



## **A Cross-sectional Study of the Prevalence of Asymptomatic Bacteriuria among HIV-positive and HIV-negative Expectant Mothers in a Tertiary Health Centre in South Eastern Nigeria**

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### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author LCI designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors HUE and JOE managed the literature searches, analyses of the study, performed the spectroscopy analysis. Authors TCO, CKO and JCU carried the statistical analysis. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Background:** Asymptomatic Bacteriuria (ASB) in pregnancy is associated with potential urinary and obstetric complications. The diagnosis and management of ASB in expectant mothers is in keeping with safe motherhood initiative.

**Aim:** To determine the prevalence and pattern of ASB among HIV-positive and HIV-negative pregnant women in Enugu State, South Eastern Nigeria.

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**Methods:** This was a comparative analytical study among HIV-positive and HIV-negative pregnant women at the University of Nigeria Teaching Hospital, Enugu State. 'Clean catch' urine samples of these women collected and analysed. Statistical analysis was performed using the Chi-square and student's t tests as appropriate. A P-value of less than 0.05 was considered statistically significant.

**Results:** Among the two hundred and forty HIV-positive women, (23.3%) had significant ASB while (10.4%) of an equal number of HIV-negative women had significant ASB. The difference was statistically significant ( $p=0.013$ ). A higher proportion of HIV-positive women, (22.5%) with CD4 cell count of  $\leq 500/\text{mm}^3$  had significant ASB ( $p=0.015$ ). *Escherichia coli* was the commonest isolate in both groups of women. The isolates were generally sensitive to amoxicillin-clavulanic acid, nitrofurantoin and cefuroxime in the two groups. The sensitivity to sulfamethoxazole-trimethoprim was low; HIV positive (21.5%), HIV negative (16.0%). Among those treated, none had overt UTI in pregnancy.

**Conclusion:** Screening, treatment and follow up for ASB in pregnancy are necessary especially in HIV positive women with CD4 cell count less than  $500/\text{mm}^3$ . This should be included during counselling in all antenatal protocols.

*Keywords: HIV; pregnant; women; asymptomatic; significant; bacteriuria.*

## 1. INTRODUCTION

The concept of asymptomatic significant bacteriuria (ASB) was introduced by Kass in 1956 [1]. Asymptomatic bacteriuria is the isolation of specified quantitative count of bacteriuria in an appropriately collected urine specimen obtained from a person without symptoms or signs referable to urinary infection [2,3].

ASB is common but the prevalence varies widely with age, sex, and the presence of genito-urinary abnormalities [3]. Women, particularly pregnant women are more at risk than men due to pregnancy, short urethra and easy faecal contamination of urinary tract [3]. During pregnancy, the hormonally induced dilatation of the renal pelvis and ureters and the mechanical obstruction of the distal ureters by the gravid uterus result in urinary stasis thus promoting bacterial colonization [4].

In Nigeria the prevalence of ASB among pregnant women range from 1.7% to 78.7% [5,6,7].

If left untreated, as many as 30-50% will develop symptomatic urinary tract infections (UTI) often, in the form of pyelonephritis [3]. ASB and pyelonephritis have been associated with adverse pregnancy outcomes such as preterm birth, premature rupture of membranes, pre-eclampsia, abortions and low birth weight [3,4].

Risk factors include multiparity, previous medical history of UTI, immune suppressive conditions such as diabetes mellitus and Human Immune

deficiency Syndrome (HIV) [8,9,10]. A study in South Africa found a higher prevalence of ASB among HIV-positive, 18.6% compared with HIV-negative, 12.9% pregnant women [4].

