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Original Research Article

Asymptomatic bacteriuria in HIV positive and negative pregnant women at Federal University Teaching Hospital Lafia: a comparative study

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ABSTRACT

Background: Pregnant women with asymptomatic bacteriuria (ASB) are at increased risk of complicated urinary tract infection. The prevalence and risk of progression may be higher with background human immunodeficiency viral (HIV) infection. The aim of the study was to compare the prevalent microbial isolates and antibiotic sensitivity pattern in asymptomatic bacteriuria among HIV positive and negative antenatal clients in Federal University Teaching Hospital (FUTH), Lafia.
Methods: A cross sectional comparative study carried out among 60 HIV positive and negative antenatal women respectively at the obstetric unit of FUTH, Lafia, Nasarawa state. Relevant Socio-demographic and clinical data were collected using structured proforma. 'Clean catch' midstream urine samples were collected from each subject and microbial culture and sensitivity test were carried out and analysis done using SPSS version 22. A p-value of less than 0.05 was accepted as statistically significant.
Results: The overall prevalence of ASB in both groups was 53.3% with a higher prevalence of 54.7% HIV positive compared to the prevalence of 45.3% in HIV negative pregnant women. *Echerechia coli* were the commonest organisms isolated and majority of the organism isolated were gram negative. Ciprofloxacin was the antibiotics with the best sensitivity. However, there is a marked resistance of greater than 50% of all the drugs.
Conclusions: Though there is high prevalence of ASB in HIV positive women than the HIV negative pregnant women, there was no statistically significant difference in prevalence, microbial isolates and antibiotic sensitivity in the two groups.

Keywords: Asymptomatic bacteriuria, HIV, Pregnant women, Nigeria

INTRODUCTION

Urinary tract infections (UTIs) are the most common bacterial infections in pregnancy.[1,2] Bacteriuria is defined as the presence of $>10^5$ colonies of a single pathogen per milliliter of clean catch urine.[2] UTI can either present as asymptomatic bacteriuria (ASB) or symptomatic acute cystitis and acute pyelonephritis.[1] Asymptomatic bacteriuria (ASB) is a condition in which urine culture reveals a significant growth of bacteria greater than or equal to 10^5 colony-forming unit per milliliter (ml) of urine taken from a clean catch midstream urine but without the patient showing symptoms.[2,3] There is usually an increase in the progression from asymptomatic to symptomatic bacteriuria.[3]

Asymptomatic bacteriuria 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, their short urethra and easy faecal contamination of urinary tract.[4] 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]

The concept of asymptomatic significant bacteriuria (ASB) was introduced by Kass in 1956.[6] Several studies have shown different prevalence rates of asymptomatic bacteriuria in pregnancy with range from 3-10% in most developed countries.[5,7-9] This wide variation in the prevalence of asymptomatic bacteriuria is explained by differences in the population characteristics, and most importantly, differences in screening methodology and criteria for the diagnosis of asymptomatic bacteriuria in these studies, which in most cases are at variance with the accepted standard.[7]

The incidence of asymptomatic bacteriuria in the obstetric population has an average of 2-11%.[1] This predisposes to the development of pyelonephritis, which leads to obstetric complications like preterm labour and low birth weight infants.[3,7] Asymptomatic bacteriuria has been reported to be associated with an increased risk of symptomatic urinary tract infection.[5]

While uncomplicated UTIs occur most often in young healthy adult women and are easy to treat, UTI can have a complicated course and be more difficult to treat with frequent recurrence in other groups of patients.[5] Complicated UTIs, are infections associated with factors that increase the chances of acquiring bacteria and decrease the efficacy of therapy.[8] These factors include metabolic derangement, hormonal changes, impaired immunity, functional and structural abnormalities.[12] Pregnant women, diabetics, HIV/AIDS patients, transplant recipients, patients with urinary calculi, renal/bladder abscesses, spinal cord injury and indwelling catheters are more prone to developing complicated UTI and it may also be due to multidrug-resistant bacteria or unusual pathogens, such as yeast.[9] Complicated UTIs may involve both lower and upper tracts and their primary significance is that they increase the rate of therapy failures.[9,10]

