



Pattern of microbial isolates and microbial sensitivity among HIV positive pregnant women with asymptomatic bacteriuria in Zaria, Nigeria

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ABSTRACT

Background: Asymptomatic bacteriuria in pregnancy is a common condition affecting pregnant women because of both anatomical and physiological changes in pregnancy. This condition appears to be commoner in people living with HIV because of the added immunosuppression caused by the virus. **Aim:** The study was to identify the pattern of microbial isolates and microbial sensitivity among HIV positive pregnant women with asymptomatic bacteriuria in Zaria, Nigeria. **Methods:** This was a prospective cross sectional study among symptom-free HIV positive pregnant women attending the prevention of mother to child transmission of HIV (PMTCT) antenatal clinic in Zaria, Nigeria between 1st March and 31st August 2011. A structured, close-ended questionnaire was administered and mid-stream urine samples were obtained and processed within 2 hours of collection in the laboratory. **Results:** A total of 220 consenting, asymptomatic, HIV positive pregnant women were screened for bacteriuria out of the 240 eligible women who were approached to participate in the study. Sixteen (16) women were positive for significant bacteriuria, giving a prevalence of 7.3%. A total of six (6) different isolates were isolated with *Staphylococcus aureus* (8) making 50%, *E. Coli* (4) 18.8%, *Klebsiella spp* (2) 12.5%, *Streptococcus spp* (2) 12.5% and *Proteus spp* (1) 6.2%. All the isolated organisms were sensitive to gentamycin, cefuroxime, ceftazidime, ciprofloxacin and nitrofurantoin. **Conclusion:** This study found *Staphylococcus aureus* as the most common organism isolated in the urine of asymptomatic patients with HIV infection in pregnancy. The isolated organisms were sensitive to gentamycin, cefuroxime, ceftazidime, ciprofloxacin and nitrofurantoin.

Key words: HIV, Asymptomatic bacteriuria, Microbial isolates, mid-stream urine, immunosuppression

INTRODUCTION

HIV and AIDS pandemic is one of the serious health crises in Nigeria^[1,2] and indeed the world. HIV attacks the white blood cells called

CD4 cells that protect the body from illness.^[1] Over time, the body's ability to fight common infection is lost and the person becomes immunocompromised thus is susceptible to opportunistic infections.



Infections of the urinary tract are one of the commonest infections affecting pregnant women for which antibiotics are prescribed.^[2-5] These infections can be overt: cystitis, acute pyelonephritis or occult, asymptomatic bacteriuria.

Asymptomatic bacteriuria is defined as the quantitative growth of bacteria greater than or equal to 10^5 colony forming units per milliliter of urine of the same organism, on aseptically collected midstream urine specimen in the absence of symptoms.^[6] This condition appears to be commoner in people living with HIV infection because of the added immune suppression caused by the virus.^[3,7]

Microorganisms causing urinary tract infection, UTI, vary in their sensitivity to antimicrobials. In most studies the commonest urinary isolate was *E. coli*.^[3,4,8-11] However, few studies reported *Staphylococcus aureus* as the commonest.^[12] In Zaria, Ehinmidu isolated *Pseudomonas aeruginosa* as the commonest isolate.^[13] The knowledge about UTI-causing pathogens and their sensitivity patterns would guide clinicians in making an appropriate choice of empirical treatment especially in HIV positive pregnant women to reduce the possible development of drug resistant.

The study was to identify the pattern of microbial isolates and microbial sensitivity among HIV positive pregnant women with asymptomatic bacteriuria in Zaria, Nigeria.

METHODOLOGY

This was a cross sectional study, carried out among consecutive, consenting HIV positive pregnant women attending the PMTCT antenatal clinic of ABUTH, Zaria, Nigeria between 1st March and 31st August 2011. During the 6 months period, a total of 2,672 pregnant women were seen in the antenatal clinic, out of this 141 were new enrollees to the PMTCT program. A total of 220 consenting, asymptomatic, HIV positive pregnant women were screened for bacteriuria out of the 240 eligible women who were approached to participate in the study. Two specimens were reported as contaminants, 5 questionnaires were discarded because of improper completion and 13 specimens were declared missing in the laboratory (period coincided with

industrial action by laboratory scientists). Therefore a total of 20 subjects were discarded during the course of the study. Thus only 220 subjects ultimately constituted the study population and were analyzed.