ASB is a microbiological diagnosis using urine specimen that has been collected aseptically and transported to the laboratory timely to limit bacterial multiplication [3]. The usual quantitative definition is  $\geq 10^5$  colony forming unit (cfu)/ml in two consecutive urine specimens [11]. Eigbofoh et al. [12] in their study among pregnant women compared rapid dipstick for asymptomatic bacteriuria and the gold standard-urine microscopy, culture and sensitivity, and found that dipstick had a poor correlation with urine culture, although specificity was high to rule out UTI. Various studies have shown the variation in frequency of isolates and susceptibility patterns even within the same country thus emphasizing the need for constant surveillance of most causative species of ASB to prevent the deleterious effects in pregnancy [8,9]. There are several studies on asymptomatic bacteriuria in pregnant women generally but literature search did not yield any result on special population of HIV-positive pregnant women in our environment.

The aim of this study is to compare the prevalence and pattern of asymptomatic bacteriuria among HIV-positive and HIV-negative women in Enugu, Nigeria.

## 2. METHODS

This was a comparative analytical study carried out at the antenatal clinic of the University of Nigeria Teaching Hospital (UNTH), Ituku-ozalla,

Enugu, South Eastern Nigeria from 1<sup>st</sup> June 2014 to 31<sup>st</sup> August 2014.

Approval for this study was obtained from the Ethical Committee of UNTH, Enugu and informed verbal consent from the respondents. Confidentiality of the patients' identity was guaranteed by the use of numbers and codes.

## 2.1 Study Design and Sample Selection

The population consists of two sample groups of expectant mothers selected on a 1:1 ratio. Group A consists of consecutive HIV-positive women while Group B was made up of equal number of consecutive HIV-negative attendees at the ante natal clinic.

The two groups were compared with regards to socio-demographic characteristics that included among others, age, marital status, parity, gestational age, urinalysis, prevalence of asymptomatic significant bacteriuria, culture of isolated organisms, antibiotics given and outcome of treatment.

In this study, Premature Rupture of Membranes (PROM) was defined as the rupture of the fetal membranes before the onset of labour. Preterm labour (PTL) was onset of labour before 37 completed weeks while low birth weight (LBW) was any baby that weighed less than 2500 g at birth.

The inclusion criteria were pregnant women who are HIV-positive and pregnant women who are HIV-negative. The exclusion criteria include women with obvious urinary tract infection (UTI) in pregnancy or renal disease and patients who declined consent. Others excluded are hypertensive, anaemic, diabetic and patients on antibiotics.

The study was conducted on consenting patients using a structured questionnaire for the socio demographic variables and a pretested data extraction form for the collection of the clinical parameters. The questionnaire was administered by four trained medical officers.

The sample size was calculated using the formula below for estimated population size of less than 10,000 [13].

$$N=Z^2 P (1-P)/D^2$$

Where N=minimum sample size,  
Z=1.96 at 95% confident limit,  
D=margin of error tolerated =5%,

P=prevalence of 15.1% in a similar study in UNTH [14].

$$N= \frac{1.96^2 \times 15.1 \times 84.9}{5^2}$$

N=197. A sample size of 200 for each group was adequate.

Adding 20% of the minimum sample size for the expected non-response rate, a sample size of 250 for each group was obtained.

## 2.2 Specimen Collection

The patients were taught how to collect the urine specimen by the 'clean catch' mid stream method. Each patient collected two consecutive urine samples into two sterile containers. The specimens were sent immediately to the microbiology laboratory for urinalysis, microscopy and culture. Urinalysis was done using Multistix strips made by Bayer (Germany).

Samples were cultured on Cystine Lactose Electrolyte-Deficient (CLED) agar using calibrated loop delivering 0.002 ml of urine. Incubation was done at a temperature of 35-37°C for 24 hours with Binder B28 incubator (WTB BINDER GMBH GERMANY). Colony counts greater than or equal to 100,000 bacteria per ml in each of the two samples were considered significant.

Sensitivity was determined with standard antibiotics discs using Disc diffusion method [15].

All positive cases were treated with antibiotics to which the organism is sensitive and that is safe in pregnancy for seven days.

