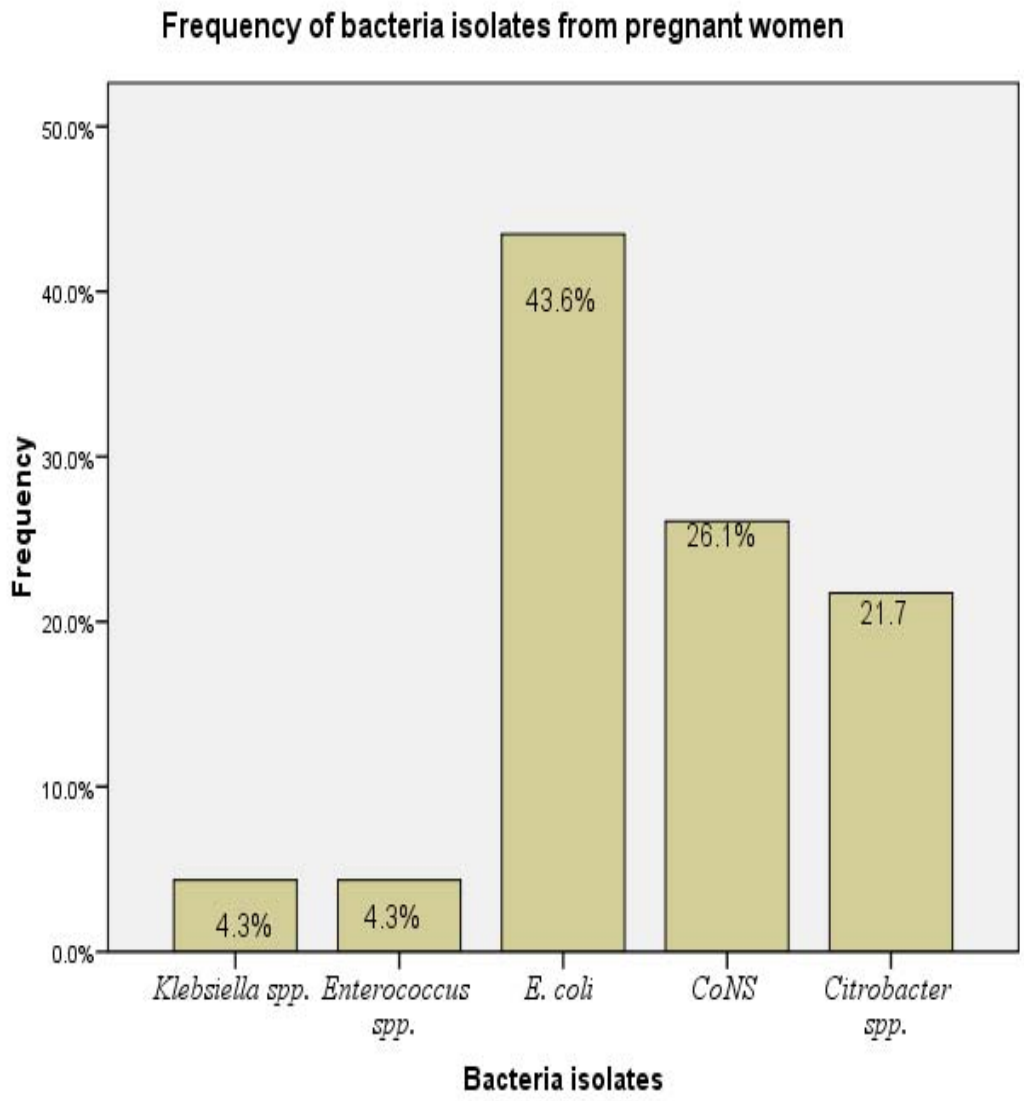

Figure 1: Frequency of bacteriuria isolate among pregnant women

With respect to gram negative isolates, the antimicrobial susceptibility pattern showed Gentamicin and Norfloxacin to be the most effective antibiotics whereas Ciprofloxacin was found to be the most resistant antibiotic. On the other hand, for overall gram positive isolates, Gentamicin and Norfloxacin showed the most sensitivity rates while the most resistant drugs were Chloramphenicol, Ampicillin and Amoxillin. **(Table 3)**

Table 3: Antimicrobial susceptibility pattern of bacteria isolates from urine culture of pregnant women

