PREVALENCE OF SIGNIFICANT ASYMPTOMATIC BACTERIURIA IN HIV-POSITIVE INDIVIDUALS UNDER ANTIRETROVIRAL THERAPY

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ABSTRACT

Human immune deficiency virus patients are likely predisposed to urinary tract infections due to the suppression of their immunity and women in this category tend to get infected more often because of their anatomy. A research to assess the prevalence of significant asymptomatic bacteriuria among HIV-positive patients currently undergoing HIV antiretroviral therapy (HART) was carried out. Fifty HIV-patients on ART in a private comprehensive referral hospital in Uyo, Nigeria were screened by re-validating their post therapeutic serological status using ELISA kit, "determine". Mid-stream urine samples were obtained and analyzed by microscopy, culture and antibiotic susceptibility testing. All the patients were still seropositive and showed significant asymptomatic bacteriuria (log CFU/ml > 5). Sex distribution of the patients showed more female than men. Fifty percent of the patients were married, while 26 % of the patients were in the largest age range, 36-40 years. *Escherichia coli* (36%) was the most predominant bacterium isolated followed by *Staphylococcus aureus* (34 %), *Klebsiella pneumoniae* (16 %) and *Pseudomonas aeruginosa* (14 %). The bacterial isolates from patients on antiretroviral therapy showed 10-80 % resistance to Cefixime, Amikacin, Gentamycin, Imipenem, Ciprofloxacin, Ampicillin, Oxacillin, Streptomycin, Amoxicillin and Tetracycline, and also had a high MAR index ≥ 0.25. This study has shown that HIV-positive patients on HART have highly significant asymptomatic bacteriuria despite HIV chemotherapeutics. There is need to monitor patients' drug compliance, viral load and possible HART resistance, as well as regular urine analysis to enable prompt measures to be taken in order to avoid difficult-to-treat urinary tract infection

INTRODUCTION

Asymptomatic bacteriuria (ASB) implies the detection of significant bacterial counts of $\geq 10^5$ colony-forming units per milliliter (CFUs/ml), in the urine of individuals showing no infection symptoms or signs (Nicolle *et al*., 2005; Nelius *et al*., 2011; Banu and Jyothi, 2013). Urinary tract infections (UTIs) are however rampant among immunocompromised or immunosuppressed patients especially those with HIV/AIDS (Schönwald *et al*., 1999). Urinary tract infections are very common infections especially in localities with defective toilet hygiene. Frequently reported diagnosis revealed that most UTIs in Nigeria have bacteria as the aetiological agent, which might be accompanied with complications like pyelonephritis and sepsis in immunocompromised patients (Barber *et al*., 2013; Kayima *et al*., 1996).

Human immunodeficiency virus (HIV) is the aetiological agent of the Acquired immunodeficiency syndromes (Prescoott *et al.*, 1999). HIV is one of the human T-cell lymphotrohic retrovirus human T-cell leukemia (Fleming,1984; Abimiku *et al*.,1994). The human immunodeficiency virus (HIV) has emerged as a global health problem, with serious medical, economic and social implications. The human immunodeficiency virus (HIV) epidemic continues to be a burden globally and presents serious public health problems in developing countries including in Nigeria. Low CD4 counts have been said to put HIV-positive patients at higher risk of bacteriuria (Banu and Jyothi, 2013). Hoepelman *et al*. (1992) also noted a similar

finding. The prevalence of asymptomatic bacteriuria in HIV-positive individuals varies depending on the population studied (Banu and Jyothi, 2013; Widmer *et al*., 2010; De Pinho *et al*., 1994 and Awolude *et al*., 2010). De Pinho *et al.* (1994) for example, reported a prevalence of 3% in asymptomatic HIV-positive individuals and 13% in those with AIDS.

HIV epidemic affects female severely in the Sub-saharan African sub-region and women of reproductive age make up 57% of adults living with HIV (Uneka *et al.*, 2007). People living with human immune deficiency virus are likely to be predisposed to urinary tract infections due to the suppression of their immunity and women in this category tend to get it more often because of their anatomy (Bakke and Diagranes, 1991; Kayima *et al.*, 1996). With the advent of high antiretroviral therapy (HART) which has shown to have an indirect effect, this has improved considerably the health and life expectant of people who are HIV positive (Spence *et al.*,1995). Masliah *et al.*, (2002) reported the cause of death of HIV-infected persons revealed a decrease in opportunistic infections but an increase in bacterial infections among patients on HAART. A study at the London Royal Hospital, London revealed (UTIs) to be more commonly among elderly patients compared to younger age patients with HIV infection (Evans *et al.*, 1995) while another study by Ibadin *et al*. (2006) revealed HIV/AIDS to be predisposing factor for the increased incidence of urinary tract infection.