Blood samples for the HIV-positive patients were also drawn for the CD<sub>4</sub> count which was analysed using Partec Cyflow counter made in Germany. The patients were followed up till delivery.

## 2.3 Data Analysis

The data were entered into a computer using Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) computer software version 15.0 for windows. Chi square ( $\chi^2$ ) and Student's t tests were used as appropriate to compare associations between proportions and categorical variables. A P-value less than 0.05 were considered statistically significant.

### 3. RESULTS

Four hundred and eighty attendees at the antenatal clinic were chosen. Among these were two hundred and forty women who were HIV-positive and equal number who were HIV-negative.

The characteristics of the two groups are illustrated in Table 1.

The mean age for the HIV positive women was 21.4±3.6 years (range 15-45 years) while that of the HIV negative women was 20.6±2.7 years (range 16-44 years). There was no significant difference in maternal age (P=0.071), parity (P=0.074) and gestational age (P=0.059) in both groups of women. The association between maternal age, parity, gestational age and significant ASB in both groups was also not statistically significant. HIV-positive women, (22.5%) with CD4 cell count of ≤ 500/mm<sup>3</sup> had significant ASB as against (0.8%) with CD4 cell count > 500/mm<sup>3</sup> ( $X^2 = 17.284$ ,  $df = 7$ ,  $P = 0.015$ ). The mean CD4 count was 261.45±416.17/mm<sup>3</sup> and range 214.27 – 573.41/mm<sup>3</sup>.

Urinalysis in both groups of women revealed no significant difference (p=0.061).

The isolated organisms in both groups of women were similar (Table 2). In HIV positive mothers, *Escherichia coli* (7.5%) was the commonest isolate followed by *klebsiella* (6.7%) and

*Staphylococcus aureus* (4.1%). *Escherichia coli* (3.3%), *Klebsiella* (2.9%) and *Staphylococcus aureus* (1.7%) were also isolated in HIV negative mothers.

The sensitivity pattern was also similar in both groups of women as shown in Table 3. Among the HIV positive and HIV negative mothers, the isolates were sensitive to amoxicillin-clavulanic acid (95%) vs (84%), nitrofurantoin (75%) vs (68%) and cefuroxime (64.2%) vs (60%).

The sensitivity of bacterial isolates to ampicillin and amoxicillin in HIV positive mothers were (54%) and (52%) respectively while in HIV negative women, ampicillin (52%) and for amoxicillin (48%). The sensitivity to sulfamethoxazole-trimethoprim was generally low; HIV positive mothers (21.5%) and HIV negative mothers, (16.0%). Among the 81 patients with significant ASB who were treated for a week with antibiotics, none was lost to follow up. None of these patients had overt urinary tract infection. There was no statistical difference in the proportion of women that had PROM (p=0.071), LBW (p=0.069) and PTL (0.080) in pregnancy in both groups of women (Table 4).

### 4. DISCUSSION

The prevalence of ASB though common in both groups, was significantly higher in HIV-positive women compared to HIV-negative women (23.3% vs 10.4%).

Table 1. Clinical parameters of patients

Parameter	HIV-positive	HIV-negative	P value
*Maternal age (years)	21.4±3.6	20.6±2.7	0.071
Maternal age ≤ 35 years	92.3%	91.5%	0.063
*Parity	4.6±1.2	3.9±1.1	0.074
*Gestational age (weeks) at period of study	20.5±1.9	19.4±2.1	0.059
Significant ASB	23.3%	10.6%	0.013

\*Mean values

Table 2. Bacterial colonization pattern based on culture and microscopy

Bacterial isolate	HIV-positive no (%)	HIV-negative no (%)
<i>Escherichia coli</i>	18 (7.5)	8 (3.3)
<i>Klebsiella</i>	16 (6.7)	7 (2.9)
<i>Staphylococcus aureus</i>	10 (4.1)	4 (1.7)
<i>Streptococcus faecalis</i>	6 (2.5)	4 (1.7)
<i>Enterobacter</i>	3 (1.3)	0
<i>Proteus mirabilis</i>	3 (1.3)	2 (0.8)
Total	56 (23.3)	25 (10.2)