Informed consent was obtained from each participant. Inclusion criteria were all HIV positive pregnant women who gave a verbal consent to participate in the study, absence of symptoms of overt urinary tract infection like, fever, loin pains, dysuria, frequency, urgency, nocturia. Those who withheld consent were excluded from the study, as well as all HIV positive pregnant women who were currently on antibiotics or utilized antimicrobial treatment in the past 2 weeks. Those who had symptoms of acute pyelonephritis or presence of fever and any 2 of dysuria, nocturia, loin pains, frequency or urgency, those diagnosed to have UTI, whether treated or not in the index pregnancy and those diagnosed with chronic renal disease, sickle cell disease or diabetic mellitus were also excluded.

The study questionnaire was directly filled by the literate subjects, while the less literate ones had theirs completed by the researcher. Additional study information was sourced from the patients' case files: the most recent CD4 cell count, current viral load and the current antiretroviral drugs the patient was using.

A single midstream urine specimen was collected from eligible subjects. The container was tightly closed with its lid and submitted to the researcher, who appropriately labeled it. All the urine specimens collected were transported from the antenatal clinic in an ice packed container to the laboratory and processed within two hours of collection. On arrival in the laboratory, the samples were registered. They were then moved to the inoculation room with minimal delay. Using an aseptic technique, microscopy, culture and sensitivity was carried out on all urine samples collected.

The inoculated plates were then incubated in air at 37°C for 18-24 hours after which the bacterial colonies were then counted and multiplied by 1000 to get the value in CFU/ml. "Significant growth" was adjudged from counts of greater or equal to 100,000 CFU/ml. The significant growths were then processed further by performing a gram stain. For gram

positive organisms, catalase and coagulase tests were performed for identification. For gram negative organisms, Triple Sugar Ion (TSI), urea, citrate, indole and oxidase tests were done to identify the organisms.

A sensitivity testing was also done on Significant Isolates using the Clinical Laboratory Standard Institute (CLSI) technique^[14] and the results were interpreted as sensitive or resistant by measuring the zone diameters of inhibition. Having done the inoculation, the sample of urine was well mixed inside the universal bottle and dispensed into a centrifuge test tube and spun at 3,000 revolutions per minute (rpm). The supernatant was decanted, the sediment or deposit are then tapped on a clean grease free slide, covered with a cover slide and visualized under the microscope for identification of cells using $\times 10$ (panoramic view) and $\times 40$ (count the cells) eye piece of the microscope. Significant pyuria was when 3-5 white blood cells (WBC) are seen per high power field (phf).

The results were then issued out to the patients who were treated with the prescription of antimicrobials to which sensitivity of the bacterial organisms were indicated.

Subjects that were used for this study did not bear any cost for investigation or treatment. Those who returned negative results for asymptomatic bacteriuria were reassured and continued the PMTCT programme as per the National guidelines, while those with significant bacteriuria were treated with appropriate antimicrobials and followed up till delivery, with the conduct of repeat urine microscopy and culture at their subsequent trimesters of pregnancy, also at no cost to the patients.

The ethical approval for the study was sought and obtained from the Hospital Research and Ethical committee. Ethical standards were adhered to in the collection, handling and processing of samples throughout the conduct of the study.

Statistical analysis

A database was developed using the computer software, Statistical Package for the Social Sciences (SPSS) version 17. Cleaning of the completed questionnaires was done before

entering the data into the database, a further cleaning was also done on the electronic data. This was then followed by descriptive analysis and interpretation.