Nigeria has a high burden of HIV infection with the 2013 prevalence estimate as 3.2% (Olowe *et al.*, 2015) and considering its large population size, this constitutes a significant population of individuals who are at risk of UTI and other complications following ASB. The present research was conducted to assess the prevalence of significant asymptomatic bacteriuria among HIV-positive patients currently undergoing HIV antiretroviral therapy (HART) in a private comprehensive referral hospital in Uyo, Nigeria

METHODOLOGY

This study was carried out in Uyo, Local Government Area which is the capital of Akwa Ibom state. Specifically it was carried out on patients attending a private hospital with capacity of being classified as a secondary health facility. The hospital where participants were recruited, provides both tertiary and secondary health care including HIV/AIDS care and support. Approval for the study was sought and obtained from the Chief Medical Director and Medical Team in charge of the hospital and identity of the subjects was kept confidential.

A total of 50 HIV-positive individuals receiving free HIV care at the HIV treatment center (IHVN) were recruited. Consecutive attendees on routine clinic visit who consented within the study period were enrolled. Sample size was determined based on the prevalence obtained from a similar study (Awolude *et al*, 2010) resulting in a minimum sample size of 50.

Informed consent was obtained from the patients before inclusion in the study. The nature of the study, the minimal risk involved in the procedure, and advantage of the research were explained to the patients. Those who were on antibiotics at the time of recruitment were excluded from the study.

Questionnaire was administered to each individual to obtain demographic and other social (predisposing) information of interest such as age, marital status, number of sex partners, suspected infidelity by spouse/sex partner and level of education. Blood and urine samples were collected from all participants. Appropriately labeled universal bottles were given to each participant for the collection of clean catch midstream urine specimen following explanation of the procedure for sample collection.

HIV-1 and HIV-2 Antibodies Assay

The blood collected was used for the rescreening for the presence of HIV-1 and HIV-2 antibodies in the blood samples collected, using a World Health Organization (WHO) approved kit called “determine” which is an Eliza-based kit. The kit was designed primarily to test HIV-1 and HIV-

2 antibodies in the blood. The Eliza-based kit is both sensitive and specific (99-100%) and was used according to manufacturer instruction. The kits were stored at 4ºC.

Bacteriuria Analysis

A measure of about 10 to 15ml of well mixed mid-stream urine collected were put in a test tube and placed in the centrifuge. It was centrifuged at low speeds of about 2,000 and 3,000 rounds per minute for about 7 min and the supernatant decanted (to retain 0.2-0.5 ml) inside the tube. The tube was shaken to mix the sediments and supernatant retained in the test tube. A sterile pipette was used to collect and place a drop of the re-suspended sediment on the microscopic slide and a cover slip was placed over the slide and observed under the microscope for pus cells, RBC, and other organisms. The bacterial enumeration in the urine sample obtained was carried out aseptically on Nutrient agar and MacConkey agar using the pour plate method in accordance with Cheesbrough (2006). The inoculated plates were incubated at 37^{o}C for 24 h. After 24 h of incubation, the colonies in each plate was then counted using Gallankamp colony counter (Biomereux, France).

Diagnosis was made based on significant bacteriuria which is greater than or equal to 10^5 colony forming units per milliliter (CFU/ml) of the same organism. Colonial morphology, Gram stain, standard biochemical tests, methyl red and Vogues-Proskauer test, citrate utilization test, coagulase test, catalase test, urease test, motility test, oxidase test and indole test were used to identify the organisms.

Antimicrobial Susceptibility Assay

Antimicrobial susceptibility testing was done using the multiple predetermined concentration of antibiotics impregnated filter discs according to Clinical Laboratory Standards Institute (CLSI, 2017) guidelines for antimicrobial susceptibility testing of the isolates for the following antibiotics: chloramphenicol, amoxicillin, cefuroxime, augmentin, gentamycin, sparfloxacin, cotrimoxazole, ciprofloxacin, and erythromycin

RESULTS

Fifty (50) HIV-positive individuals were enrolled. With respect to gender (sex), female showed higher seropositive than male. Within the sex group 44 females' patients were seropositive and this accounted for about 88 % (n=50) of the entire HIV-positive patients considered. Within the age group, they were highest seroprevalence (ratio of HIV positive) among the age group of (36-40) years with 26% (n=50) seroprevalence. The youngest age group (16-20) years were the least 4% (n=50). Other age group and their numbers as well as the percentage are shown in Table 1.

