

**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 labour and preterm rupture of the membranes. Additionally, it has been associated with clinical conditions such as anaemia, preterm birth, low birth weight and perinatal mortality.

Aim: This study, therefore, determined the prevalence of ASB among pregnant women attending the 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 the prevalence of ASB was significant (Pearson $\chi^2 = 4.88$, 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 Amoxicillin 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.

16

17 1. INTRODUCTION

18

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

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

37 The significance of ASB relates to its potential to cause acute pyelonephritis, preterm labour
38 and preterm rupture of the membranes, other clinical condition includes; anaemia, preterm
39 birth, low birth weight and perinatal mortality (J. Akerele, Abhulimen, & Okonofua, 2001;
40 Patterson & Andriole, 1997). A study carried out on ASB showed a prevalence between 2-
41 7% (Okpere, 2003) but may be as high as 10% in a high-risk population (Kacmaz, Cakir,
42 Aksoy, & Biri 2006). Furthermore, the prevalence of ASB conditions may be as twice as
43 common in pregnant women with sickle cell trait and three times as common in pregnant
44 women with diabetes mellitus status (Kinningham, 1993). Studies done so far show that the
45 prevalence rate of ASB in developing countries is higher than that in the developed states
46 (Hooton , Winter, Tiu , & Stamm 1995).

47 A hospital-based study carried out in some developed states including Canada showed a
48 prevalence rate of 4-7% as compared to that of developing countries like Nigeria (i.e. Benin
49 City and Sagamu) and Ethiopia. In Ghana, a study conducted in Komfo Anokye Teaching
50 Hospital (KATH) and University of Cape Coast (UCC) hospital showed prevalence rates of
51 7.3% and 56.5% respectively (P. Akerele, Abhuliren, & Okonofua, 2001.; A. Alemu *et al.*,
52 2013; A. Boye *et al.*, 2012; Turpin, Minkah, Danso, & Frimpong, 2007). Another report also
53 showed that ASB occurs between 5-9% for both non-pregnant and pregnant women and if
54 left untreated, can progress to symptomatic UTI's including acute cystitis and pyelonephritis;
55 this progress to symptomatic UTI's is likely to be more in pregnant women than non-
56 pregnant women. However, other conditions that increase the incidence include low socio-
57 economic status, grand multi-parity and advance in age. A recommendation was made by
58 the Infectious Diseases Society of America that there is a need to screen and treat all
59 positive cultures during pregnancy. The study, therefore, determined the prevalence of ASB
60 (Asymptomatic bacteriuria) in pregnant women attending antenatal clinic at the Korle-Bu
61 Teaching Hospital. The research work also went ahead to ascertain the antibiotic
62 susceptibility patterns of this significant bacteriuria.

63

64 2. MATERIAL AND METHODS

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66 2.1 Study design / Study site

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

72 2.2 Study participants/ Eligibility criteria

73 A total of 200 pregnant women attending antenatal clinic at KBTH within the study period
74 were recruited using convenient sampling method. Pregnant women within the ages of 15-
75 45years were included in this study, however, participants with signs and symptoms of
76 Urinary tract infections (UTI's), kidney diseases, diabetes, urological surgery, carcinoma and
77 spinal cord injuries were excluded.

78 2.3 Data Collection and Processing

79 2.3.1 Urine Sample Collection

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

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

90 2.3.2 Urine Sample Processing

91 The samples were subjected to routine microscopy and culture. A dip stick test strip was first
92 inserted into the urine samples to check for the presence of nitrites and leukocytes. The
93 urine samples were further centrifuged and their sediments were observed under a
94 microscope for the presence of pus cells, red blood cells, casts and epithelial cells. The
95 Urine samples were also cultured on Cysteine Lactose electrolyte deficiency (CLED) agar
96 media using the streak plate method by use of a calibrated inoculation loop of size
97 0.001CFU/L, the plates were then incubated at 37°C and read within 18-24 hours for
98 significant bacteriuria. Significant bacteriuria was defined as a single colony count of $\geq 10^5$
99 organisms. The organisms were isolated and identification of pathogens was done using
100 standard biochemical and sugar fermentation tests. Antibiotic susceptibility tests were also
101 conducted on them by plating on Muller Hinton agar employing the Kirby Bauer disc diffusion
102 method.