Therefore, screening and treatment of ASB in such high-risk group of patients may be considered to prevent progression to complicated UTI with adverse outcomes such as renal hypertension and chronic kidney disease.[5,11]

Human immunodeficiency virus (HIV) infection is associated with progressive immune dysfunction and appears to increase the risk for developing significant bacteriuria in patients.[12-14] In HIV infection co-morbidity with other organisms is common and this may impact on the pregnancy outcome in these patients. Such organisms may include those of asymptomatic bacteriuria.[13-15]

Various studies have shown the variation in frequency of isolates and susceptibility patterns which indicate the need for constant surveillance of most causative species of ASB to prevent the deleterious effects in pregnancy. There are several studies on asymptomatic bacteriuria in pregnant women in the North Central region of Nigeria and other parts of the world but there has been no any study in Federal University Teaching Hospital (FUTH), Lafia (Formerly known as Dalhatu Araf Specialist Hospital, Lafia). This study is aimed to compare the prevalence, microbial isolates and their antimicrobial sensitivity in asymptomatic bacteriuria in HIV positive and HIV negative pregnant women in FUTH, Lafia. The findings from this study may form the basis for recommending routine screening and treatment of ASB among HIV positive and HIV negative pregnant women in FUTH, Lafia. This will go a long way to reduce the risk of developing complications in both the mother and the unborn child of HIV positive and HIV negative pregnant women.

METHODS

Study design

This is a cross-sectional comparative study carried out at the antenatal clinic of FUTH, Lafia, Nasarawa State located in the North Central region of Nigeria. All pregnant women who presented for booking during the study period and had met the selection criteria were recruited for the study. One group were previously and newly diagnosed HIV positive pregnant women and the second group made up of equal number of HIV negative pregnant women,

Sample size determination

The sample size for cross-sectional comparative study was determined using the following formula for comparison of two proportions.

$$n = \frac{(Z^{\alpha} + Z^{\beta})^2 \{P_1(100\%-P_1) + P_2(100\%-P_2)\}}{(P_1-P_2)^2}$$

n= Minimum sample size per one group, P_1= prevalence of asymptomatic bacteriuria in HIV positive pregnant women based on previous study done in Lagos University Teaching Hospital.[15], P_2= prevalence of asymptomatic bacteriuria in HIV negative pregnant women based on previous study in the South Eastern Nigeria.[8], Z^{α} =percentage point of the normal distribution, corresponding to the significant level at 5%= 1.96, Z^{β}= percentage point of the normal distribution corresponding to 100%- the power. Power at 80%= 0.84

For this study,

P_1=31.9[15], P_2=10.4[9], Z^{α}= 1.96, Z^{β}= 0.84

$$n= \frac{(1.96+0.84)^2\{31.9(100-31.9) +10.4(100-10.4)\}}{(31.9-10.4)}$$

n = 53 antenatal patients in each group

To allow for non-response, the sample size was increased by about 10%, which is 5.3 and approximately 6. Therefore, the number of pregnant women needed to recruited in one group will be 53 + 6 = 59.

Sampling technique

Convenience sampling method was used, whereby every HIV positive pregnant woman was recruited consecutively and an equal number of HIV negative pregnant women presenting to the antenatal clinic who met the inclusion criteria were recruited. Socio-demographic characteristics, HIV status, risk factors and medical data of the participants were collected using a structured proforma and entered into a Microsoft Excel for cleaning and preparation for data analysis. At bookings these variables and clinical data of patients were collected using the proforma and features from the urine sample of every patient were tied to their form and entered using a unique identification number.

Sample collection and processing

The patients were instructed adequately by the nursing staff on how to collect clean catch midstream urine. After initial cleaning of the perineum with running water, the first part of the urine was voided and about 10mls of the midstream urine was collected into the sterile universal bottles which was correctly labeled and distributed to them. After the collection, the bottles were tightly closed and the urine samples transported to the microbiology laboratory within 30 minutes for processing.