RESULTS

A total of 220 consenting, asymptomatic, HIV positive pregnant women were screened for bacteriuria out of the 240 eligible women who were approached to participate in the study. Two specimens were reported as contaminants, 5 questionnaires were discarded because of improper completion and 13 specimens were declared missing in the laboratory (period coincided with industrial action by laboratory scientists). Therefore a total of 20 subjects were discarded during the course of the study. Thus only 220 subjects ultimately constituted the study population and were analyzed. During the six months (1st March - 31st August, 2011) period of the study, a total of 2,672 Antenatal patients were seen in the ANC (1,542 HIV negative and 1,130 HIV positive) and 141 were new enrollees into the PMTCT program. The Clinic HIV Prevalence during the study period was therefore 5.3%. It is noteworthy that the period of this study overlapped the introduction of Nigeria's 2010 National PMTCT Guidelines, which introduced the triple ARV prophylaxis and shifted eligibility for ART treatment from CD4 of 250 to > 350 cell/mm³, among others. As such subjects categorized as being on "ARV prophylaxis comprised those on single, dual or triple prophylaxes.

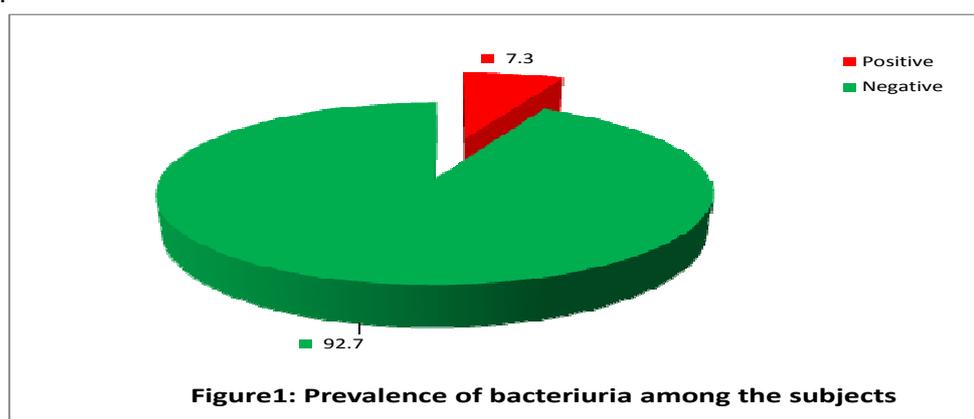
The ages of the women studied ranged between 18 and 40 years, with a mean age of 29.3 years. Most of the subjects were in the age group 25-29 years (37.7%), while the age groups 15-19 and 40-44 years were the least with 2.7%. About half of the respondents were of the Hausa/Fulani ethnic group (53.2%), closely followed by the different ethnic groups of southern Kaduna (15.9%). Also from the study, most of the subjects' highest level of educational attainment was the secondary school or higher (66.4%), while less than 10% had no formal education. More than half of the subjects were not gainfully employed and were Housewives (54.1%) and less than a quarter were civil servants. Almost all the subjects were married at the time of the study (97.3%) and 2.3% were widowed or separated. Majority of the subjects (65.5%) were in their first order