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2.4 Statistical Analysis

Data collected were entered into Microsoft Excel and further analyzed using SPSS version 22.0. Most variables were reported in frequencies and percentages while haemoglobin 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 the demographical data of 200 pregnant women enrolled in the study. Out of this, the 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 haemoglobin level was 11.45 ± 0.08 while 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, the 41-45 age group had the highest prevalence (28.6%) of ASB while the least (3.4%) was found among the 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 the prevalence of ASB was significant (Pearson $\chi^2 = 4.88$, 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 the prevalence of ASB. (**Table 1**)

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

Characteristic	Non-Bacteriuria N (%)	Bacteriuria N (%)	Total N (%)	χ^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)	4.884	0.027 ^{*a}
Marital Status					
Married	159 (90.3)	17 (9.7)	176 (88.0)		
Single	18 (75.0)	6 (25.0)	24 (12.0)	2.434	0.487
Education					
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
1 st	6 (85.7)	1 (14.3)	7 (3.5)		
2 nd	89 (90.8)	9 (9.2)	98 (49.0)		
3 rd	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}

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

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139 **Table 2** shows the dip stick examination for urine chemistry which revealed the majority of
140 the participants testing negative for both nitrite and leukocyte esterase test. However,
141 24(12.0%) and 38(19.0%) tested positive for nitrite and leukocytes esterase respectively.
142

143 **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)

144 **N: Number**

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148 **Figure 1** represents the frequency of the bacterial isolates, the predominant isolate was *E*
149 *coli* (43.6%), followed by *CoNS*, *Citrobacter spp*, *Enterococcus spp* and *Klebsiella spp* as
150 (26.1%), (21.7%), (4.3%) and (4.3%) respectively.