			ANTIBIOTICS							
ISOLATES	**N**		**GEN**	**NOR**	**AMO**	**OFLO**	**TET**	**CRP**	**AMP**	**COT**
Gram Negative										
CoNS	6	S	6(100.0)	6(100.0)	1(16.7)	5(83.3)	1(16.7)	0(0.00)	2(33.3)	5(83.3)
		R	0(0.00)	0(0.00)	5(83.3)	1(16.7)	5(83.3)	6(100.0)	4(66.7)	1(16.7)
Gram Positive										
Citrobacter	5	S	5(100.0)	5(100.0)	0(0.0)	3(60.0)	2(40.0)	0(0.0)	0(0.0)	4(80.0)
		R	0(0.00)	0(0.00)	5(100.0)	2(40.0)	3(60.0)	5(100.0)	5(100.0)	1(20.0)
E.coli	10	S	10(100.0)	10(100.0)	0(0.0)	3(30.0)	3(30.0)	0(0.0)	0(0.0)	8(80.0)
		R	0(0.0)	0(0.0)	10(0.0)	7(70.0)	7(70.0)	10(100.0)	10(100.0)	2(20.0)
Enterococcus	1	S	1(100.0)	1(100.0)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100.0)
		R	0(0.0)	0(0.00)	1(100.0)	1(100.0)	1(100.0)	1(100.0)	1(100.0)	0(0.00)
Klebsiella spp.	1	S	1(100.0)	1(100.0)	0(0.0)	1(100.0)	0(0.00)	0(0.00)	0(0.00)	1(100.0)
		R	0(0.0)	0(0.0)	1(100.0)	0(0.00)	1(100.0)	1(100.0)	1(100.0)	0(0.00)
TOTAL	17	S	17(100.0)	17(100.0)	0(0.0)	7(41.2)	5(29.4)	0(0.00)	0(0.00)	14(82.4)
		R	0(0.0)	0(0.0)	17(100.0)	10(58.8)	12(70.6)	17(100.0)	17(100.0)	3(17.6)

N-Number: S-Sensitivity: R-Resistance: CoNS- Coagulase Negative Staphylococcus: E. coli- Escherichia coli: GEN-Gentamicin: NOR- Norfloxacin: AMO-Amoxicillin: OFLO-Ofloxacin: CIP-Ciprofloxacin: TET-Tetracycline: CRP- Chloramphenicol: AMK-Amikacin: AMP-Ampicillin: COT: Cotrimoxazole and CEF-Ceftriaxone

Prevalence of ASB revealed from the study among pregnant women was 11.5% [95%CI: 7.4% - 16.8%] which was coherent with a similar study conducted in Dhaka by Jubaida and colleagues whose study found a prevalence of 10.2% (Jubaida *et al.*, 2013). However, other studies revealed contrast results; a recent similar study conducted in Iran revealed a prevalence of 6.5% for significant ASB which is relatively lower compared to the prevalence from this study (Etminan-Bakhsh, Tadi, & Darabi, 2017). This relatively low prevalence might be due to screening and treatment being carried out irrespective of symptoms by the subjects which is not usually practiced in our setting. Again, a study by Labi and colleagues predicted a far lower prevalence of 5.5% per their study (Labi, Yawson, Ganyaglo, & Newman, 2015). Other studies showed relatively higher prevalence rates compared to that from this study (J. Akerele *et al.*, 2001; A. Boye *et al.*, 2012; Imade, Izekor, Eghafona, Enabulele, & Ophori, 2010,; Mokube , Atashili, Halle-Ekane , Ikomey, & Ndumbe 2013) who reported 29.5%, 86.6%, 56.5%, 23.5%, and 45.3% respectively. Inconsistency in the

prevalence rates from these various studies may be due to differences in the socio-demographic characteristics of the study population.

Out of the overall prevalence, E*scherichia coli* emerged the predominant bacteria isolated with a prevalence of 43.6%. This result is comparable with other studies which also revealed *E. coli* as the most prevalent isolate (Kerure, Surpur, Sagarad, & Hegadi, 2013; Senthinath *et al.*, 2013; Sheiner, Mazor-Drey, & Levy, 2009). On the contrary, the result is dissimilar to a study conducted in Nigeria where *Klebsiella specie*s was the most prevalent organism isolated (Izuchukwu, Oranu, Bassey, & Orazulike, 2017). Another study found S. *aureus* to be the predominant bacterium isolated (Olamijulo, Adewale, & Olaleye, 2016). There was a significant association between marital status and asymptomatic bacteriuria (***P=0.027***), with the unmarried group having a relatively higher ASB compared to the married group, a finding different from a study by Olamijulo *et al.* (2016). The high amount of bacterial isolates (BI) among the unmarried group could be associated with sex activities before and after women conceived.

The laboratory analysis for Antibiotic Susceptibility Test (AST) showed that all five (5) isolates were most susceptible to Gentamicin and Norfloxacin. However, among the twelve (12) different drugs used for the study, the most resistant drugs were Ciprofloxacin, Chloramphenicol, Ampicillin and Amoxillin; Ciprofloxacin showed high resistance among the gram positive organisms whiles the rest predicted relatively low resistances among gram positive organisms. However, comparatively the highly susceptible antibiotics (Gentamicin, Norfloxacin, Ofloxacin, Cotrimoxazole and Ceftriaxone) were also found to be highly effective from studies by Okalla, Shu, Etoga, Mengue, and Adiogo (2018) in Cameroon, Alemu, Dagnew, Alem, and Gizachew (2013) in Ethiopia, Onanuga, Omeje, and Eboh (2018) in Bayelsa state Nigeria, A. Boye *et al.* (2012) and Turpin *et al.* (2007) in Ghana, and Mokube, Atashili, Halle-Ekane, Ikomey, and Ndumbe (2013) in Buea State.