The culture and sensitivity of the urine samples was done at the Department of Medical Microbiology Laboratory. A quantity (10 mL) each well-mixed urine sample was centrifuged at 2000 x *g* for 5 min. Supernatant was discarded and a drop of the deposit was examined microscopically at high magnification for the presence of pus cells, red blood cells, epithelial cells, casts, crystals, yeast-like cells, *Trichomonas vaginalis* and *Schistosoma* ova. Bacterial isolates were confirmed by standard microbiological methods. The antibiotic susceptibility of each isolate was tested manually according to NCCLS recommendations for disc diffusion.

Well-mixed un-centrifuged urine was cultured on blood agar plate for colony counts using calibrated 0.001ml loop. MacConkey agar plate also inoculated for isolation of colonies. The blood agar plate was incubated in 5% CO_2 for 18-24hours to enhance the growth of gram-positive organisms while the MacConkey agar plate was incubated at ambient air overnight at 35 to 37^{o}C for 18 to 24 hours. Colony count of $\geq 10^5$/ml of single isolate was considered significant for diagnosing asymptomatic bacteriuria. For every sample that was positive a repeat sample was collected and reprocessed within the shortest time possible and if the second sample is negative the result was then interpreted as negative. Participants with positive culture results were referred to their attending physician for treatment with a course of antibiotics based on the sensitivity pattern.

Identification of isolates: This was based on the following: colony morphology, gram stain, biochemical tests, oxidase test and motility.

Biochemical tests: Microbact® (Oxoid, Basingstoke, UK) kit was used in the identification of gram-negative organisms to species level. The organisms were emulsified in normal saline, matched with the recommended McFarland's standard and were added to the micro wells. The colour changes were read and fed into the software which will identify the organism. The manufacturer's instructions were strictly adhered to. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control for the Microbact kit. Catalase and coagulase tests were used to differentiate Staphylococcus species. Quality control of each test was carried out with *Staphylococcus aureus* ATCC 25923 organisms.

Antibiotic susceptibility testing: All isolates were tested for antimicrobial susceptibility. This was done using the Modified Kirby-Bauer disc diffusion method. Discrete colonies were emulsified in 3-5mls of normal saline and the turbidity of the resulting suspensions were matched against a 0.5 Mcfarland Standard.

The suspension was streaked on Mueller Hinton agar using a sterile swab stick. 6 discs were placed on a 90mm plate. The plates were incubated in ambient air at 35-37^{0} C for 24 hours. The zones of inhibition were read the next day using a ruler or calipers. Zone sizes were interpreted as susceptible, intermediate or resistant using the Clinical LABORATORY Standards Institute (CLSI) standards interpretive tables.

Data analysis

Collected data were entered into Microsoft excel, cleaned and transmitted into Statistical Package for Social Science (SPSS) version 25.0. Continuous data such as age were computed as means and standard deviations. Categorical data such as presence of ASB, currently on HAART, distribution of ASB etc were computed as frequencies and percentages. Chi-square test was used for association between categorical variables. Statistical significance was adjudged to be at p-value <0.05.

RESULTS

Majority of the women in both groups fall into age groups of 30-34 years with 17 (28.3%) and 22 (36.7%) for Hv positive and negative respectively followed by age group 35and above with 16 (26.7%) and 11 (18.3%) respectively (Table 1).

The prevalence of ASB was higher among the HIV positive group 35 (54.7%), compared to the HIV negative group 29 (45.3%) but the difference was not statistically significant. The overall prevalence is 64 (53.3%) (Table 2).

The antibiotic with the best sensitivity in both groups of women were Ciprofloxacin 33 (27.5%), Levofloxacin 25 (20.8%), Gentamycin 22 (18.3) and Amoxil and Augmentin having the same sensitivity 16 (13.4%) (Table 3).

Table 1: Age distribution of the study population.