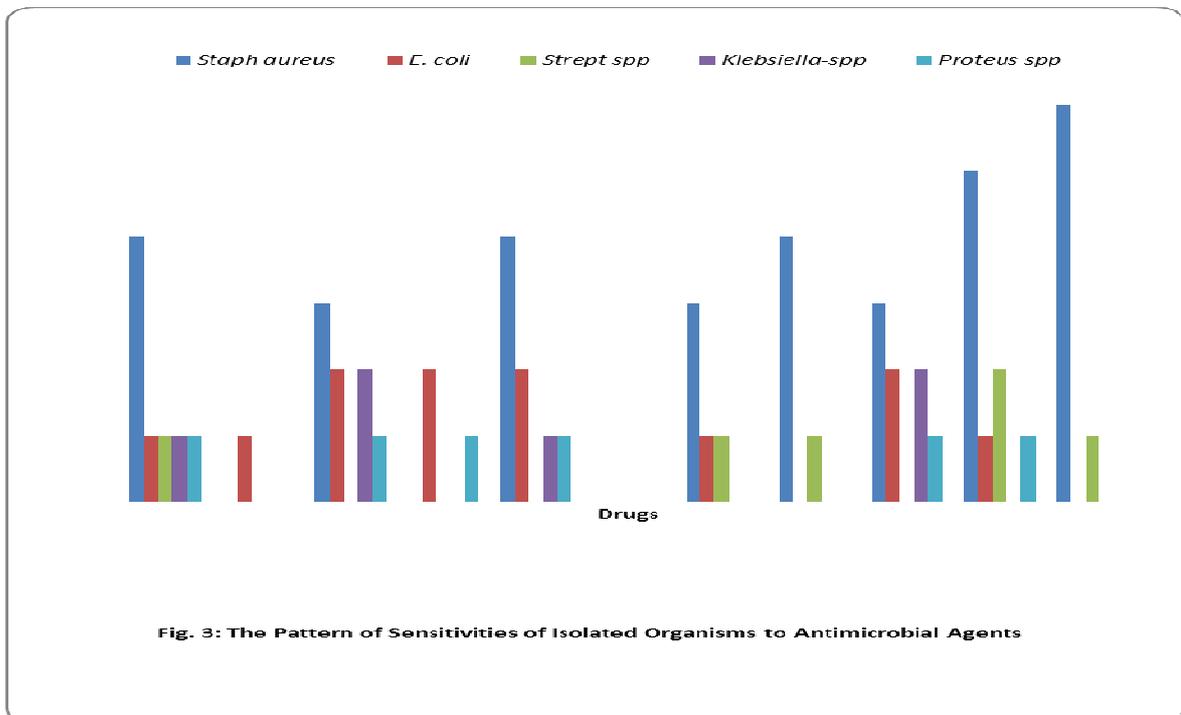
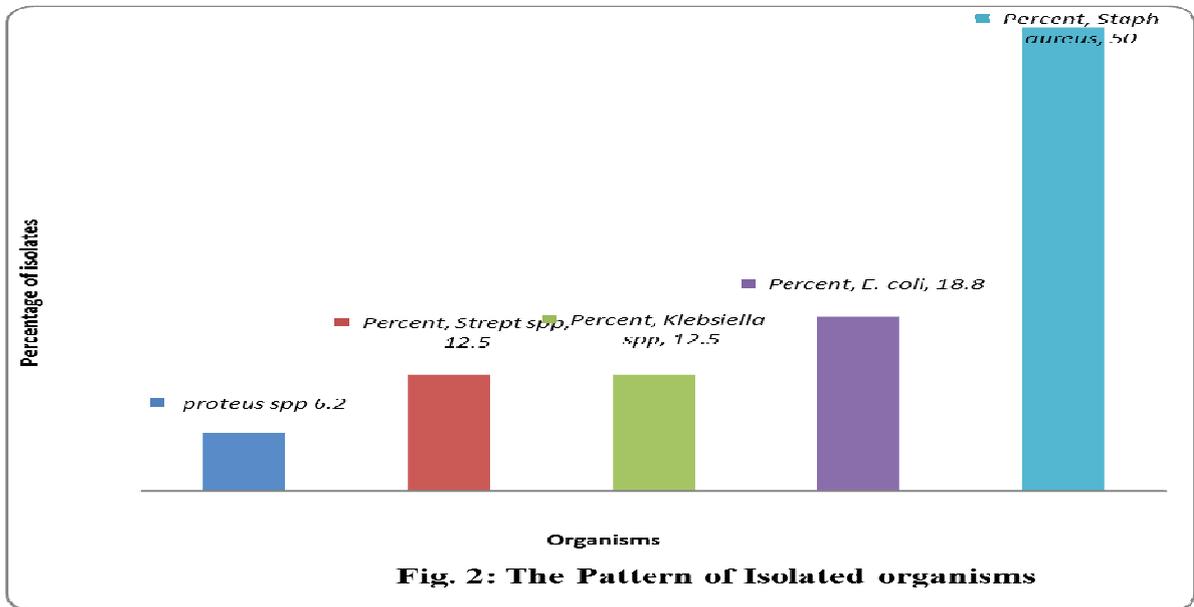
of marriage, while 34% had been in a number of previous marriages, while less than 1% were not married. The highlights of the socio-