**Table 3. Susceptibility and resistance pattern to antibiotics**

Antibiotics	Sensitive		Resistant	
	HIV-positive no (%)	HIV-negative no (%)	HIV-positive no (%)	HIV-negative no (%)
Amoxicillin-clavulanic acid	52 (95%)	21 (84%)	8 (5%)	4 (16%)
Nitrofurantoin	42 (75%)	17 (68.0%)	14 (25%)	8 (32.0%)
Cefuroxime	36 (64.2%)	15 (60.0%)	36 (35.8%)	10 (30%)
Ampicillin	30 (54%)	13 (52%)	26 (46%)	12 (48%)
Amoxicillin	29 (52%)	12 (48%)	27 (48%)	13 (52%)
Sulfamethoxazole-trimethoprim	12 (21.5%)	4 (16.0%)	44 (78.5%)	21 (84%)
Total number of bacterial isolates	56	25		

**Table 4. Complications in pregnancy**

Parameter	HIV-positive	HIV-negative	P value
PROM	4.9%	3.7%	0.071
LBW	3.7%	3.7%	0.069
PTL	6.2%	4.9%	0.080

PROM: Premature rupture of membranes; LBW: Low birth weight; PTL: Preterm labour;  
ASB: Asymptomatic bacteriuria

In Tyne berg South Africa, the overall prevalence of ASB was **(8.3%)**, with no statistical difference found between the **(9.2%)** in HIV-positive and **(7.9%)** in HIV-negative patients [4]. A study in Kano Nigeria reported similar prevalence of urinary tract infections (UTI) in the AIDS patients, **(25%)** compared to the control non AIDS patients of **(10%)** [16]. The higher prevalence of ASB in HIV-positive women may be related to the suppression of their immunity.

The prevalence of **(23.3%)** reported among the HIV-positive women in this study was comparable to results from other parts of Nigeria; Kano **(25%)** and Calabar **(25.3%)** [16,17]. The prevalence in our study was higher than **(8.3%)** and **(9%)** reported in South Africa and Iran respectively [4,18]. The difference in prevalence rates may be related to the diverse population characteristics, methods of screening and criteria for diagnosis. In Ilorin Nigeria, a higher prevalence of **(40%)** was reported [19]. This was attributed to the fact that only a single urine culture was used whereas in most studies, the diagnosis required two or even three consecutive positive urine cultures [1,11].

In this study, there was no significant effect of maternal age, parity and gestational age on the development of significant ASB in both groups of women. This is in consonance with other reports [8,20]. In their study however, Nwadioha et al. [16] observed that UTI was common in age fifty years and above in both the tests and controls and this was attributed to voiding dysfunction.

Our study showed that (92.3%) of the HIV-positive women and (91.5%) HIV-negative women were below the age of 35 years and this could have reduced the likelihood of developing ASB observed with increasing age in other studies [16,20]. Furthermore, the mean gestational age in both groups of women were relatively low resulting in less ureteral pressure and stasis unlike the gravid uterus at term.

*Escherichia coli* were the commonest isolate followed by *Staphylococcus aureus* in both HIV-positive (7.5%) and HIV negative (3.3%) mothers. This is at variance with Nwadioha et al. [16] report which showed higher proportions of *Escherichia coli* in HIV positive (30%) and HIV negative (65%) patients. Of the (21.2%) HIV positive patients who had significant bacteriuria in Lagos Nigeria, *Escherichia coli* constituted (42.42%) [21] while in Ibadan Nigeria (48%) of 25 cultures showing significant bacteriuria grew *Escherichia coli* [8]. *Escherichia coli* is reportedly the most common micro organism in the vaginal and ano-rectal areas and its multiplication is favoured by poor personal hygiene and stasis of urine frequently seen in pregnant women [2,4]. In other studies in Nigeria, *Staphylococcus aureus* showed the following at Ibadan **(28%)**, Calabar **(87.2%)** and Benin **(27.2%)** suggesting the possibility of nosocomial infections and the risk of opportunistic infections to which HIV patients are particularly prone to [8,17,22]. In another study in Kano Nigeria, *Proteus mirabilis* **(49%)**, a major soil contaminant and rare isolate in the past was a major uropathogen among antenatal attendees