Variables	HIV status			Test statistic	P value
	Positive (n=60)	Negative (n=60)	Total (n=120)		
Age (years)	**N (%)**	**N (%)**	**N (%)**		
<20	5 (8.3)	9 (15.0)	14 (11.7)	X^2=1.241	0.538
20-24	10 (16.7)	8 (13.3)	18 (15.0)		
25-29	12 (20.0))	10 (16.7)	22 (18.3)		
30-34	17 (28.3)	22 (36.7)	39 (32.5)		
35 and above	16 (26.7)	11 (18.3)	27 (22.5)		

Table 2: Prevalence of ASB among the HIV positive and negative pregnant women.

Asymptomatic bacteriuria	Present (%)	Absent (%)	Total (%)
HIV positive	35 (54.7)	25 (44.6)	60 (100.0)
HIV negative	29 (45.3)	31 (55.4)	60 (100.0)
Total	64 (53.3)	56 (46.7)	120 (100.0)

X2=5.040, p=0.411

Table 3: Antibiotic sensitivity pattern for the isolated group of organisms.

Variables	HIV positive		HIV negative		Total		
Antibiotic	**Sensitive N (%)**	**Resistant N (%)**	**Sensitive N (%)**	**Resistant N (%)**	**Sensitive N (%)**	**Resistant N (%)**	**P value**
Amoxil	11(18.3)	49 (81.7)	5 (8.3)	55 (91.7)	16 (13.3)	104 (86.7)	0.107
Augmentin	14 (23.3)	46 (76.7)	2 (3.3)	58 (96.7)	16 (13.3)	104 (86.7)	0.001
Levofloxacin	17 (28.3)	48 (71.7)	8 (13.3)	52 (86.7)	25 (20.8)	95 (79.2)	0.043
Peflacine	8 (13.3)	52 (86.7)	7 (11.7)	53 (88.3)	15 (12.5)	105 (87.5)	0.783
Rifampicin	3 (5.0)	57 (95.0)	5 (8.3)	55 (91.7)	8 (6.7)	112 (93.3)	0.464
Nalidixic acid	1 (1.7)	59 (98.3)	2 (3.3)	58 (96.7)	3 (2.5)	117 (97.5)	0.559
Streptomycin	8 (13.3)	52 (86.7)	5 (8.3)	55 (91.7)	13 (10.8)	107 (89.2)	0.378
Chloramphenicol	4 (6.7)	56 (93.3)	8 (13.3)	52 (86.7)	12 (10.0)	108 (90.0)	0.224
Amplicox	1 (1.7)	59 (98.3)	2 (3.3)	58 (96.7)	3 (2.5)	117 (97.5)	0.559
Norfloxacin	2 (3.3)	58 (96.7)	3 (5.0)	57 (95.0)	5 (4.2)	115 (95.8)	0.648
Septrin	5 (8.3)	55 (91.7)	5 (8.3)	55 (91.7)	10 (8.3)	110 (91.7)	1.000
Ceporex	7 (11.7)	53 (88.3)	2 (3.3)	58 (96.7)	9 (7.5)	111 (92.5)	0.083
Tarivid	8 (13.3)	52 (86.7)	3 (5.0)	57 (95.0)	11 (9.2)	109 (90.8)	0.114
Ciprofloxacin	20 (33.3)	40 (66.7)	13 (21.7)	47 (78.3)	33 (27.5)	87 (72.5)	0.152
Gentamycinn	14 (23.3)	46 (76.7)	8 (13.3)	52 (86.7)	22 (18.3)	98 (81.7)	0.157
Ampicillin	2 (3.3)	58 (96.7)	0 (0.0)	60 (100.0)	2 (1.7)	118 (98.3)	0.154

Echerechia coli were the commonest organism isolated in both HIV positive and HIV negative women. 21 (36.6%) and 15 (25.0%) respectively and 25 (43.8%) and 31 (51.7%) did not culture any organisms in both groups respectively (Figure 1).