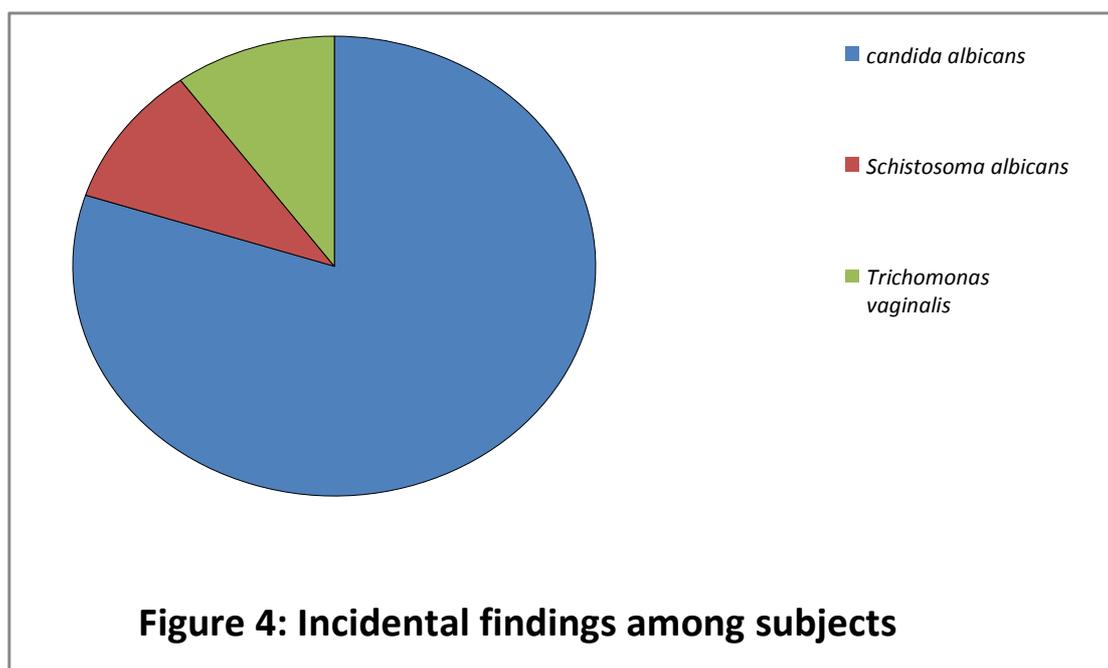
demographic profile of the respondents is presented in table 1 below.

Table 1: Demographic variables of the subjects in frequencies and percentages

Variables	Variable options	Frequency n=220	Percent
Age (yrs)	15-19	6	2.7
	20-24	27	12.3
	25-29	83	37.7
	30-34	64	29.1
	35-39	34	15.5
	40-44	6	2.7
Ethnicity	Hausa/Fulani	117	53.2
	Igbo	18	8.2
	Yoruba	9	4.1
	Southern Kaduna	35	15.9
	Edo	5	2.3
	Igala	9	4.1
	Others	27	12.3
Highest Education Level Attained	None	14	6.4
	Quoranic	23	10.5
	Primary	37	16.8
	Secondary	68	30.9
	Tertiary	78	35.5
Occupation	House wife	119	54.1
	Civil servant	39	17.7
	Petty trader	36	16.4
	Seamstress	12	5.4
	Student	13	5.9
	Applicant	1	0.5
	Order of marriage	Not married	1
	First	144	65.5
	Second and above	75	34.0







Out of the 220 women examined for asymptomatic bacteriuria, 16 were positive for significant bacteriuria, giving a prevalence of 7.3% among the HIV positive pregnant women studied. This is represented in figure 1 above.

The bacteria isolated are shown in figure 2 above. The dominant bacteria were *Staphylococcus aureus* (50%) and *E. coli* (18.8%). The other isolates were *Klebsiella spp* 12.5%, *Streptococcus spp* 12.5% and *Proteus spp* 6.2%. There were no mixed cultures isolated in all the samples.

Among the study subjects, almost a third (61) had significant pyuria (WBC ≥ 3 phf) without significant bacteriuria. Additionally, 81.3% (13) of the HIV positive pregnant women with significant bacteriuria had concomitant significant pyuria.

The isolated organisms were subjected to drug sensitivity test; a total of 11 different drugs were used. Figure 3 above is a composite representation of the different sensitivity pattern of the different isolated organisms to the tested antimicrobial agents.