Table 1: Age Distribution among HIV- Positive Patients

Age Group	No. of Positive Patient	Percentage (%)
16-20	2	4
21-25	4	8
26-30	5	10
31-35	9	18
36-40	13	26
41-45	5	10
46-50	5	10
51-55	3	6
56-60	1	2
61-65	1	2
66-70	1	2
Unknown	1	2
Total	50	100

The social statistics of the sampled patients was noted and it varied with marriage or relationship status. It was observed that singles were 28%, while 25% were legally married by customs and

traditions. Information from the patients showed that about 10% were divorcee which already had children while 12% were widows. This is shown in Figure 1.

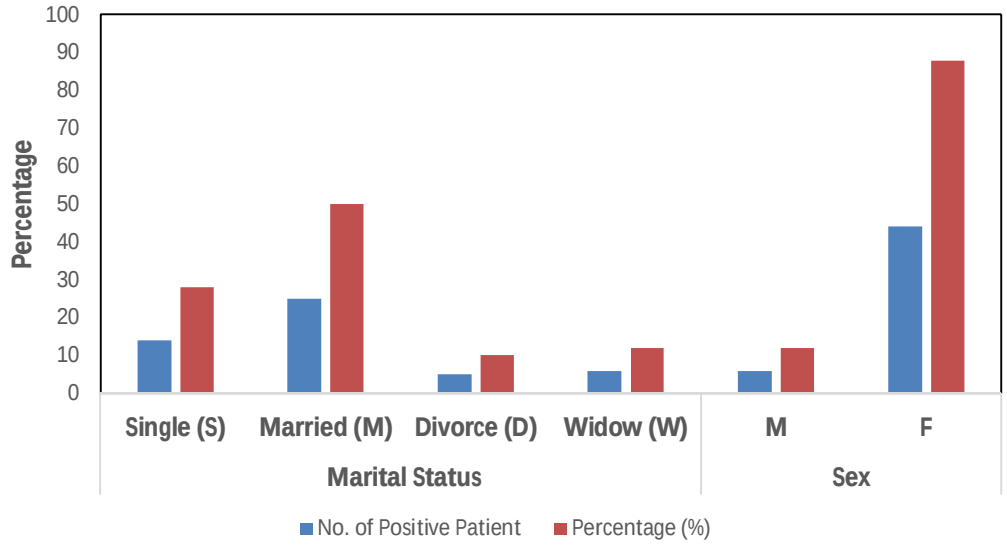

Figure 1: Gender and Marital Status of the HIV-positive patients

With respect to education and employment status, there were more self-employed individuals (76%; n=50) than the unemployed, students were the least (6%; n=50) observed under the employment status. Educational background was further grouped into different levels like the primary levels (18%; n=50), secondary level had (48%; n=50) followed by the tertiary levels (university, colleges, and polytechnics) which were 4%. This is shown in Figure 2.

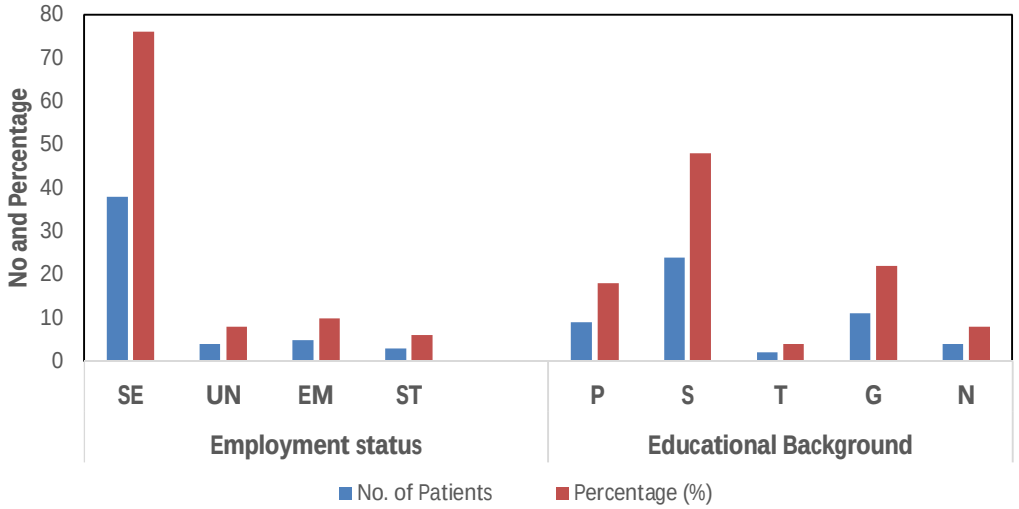

Figure 2: Educational Background and Employment status of the Patients **(**SE = Self Employed, UN = Unemployed, EM = Employed, ST= Student, P = Primary Level, S = Secondary Level, T = Tertiary Level, G = Graduate, N = None)