Gram staining status assessment revealed only S. *aureus* was gram positive while others were gram negative and *E. coli was* the commonest organism cultured in 36(30.8%) followed by *Providencia spp* 14 (11.9%), *Prot. Mirabilis* 9 (7.6) *S. aureus* 3 (2.5%) (Figure 2).

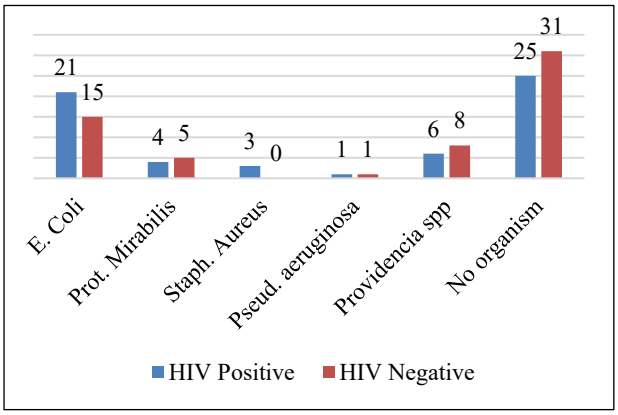

Figure 1: Distribution of organisms cultured in both groups.

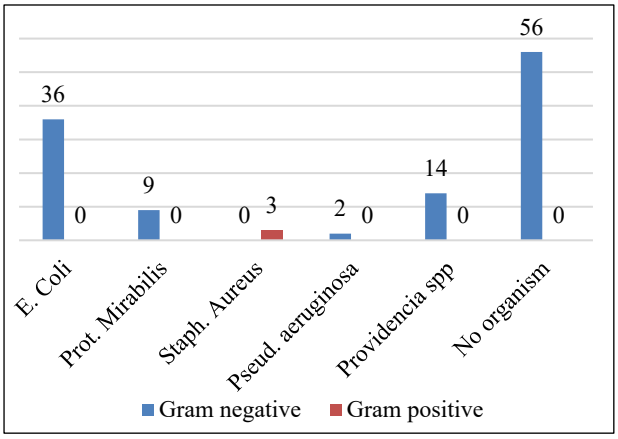

Figure 2: Bacterial isolates from culture and microscopy in both groups of patients.

DISCUSSION

In this study, the overall prevalence of ASB is 53.3% with HIV positive pregnant women having a higher prevalence of 54.7% against HIV negative women having 45.3%. This prevalence is comparable to a study at the Lagos State University Teaching Hospital (LASUTH) which show that 21.1% of the population had significant bacteriuria, while 57.8% of those with significant bacteriuria had asymptomatic bacteriuria.[5] Lower prevalence were recorded in Nnewi et al (19.5%) and in some African countries like Ethiopia (19.9%), Ghana (17.5%) and Turkey (8.5%).[6,16-18] In HIV positive pregnant women the prevalence of ASB in this study is 58.3%. This is far higher than study done in Ibadan (15.5%) and 18.1% in Lagos, at a large Prevention of Mother to Child Transmission (PMTCT) of HIV clinic.[10,11] This is also higher than that of Sagamu (31.3%).[7]

Comparing ASB in HIV positive and negative pregnant women in this study, there is no statistical significant difference in the prevalence of ASB between HIV positive (58.3) and HIV negative (48.3) pregnant women (p=0.411). A study done in Enugu, showed a statistically significant difference in the prevalence of ASB between the HIV positive (23.3%) and HIV negative (10.4%) groups and this was attributed to the suppression in their immunity.[8] However, in this study the high p-value may be attributed to the low sample size. Another study done in Tyreberg South Africa showed no statistical difference in prevalence of ASB between HIV-positive (9.2%) and HIV-negative (7.9%) subjects possibly because the HIV positive patients used in this study were not immune-compromised even though they were not on anti-retroviral therapy at the time of diagnosis.[21,22]

The strength of the research includes identification of bacterial colony with the use of a Microbact kit® (Oxoid, Basingstoke, UK) together with gram staining and microscopy and colonial morphology and not just by using a few commonly available biochemical tests and colonial morphology alone.[4,23] The microbact kit though expensive, helps to identify gram negative rods to specie level. Also, two consecutive urine samples were analyzed before making a diagnosis to limit the possibility of contamination and analysis was commenced within 30minutes of collection to limit bacterial growth.