On the contrary, Sujatha and Nawani (2014) and Alex Boye *et al.* (2012) found Tetracycline, Ampicillin, and Amikacin as susceptible to both gram positive and negative organisms. Ferrieres, Hancock, and Klemm (2007) stated that clear alternatives as a first line drug against UTI before culture and sensitivity were Gentamicin and Norfloxacin but Gentamicin was relatively safer in pregnancy and effective against most urinary tract infections (Kenechukwu , Chinekwu , Davidson , & Golibe 2005; Miglioli , Silini , Carzeri , Grabocka , & Allerberger 1999) but may cause nephrotoxicity and negative fetal conditions when used adversely and close to term during pregnancy (Noel , Tufon, Waindim, Akwo, & Bong, 2013). The high resistance among Penicillin related antibiotics might be associated with high rate of its abuse unlike the aminoglycosides and the fluoroquinolones whose low resistance may be due to their less availability and the wide range of antibiotic therapy.

The number of leukocyte esterase positive reactions of the urine dip stick was greater than nitrite reactions which is mostly used in most hospitals, clinics and some health centers for bacteria identification during routine urine examination. Detection of presence of bacteria by nitrite reaction using the urine dipstick was highly specific but lacked sensitivity resulting in consequence of having a number of false negatives. A method comparative study by Habiba Ibrahim and Yunusa, 2015 revealed similar results supporting the false negativity of the urine dipstick in detecting bacteria in urine. However, other studies showed contrasting results to that from this study (Okusanya, Aigere, Eigbefoh, Okome, & Gigi, 2014; Shelton, Boggess, Kirvan, Sedor, & Herbert, 2001). Clearly this shows that the dip stick cannot identify non-nitrate producing bacteria as well as urine that stays in the bladder for a short period before it is voided, thereby making it present with shortfalls as a routine test kit.

The results of this study support the suggestion that urine culture should be the method of choice for screening among pregnant women. Therefore, ASB identification among pregnant women should be treated as per antimicrobial sensitivity pattern of the isolate to prevent overlook of some bacterial growth thereby leading to maternal and perinatal morbidity.

Another observation was the presence of 23 Bacteria isolates among the Christian participants compared to none among the Muslims. This might be attributed to effective rinsing of the vulva following every episode of urination among Muslims, a practice in the religion. Furthermore, high bacteriuria was found among the 41-50 age group. This general revelation of ASB isolation being more in the older women compared to young females might be due to the action of hormones leading to increased deposition of glycogen within the smooth muscles. This then attracts lactobacillus activity which is glycogenphilic and thus colonizing the vaginal epithelium, making it acidic and hence preventing invasion of the region by other pathogens. Therefore, decreasing glycogen deposition, and reduction in the lactobacillus colonies as part of ageing process enhance bacterial adherence and invasion by pathogens, making them more susceptible to UTI (Eschenbach *et. al.*, 1992). However, a similar study found high bacteria isolates among 21-25 age group which was likely to be an active sex group (A. Boye *et al.*, 2012). The anatomical relationship of the female urethra to the vagina makes it liable to trauma during sexual intercourse and this can increase bacteriuria.(A. Boye *et al.*, 2012).

Prevalence of ASB was found to be high among pregnant women in their 1st and 3rd trimesters from this study. A previous study carried out by Olusanya and Olutiola (1984) also confirms the high prevalence among the third trimester group whiles another study also found a high ASB isolates among the 2nd trimester group (A. Boye *et al.*, 2012). Finally, hemoglobin was found to be significantly associated with bacteriuria (***P=0.018***). This clinical condition might be due to the production of polysaccharides, endotoxins and particularly hemolysis by most of the bacteria which causes tissue such red blood cells damage.

4. CONCLUSION AND RECOMMENDATIONS

Overall prevalence of asymptomatic bacteriuria was 11.5% [95%CI: 7.4% - 16.8%] among the referral pregnant women attending KBTH with *E. coli* 43.6% being the predominant isolate followed by CoNS 26.7% which were all susceptible to Gentamicin and Norfloxacin. The study showed clearly that urine dip stick method is not a “gold” process for bacteria identification since it cannot isolate most of the gram positive bacteria.

The study recommends that screening and treatment of asymptomatic bacteriuria in pregnancy should be an integral part of obstetric care and should be included in all antenatal guidelines. Also, all urine samples from pregnant women should be cultured for ASB isolation and hence an appropriate drug selection via AST process. Furthermore, urine culture should serve as the gold standard as against the urine dipstick method and be practiced among all facilities to help reduce incidence of misdiagnosis. Lastly, from this study we recommend and advocate that all Penicillin related antibiotics should not be used for the treatment of ASB.

LIMITATION

Some participants might have not been able to adequately follow the mid-stream urine collection.

COMPETING INTERESTS

The authors hereby declare that there are no competing interests regarding the publication of this article.

CONSENT (WHERE EVER APPLICABLE)

Written informed consent was obtained from participants before the study took place and these documents are preserved by the authors.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

Ethical clearance was sought from the Institutional Review Board of the University of Cape Coast (IRB/UCC) and administrators of the Hospital before the study was commenced. In addition, all pregnant women recruited into the study gave their informed consent after thorough explanation of the rationale of the study.

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