With respect to bacteriuria among the HIV- patients, all the 50 patients examined showed significant bacteriuria, with the count in log (CFU/mL) that ranged from 5.6 to 7.2. The results of cultural, morphological, biochemical tests carried out and probable identification showed that in terms of the occurrence of individual bacterial species among the patients, *Escherichia coli*

occurred in 36 %, *Staphylococcus aureus* in 34 %, *Pseudomonas aeruginosa* in 16% and *Klebsiella pneumoniae* which occurred least were 14% as shown below in Table 2.

Table 2: Occurrence of bacterial species among the patients

Bacteria	Number	Frequency of Occurrence %
Escherichia coli	18	36
Staphylococcus aureus	17	34
Pseudomonas aeruginosa	8	16
Klebsiella Pneumonia	7	14
Total	50	100

Based on the antibiotic susceptibility profile of bacteria isolated from the urine, the results that showed the percentage of the bacteria that were resistant and sensitive to the multidisc antibiotics used have been shown below based on the classifications of the disc used. Incidentally, it was observed that there was no zone of inhibition that interpreted into the intermediately resistant range for all the antibiotics.

Table 3. Percentage antibiotics susceptibility profile of gram-negative bacteria from HIV-patients' urine

Antibiotics	*Escherichia coli*		*Klebsiella pneumoniae*		*Pseudomonas aeruginosa*	
	S %	R %	S %	R %	S %	R %
GEN	80	30	80	20	90	10
IMEP	70	40	80	20	80	20
CIP	80	30	80	20	70	30
AMP	10	90	20	80	10	90
OXA	30	70	20	80	10	90
STR	20	80	20	80	20	80
AMX	10	90	70	30	30	70
TET	10	90	70	30	10	90
CE	30	70	70	10	90	10
AMI	20	80	90	90	10	85

Key: S % = Percentage Sensitivity, I % = Percentage Intermediate, R % Percentage Resistance, GEN= gentamycin, IMEP= imipenem, CIP = ciprofloxacin, AMP = ampicillin, OXA = oxacillin, STR= streptomycin, AMX = amoxicillin, TET= tetracycline, CE = cefixime, AMI = amikacin

Table 4: percentage antibiotics susceptibility pattern of gram-positive isolates of bacteria in HIV urine

Antibiotics	*Staphylococcus aureus*	
	S %	R %
GEN	20	80
IMEP	80	20
CIP	75	25
AMP	10	90
OXA	30	70
STR	20	80
AMX	15	85
TET	20	80
CE	80	20
AMI	80	20

Key: S % = Percentage Sensitivity, I % = Percentage Intermediate, R % Percentage Resistance, GEN= gentamycin, IMEP= imipenem, CIP = ciprofloxacin, AMP = ampicillin, OXA = oxacillin, STR= streptomycin, AMX = amoxicillin, TET= tetracycline, CE = cefixime, AMI = amikacin

Multiple antibiotic resistance (MAR) indices among all the four bacterial isolates (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella Pneumonia*) were higher than

0.2. Most of the strains of *E. coli* showed predominantly MAR index 0.5, while strains of *P. aeruginosa* showed least MAR indices that ranged from 0.25 to 0.3. This is shown in Figure 3.

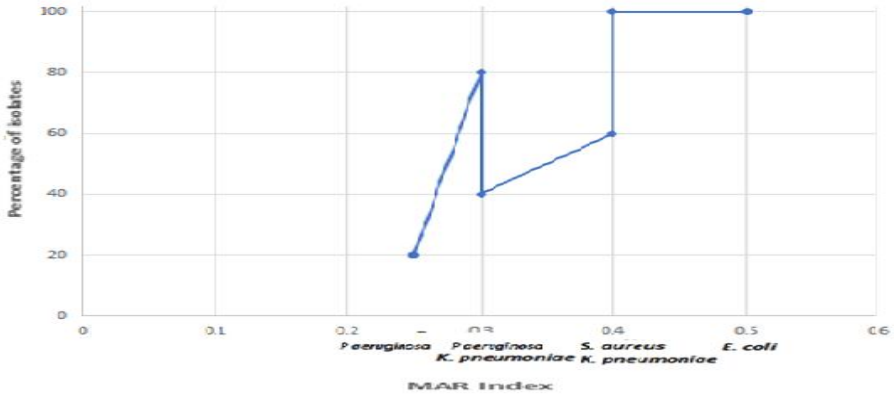

Figure 3: Observed MAR Indices and corresponding Percentage of Bacterial Isolates