The Gram status assessment revealed only S. *aureus* was gram positive while others were gram negative and *E. coli was* the commonest organism cultured in 36(30.8%) followed by *Providencia spp* 14 (11.9%), *Prot. mirabilis* 9 (7.6) *S. aureus* 3 (2.5%) which is gram positive, and *Pseud aeruginosa* 2 (1.7). This agreed with foreign studies in Ghana which *Escherichia coli* was 36.8% (7/19) and was ranked as the most prevalent isolated organism followed by *Klebsiella* spp. (26.3%) and also in Uganda which most common isolates in descending order were *E. coli* (n=13, 46.4%) and *S. aureus* (n=9, 32.1%).[18,23,24] This is also similar to an Iranian study that support the fact that *E. coli* is the commonly isolated organism.[26] Nigerian study done in Jos and Ogun also isolated *Escherichia coli* as the commonest organism that causes asymptomatic bacteriuria in pregnant women.[20,22,29] However, many studies done in Nigeria isolated *Staphylococcus aureus* as the commonest cause of ASB These studies were done in Benin (54.5%), Ilorin (72%) Abakaliki (45.9%) and in Ibadan (41.3%).[10-12,17] The *E. coli* was also observed to be the commonest organism causing ASB in both the HIV positive and the HIV negative pregnant women. However, the staph. Aureus was common in HIV positive and none was found in HIV negative pregnant women and this may be due to the small sample size.

Antibiotics with the best sensitivity in this study are Ciprofloxacin 33 (27.5%), Levofloxacin 25 (20.8%), Gentamicin 22 (18.3%), Amoxycillin 16 (13.35) and this is similar to a study in Jos which Ciprofloxacin (85.7%) Sparfloxacin (85.7%), Augmentin (28.6%) and

Gentamicin (28.6%).[20,27] In the HIV positive patients, Ciprofloxacin is the antibiotics with the best sensitivity followed by levofloxacin Gentamycin and Augmentin while in the HIV negative pregnant women is also Ciprofloxacin 13 (21,7) but is followed by Levofloxacin, Chloramphenicol and Gentamycin which all have equal sensitivity 8 (13.5). There is a significant resistance of greater than 50% from both the HIV positive and the HIV negative pregnant women. The presence of resistant strains of bacteria may be a reflection and as a consequence of gross abuse of these antibiotics which are readily procured over the counter without prescription.

This study has few limitations. The original criterion for diagnosis, required bacteria counts of >105/ml on two consecutive clean catch samples as was done in the current research. In addition, antibiotics that were safe in pregnancy were selectively picked for the sensitivity testing as against the routine antibiotics commonly used for the general non-obstetric population which limits therapeutic options. Despite these, the convenience sampling method used could have introduced some form of bias, in addition to the study being a hospital-based study. Patients reporting to the hospital are likely to differ systematically from patients seeking alternative treatments. Therefore, it may not be totally representative of the general population.

CONCLUSION

Asymptomatic bacteriuria is quite common in this study population with an overall prevalence of 53.5% and an insignificantly higher prevalence in the HIV positive pregnant women. This signifies that there is a need to screen and treat pregnant women for ASB. The Gram negative bacteria are the common causative agents of ASB in this study. The antibiotics with the high sensitivity were Ciprofloxacin, Levofloxacin and Augmentin. However, there is gross resistance of greater than 50% in all the antibiotics used in both groups with higher resistance in the HIV negative group.

Recommendations

Routine screening for and treatment of asymptomatic bacteriuria in pregnancy should be an integral part of obstetric care especially in the third trimester and should be included in antenatal guidelines in settings where it isn't currently the practice. There is also the need to educate our pregnant women and the general population on the need to avoid abuse of antibiotics which may lead to development of resistance.

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