All the isolated organisms showed 100% sensitivity to gentamycin, 80% to cefuroxime, ceftazidime, ciprofloxacin and nitrofurantoin. *Staphylococcus aureus* that was the most commonly isolated organism in the study was

sensitive to 72.7% of the drugs tested; also *E.coli* which was the second commonly isolated organism was sensitive to 72.7% of the drugs tested.

All the isolated organisms were resistant to ampicillin. The *Klebsiella spp* were resistance to two-thirds of the drugs tested.

Incidental findings during the study period included: diagnosis of *Candida albicans* in 8 samples and *Schistosoma haematobium* and trophozoites of *Trichomonas vaginalis* in one sample each. The subjects concerned were treated for their respective diagnoses. None of the subjects with these incidental findings had significant bacteriuria (figure 4).

DISCUSSION

Urinary tract infection results from a complex interaction of host factors and the infecting organism. There are defense mechanisms preventing urinary tract infection; like the vaginal and urine PH, the normal vaginal flora, `wash out` effect of the out flowing urine and the mucopolysaccharide lining produced by the urothelium. Some of these defense mechanisms become defective in the presence of HIV infection because of the immunocompromised state produced by the infection. HIV infection in pregnancy is one of the commonest medical conditions

complicating pregnancy and is the leading global cause of death in women of reproductive age.^[1]

The prevalence of asymptomatic bacteriuria was 7.3% among HIV positive pregnant women attending the PMTCT antenatal clinic of ABUTH, Zaria, Nigeria. The most common bacterial isolates were *Staphylococcus aureus* (50%) and *E.coli* (18.8%). This was similar to that reported by Ugbogu *et al.* in 2010 and Akinloye in Ibadan Nigeria.^[12,15] Though these studies were among HIV free pregnant women. Most studies reported *E.coli* as the commonest isolate.^[2,4,8-11] The study by Awolude in Ibadan, Nigeria was particularly on HIV positive pregnant women.

In this study, the isolates were sensitive to gentamycin, cefuroxime, ceftazidime, ciprofloxacin and nitrofurantoin. This was similar to the Ibadan, Nigeria study. However, it differs from a study in Jos, Nigeria^[7] where most of the isolated organisms were resistant to the commonly used antibiotics in both the HIV negative and positive group. Ojoo *et al.* also^[16] reported resistant to the commonly used drugs, although the study was conducted on HIV positive commercial sex workers. Ehinmidu in Zaria, Nigeria, reported urinary isolates were highly sensitive to ciprofloxacin and gentamycin,^[13] as seen in this study.

All the isolated organisms were resistant to ampicillin. This was similarly reported by Awolude, and other researchers.^[3,17,18] Probable explanation for this resistance may rest in the differences in the level of exposure to antibiotics, including their abuse, which ampicillin which is cheap and readily available may be prone to abuse and subsequently the high level of resistance.

Although none of the incidental findings of *Candida albicans*, *Schistosoma haematobium* and trophozoites of *Trichomonas vaginalis* were positive for significant bacteriuria, these pathogens should also be sought for in the urine of HIV positive pregnant women. Their presence alone can unsuspectingly worsen the morbidity in these HIV positive pregnant women.

CONCLUSION

This study found *Staphylococcus aureus* as the most common isolated organism in the urine of symptom free HIV positive pregnant women. The bacterial isolates in this study showed high susceptibility to the commonly used antimicrobials and antibiotics in our environment, namely: gentamycin, cefuroxime, ceftazidime, ciprofloxacin and nitrofurantoin. There was a high microbial resistant to ampicillin. Urine microscopy culture and sensitivity study should be routinely performed at least once and as early as possible on HIV positive pregnant women and the treatment should be bacterial culture and sensitivity specific, as far as resources avail. Pathogens like *Schistosoma haematobium*, trophozoite of *Trichomonas vaginalis* and *Candida albicans* should also be sought for in the urine of HIV positive pregnant women, as were accidentally detected in this study. Their presence alone can unsuspectingly worsen the morbidity in these HIV positive pregnant patients.

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Conflict of Interest: None declared

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