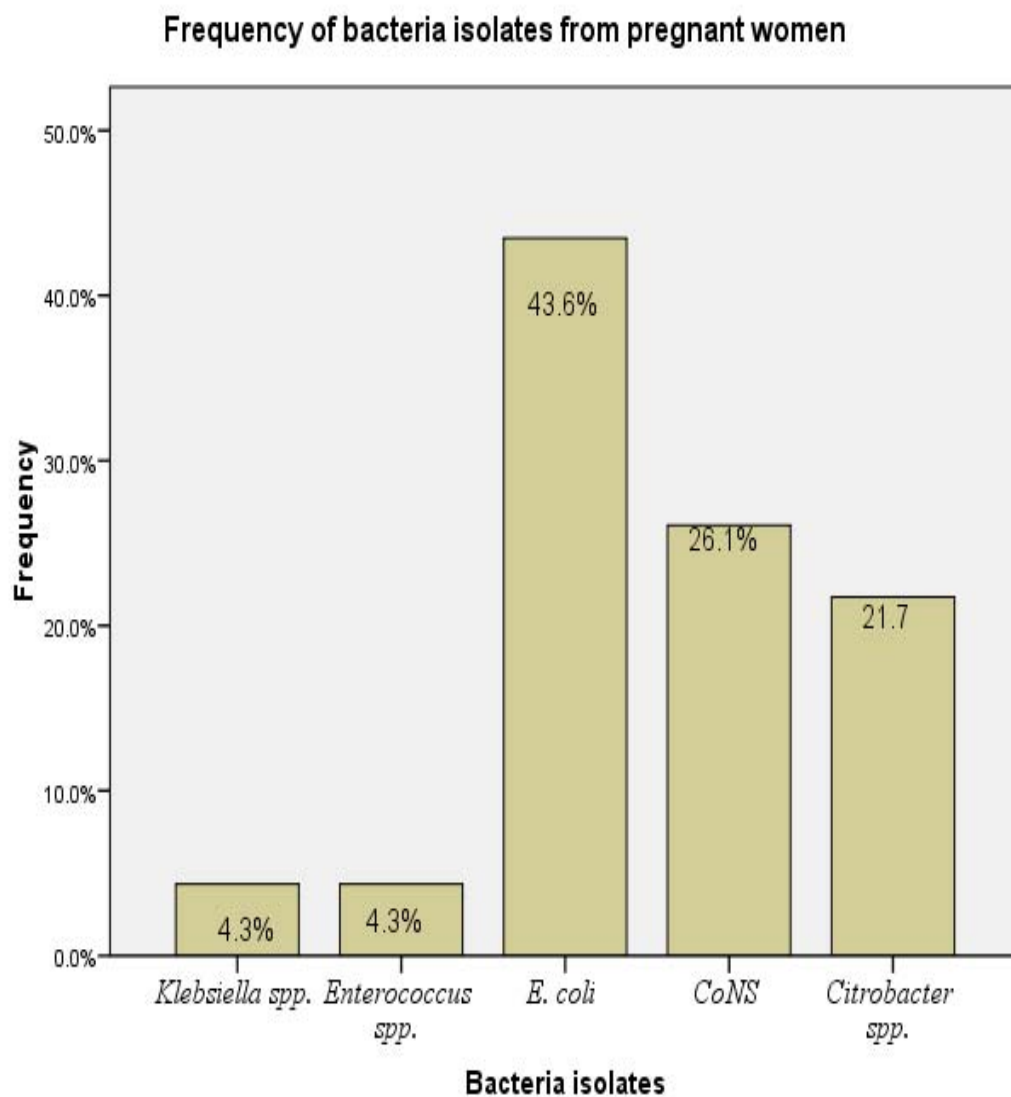


Figure 1: Frequency of bacteriuria isolates among pregnant women

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 Amoxicillin. **(Table 3)**

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

ISOLATES	N		ANTIBIOTICS							
			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)

165 **N-Number: S-Sensitivity: R-Resistance: CoNS- Coagulase Negative Staphylococcus:**
166 **E. coli- Escherichia coli: GEN-Gentamicin: NOR- Norfloxacin: AMO-Amoxicillin: OFLO-**
167 **Ofloxacin: CIP-Ciprofloxacin: TET-Tetracycline: CRP- Chloramphenicol: AMK-**
168 **Amikacin: AMP-Ampicillin: COT: Cotrimoxazole and CEF-Ceftriaxone**
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173 Prevalence of ASB revealed from the study among pregnant women was 11.5% [95%CI:
174 7.4% - 16.8%] which was coherent with a similar study conducted in Dhaka by Jubaida and
175 colleagues whose study found a prevalence of 10.2% (Jubaida *et al.*, 2013). However, other
176 studies revealed contrast results; a recent similar study conducted in Iran revealed a
177 prevalence of 6.5% for significant ASB which is relatively lower compared to the prevalence
178 from this study (Etminan-Bakhsh, Tadi, & Darabi, 2017). This relatively low prevalence might
179 be due to screening and treatment being carried out irrespective of symptoms by the
180 subjects which is not usually practised in our setting. Again, a study by Labi and colleagues
181 predicted a far lower prevalence of 5.5% per their study (Labi, Yawson, Ganyaglo, &
182 Newman, 2015). Other studies showed relatively higher prevalence rates compared to that
183 from this study (J. Akerele *et al.*, 2001; A. Boye *et al.*, 2012; Imade, Izeke, Eghafona,
184 Enabulele, & Ophori, 2010.; Mokube, Atashili, Halle-Ekane, Ikomey, & Ndumbe 2013) who
185 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, *Escherichia 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 species* 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 Amoxicillin; Ciprofloxacin showed high resistance among the gram-positive organisms while the rest predicted relatively low resistance 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 the 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 are mostly used in most hospitals, clinics and some health centres for bacteria identification during routine urine examination. Detection of the 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 the 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 the 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 while another study also found high ASB isolates among the 2nd-trimester group (A. Boye *et al.*, 2012). Finally, haemoglobin 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 as red blood cells damage.

4. CONCLUSION AND RECOMMENDATIONS

The 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 the 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, a urine culture should serve as the gold standard as against the urine dipstick method and be practised among all facilities to help reduce the 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.

292 **COMPETING INTERESTS**

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

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298 **CONSENT (WHERE EVER APPLICABLE)**

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

302

303 **ETHICAL APPROVAL (WHERE EVER APPLICABLE)**

304

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

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