who were largely house wives (57.4%) [23]. This is at variance with our study suggesting occupational risks and a changing pattern in the prevalence of organisms causing infection in the population [23].

Our study showed that in both HIV positive and negative mothers, the isolates were generally sensitive to amoxicillin-clavulanic acid (95%) vs (84%), nitrofurantoin (75%) vs (68%) and cefuroxime (64.2%) vs (60%). In Tikur Anbessa Ethiopia, it was also reported that amoxicillin-clavulanic acid, nitrofurantoin and trimethoprim-sulphamethoxazole were effective at least in (70%) of the isolates [24]. It however contrasts with the observations of Samuel et al. [25] who showed the fluoroquinolones sensitive to (83.3%) of the isolates. In Cameroon, Mokube et al. [26] noted that most of the uropathogens isolated were susceptible to cephalosporins (100%) such as cefixim, cefoxitine and cephalotin. In Uganda, Mwaka et al. [27] observed that nitrofurantoin is the single most efficacious antibiotic against all the strains of uropathogens isolated with sensitivity rate as high as 100% against *Escherichia coli* and *Staphylococcus aureus*. Though cheap, readily available and relatively safe in pregnancy, nitrofurantoin may cause hemolysis in a glucose-6-phosphate dehydrogenase deficient infant when used close to term [10]. The variations in sensitivity pattern may be related to the different pathogens isolated, emergent resistant strains and the treatment protocols of the respective hospitals. The sensitivities to commonly used antibiotics, ampicillin and amoxicillin were average and this may be a reflection of the level of abuse and misuse [16]. The observed low sensitivity to sulfamethoxazole-trimethoprim, HIV positive mothers (21.5%) and HIV negative mothers (16.0%) is probably related to its use at otherwise sub-therapeutic antimicrobial dose for the prophylactic treatment of several bacterial infections [26].

The study pattern showed that a higher proportion of HIV-positive women, (22.5%) with CD4 cell count  $\leq 500/\text{mm}^3$  had significant ASB compared to (0.8%) with CD4 cell count  $>500/\text{mm}^3$ . In Ibadan Nigeria, Awolude et al. reported significantly lower CD4 counts among HIV positive women with significant bacteriuria compared with those without significant bacteriuria (250.52 cells/ $\text{mm}^3$ ) vs (355.57 cells/ $\text{mm}^3$ ) [8]. Banu et al. [20] however found no correlation of cell counts with bacteriuria. The occurrence of significant ASB with declining CD4

cell count could be related to immune suppression in HIV patients.

PTL and PROM are associated with urogenital colonization [28]. In our study, infecting organisms were sensitive to commonly used antibiotics that are available, affordable and safe in pregnancy. After treatment, the rates of PROM, PTL and LBW were not different from that of the normal population. This is in line with other reports [4,28].

## 5. LIMITATION

This was a hospital based study. There may still be the possibility of differences between samples and the general population. There were no facilities for screening for ASB for *Ureaplasma* and *Chlamydia*.

## 6. CONCLUSION

Asymptomatic bacteriuria is common and pregnant women, especially those with CD4 cell count less than  $500/\text{mm}^3$  are at increased risk. Screening for significant ASB in pregnant women generally and HIV-positive women in particular should form a routine practice in all antenatal protocols. Antimicrobial treatment of asymptomatic bacteriuria in pregnancy will prevent overt urinary tract infections and adverse pregnancy outcomes.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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