DISCUSSION

This research evaluated the prevalence of asymptomatic bacteriuria among HIV positive individuals on antiretroviral drug therapy. We found a high prevalence rate of 26% asymptomatic bacteriuria in our study population, with at least one in five of them having this condition. This result appeared to be much higher than those reported in other parts of the country and globally as most studies report prevalence of 3–15% (Banu and Jyothiand, 2013; Widmer *et al*., 2010; De Pinho *et al*., 1994; Awolude *et al.*, 2010). Only a few studies have recorded such high prevalence as ours (Ojoo *et al*., 1996). Another study in this environment also reported a high prevalence of 18% among HIV-positive pregnant women (Ezechi *et al*., 2013). No sample was taken from HIV negative population; therefore, no comparison was made.

The usual trend in ASB is for the prevalence to be higher in the female population (Olowe *et al*., 2013), and our study also reported similar finding. We found the prevalence of ASB to be higher in women than men in keeping with reported trend which is attributed to the proximity of the urethra to the anus and its short length (Iduoriyekemwen *et al*., 2012) also in HIV/AIDS, the impaired immunity that occurs makes it easier for bacterial pathogens to adhere to the urinary epithelium (De Pinho *et al*., 1994).The prevalence of asymptomatic bacteriuria in the younger age group was almost twice as high as what was found in the older age group. This might be related to the sexual activity of the younger age group which is a recognized predisposition to bacteriuria and UTI (Hermann *et al*., 2002). It should also be borne in mind, however, that the older age group, especially the elderly, are also at risk of bacteriuria (Olowe *et al*., 2013; Hermann *et al*., 2002; Ariathianto, 2011).

Escherichia coli with a prevalence of 18 (36%) was the most prevalent organism, slightly higher in prevalence than *Staphylococcus aureus* 17 (34%), followed by *Pseudomonas aeruginosa* 18 (16%) and *Klebsiella Pneumonia* 7 (14%) which is the last. This differs from usual findings in studies on both HIV-positive and -negative individuals where *Klebsiella* spp. is more commonly isolated in hospitalized individuals; it is therefore probable that HIV-positive patients are at higher risk from infections due to hospital associated pathogens (Olowe *et al*., 2015). A similar finding with predominantly nosocomial pathogens was also reported by Gugino *et al*. (1998). In agreement with the findings of this work is the work of Nicolle (2000), De Pinho *et al*. (1994) and Awolude *et al* (2010) where *E. coli* usually predominates as the causative organism. The interference drawn in this study is limited to a small number of patients. These findings may be useful for further exploitation in large number of samples.

The antibiotic susceptibility pattern of these isolates was sought, due to reports that persons with HIV usually have urinary tract infections due to multidrug resistant organisms (Ojoo *et al*., 1996).

Our study corroborates this as most of the organisms showed moderate to high level resistance to commonly used first line antibiotics. In the present study, 10-90 % of Gram-negative organisms isolated from ART users were resistant to Ampicillin, Oxacillin, Streptomycin, Amoxicillin, Tetracycline, Cefixime, Amikacin, but were 70-80 % sensitive to Gentamycin, Imipenem, Ciprofloxacin. However, in similar study conducted in Nigeria, Benin City, isolates were only sensitive to Ciprofloxacin and Ofloxacin (Omoregie and Eghafona, 2009). High resistance to several antibiotics as well as MAR index > 0.2 implies that isolates are from high-risk sources (Suresh *et al.*, 2000) which was the case in this study, leading to high antibiotic resistance selective pressure (Suresh *et al.*, 2000).

CONCLUSION

The HIV-positive patients under ART studied in this research showed that asymptomatic bacteriuria is common in HIV-positive individuals. The serological status was still reactive despite the ART and that *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli* were the bacteria present in their urine. *Escherichia coli* was the most predominantly isolated among the HIV-positive patient under ART. Many of the isolated bacterial species were resistant to gentamycin, imipenem, ciprofloxacin, ampicillin, oxacillin, streptomycin, amoxicillin, tetracycline, cefixime, amikacin and also exhibited very high MAR index above standards.

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