



## Occurrence of Bacteria and Yeast in Urinary Tract of HIV/AIDS Patients Attending Antiretroviral Therapy (ART) Clinic at University of Jos Teaching Hospital, Nigeria

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### Abstract

Urinary tract infection is one of the infections observed among HIV patients and the cause of morbidity and hospitalisation in HIV positive individuals. The study aimed at determining the prevalence of Urinary tract pathogenic bacteria and yeast among HIV-infected individuals attending Anti-retroviral therapy (ART) clinic in Jos, Nigeria. Mid-stream urine samples collected from 240 HIV seropositive patients were cultured, isolates identified and antibiotic susceptibility profile determined using agar disk diffusion technique (Kirby-Bauer). Out of the 240 samples screened, 39(16.25%) had urinary tract infections (UTIs) and 33(16.20%) had yeast infection. The distribution of the isolates were as follows: *Escherichia coli* 12(30.77%), *Staphylococcus aureus* 10(25.64%), *Klebsiella pneumonia* 4(10.26%), *Enterobacter* sp. 4(10.26%), *Staphylococcus saprophyticus* 3(7.69%), *Citrobacter freundii* 2(5.13%), *Pseudomonas aeruginosa* 2(5.13%) and *Proteus mirabilis* 1(2.567%). Among the yeasts, *Candida albicans* showed the highest prevalence with 21(63.64%) while non-albican *Candida* sp had 12(36.36%). Age group 20-24 years with 1(33.3%) cases had the highest prevalence while bacterial infection was not detected among <19, 55-59 and ≥60 years. Females had a higher prevalence of 36(17.60%) compared to the males 3(8.33%). The antibiotic susceptibility patterns showed that gentamicin (10µg), augmentin (20µg), chloramphenicol (30µg), ciprofloxacin (5µg) and ofloxacin (5µg) were more active. The study revealed that uropathogenic bacteria and yeast were prevalent among HIV/AIDS patients. Routine screening of uropathogens should be incorporated in the management and care of HIV/AIDS patients in Nigeria.

**Key words:** Uropathogenic bacteria, Yeast cells, HIV/AIDS, Antibiotic susceptibility test.

### INTRODUCTION

Urinary tract infection (UTI) is defined as the microbial invasion of any of the tissues of the urinary tract and is a serious global health problem that affects millions of people each year (Jain *et al.*, 2015). People living with Human immunodeficiency virus (HIV) are likely to be predisposed to urinary tract infection due to the suppression of their immunity (Bakke and Digranes, 1991; Kayima *et al.*, 1996). Urinary tract infection is one of the infections observed among HIV patients and the cause of morbidity and hospitalization in HIV positive individuals (Iweribor *et al.*, 2012).

Bacterial uropathogens causing UTIs are commonly recorded among HIV/AIDS patients and the uropathogens commonly recovered were *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella* sp and *Staphylococcus aureus*. (Bigwan and Wakjissa, 2013). The advent of HIV/AIDS has resulted in many microbial agents becoming opportunistic pathogens and causing infections among individuals whose immune status has been suppressed by the infection.

These include common uropathogens such as *Escherichia coli*, *Proteus* sp., *Enterobacter* and *Klebsiella* sp, nosocomial

organisms such as *Pseudomonas aeruginosa*, *Streptococcus faecalis*, and *Staphylococcus aureus* and unusual microorganisms including *Candida* sp., *Salmonella* sp, *Acinetobacter* sp, and Cytomegalovirus (Schonwald *et al.*, 1999; Lee *et al.*, 2001; Bansil *et al.*, 2007; Ochei and Kolhatkar, 2007). UTI in HIV-positive patients tend to recur, requiring longer treatment and it is suggested that treatment should be culture-specific (Heyns *et al.*, 2005).

With the advent of Highly Active Anti-Retroviral Therapy (HAART), non-compliance to drug in-take and clinic dates may have caused UTIs to be on the increase among HIV positive individuals (Bansil *et al.*, 2007) There are limited data in this study area on UTI's among HIV/AIDS positive individuals on ART and urinary tract infection is a cause of significant morbidity among HIV infected subjects (Samuel *et al.*, 2012).

Therefore this study was aimed at isolating bacteria and yeast associated with urinary tract infections among HIV-infected individuals attending ART clinic in Jos and to determine the antibiotic susceptibility patterns of the bacterial isolates.

## Materials and Methods

### Study Area

The study was carried out at the AIDS Prevention Initiative in Nigeria-Jos University Teaching Hospital (APIN-JUTH) Jos, Nigeria.

### Study Population

Two hundred and forty (240) subjects comprising 36 male and 204 female HIV/AIDS patients aged  $\geq 18$  years attending the adult clinic of APIN-JUTH based on the calculated general prevalence of urinary tract infection among people living with HIV/AIDS in Plateau State and were randomly selected as they present themselves at the ART clinic for their regular follow up (Essien, *et al* 2013).

## INCLUSION AND EXCLUSION CRITERIA

The study included HIV/AIDS patients from  $>18$  years on ART (240 subjects) and those

patients who were not on antibiotics and consented to participate in the study. HIV/AIDS patients who were on antibiotics were excluded from this study.

## CONFIRMATION OF HIV/AIDS STATUS

A commercially available Abbott Determine HIV1 and 2 an *in-vitro* visually read qualitative immunoassay for the detection of antibodies to HIV1 and 2 in human serum, plasma or whole blood was used based on manufacturer's instruction while positive samples were confirmed using The Chembio HIV1 and 2 STAT-PAK; a single-use immune chromatographic test kit for validation of rapid HIV test results (CDC, 2014)

### Sample Collection and Processing

Clean catch mid-stream urine specimens were collected in sterile universal bottles and transported to the laboratory under ice packs for microbiological analysis as described by Cheesbrough (2010).

### Macroscopic examination of urine samples

Specimens were examined macroscopically for color; yellow (light/pale to dark/deep amber) and turbidity; clear to cloudy (Cheesbrough 2010., Edgar 2015).

### Microscopic examination of urine Specimens

Ten milliliter (10ml) of each urine specimen was aseptically transferred into a labeled centrifuged tube and spun at 1000 rpm for 5 minutes. The supernatant was decanted by completely inverting the tube and a drop of the thoroughly mixed sediment was transferred to a slide and covered with cover slip. The preparation was examined microscopically for pus cells, yeast cells, cast, crystals, epithelial cells and RBCs (Cheesbrough, 2010).

### Culture of urine specimens

Using aseptic techniques, loopful of each well mixed urine specimen was streaked on Cystein-Lactose-Electrolyte-Deficient Agar (CLED) and Sabouraud Dextrose Agar (SDA).

and incubated respectively at 37°C for 24 hours for bacterial isolation and at room temperature for 48 hours for fungal isolation. CLED Culture plates with colony count of  $1 \times 10^5$  CFU/ml only was considered significant for UTI. Isolates were identified using Gram's staining and standard biochemical test which included catalase, coagulase, urease, citrate, triple sugar iron agar and indole test (Iram *et al* 2012).

#### Staining of Yeast

Smear of suspected yeast isolates in the SDA plates was prepared by gently emulsifying colonies in a drop of Lactophenol cotton blue on a slide. The preparation was covered using a cover slip and examined microscopically initially using a low power objective lens and then switching to a higher power 40x objective for more detailed examination (Kauffman, 2005)

#### Germ Tube (GT) test

*Candida albicans* was differentiated from other yeast using germ tube test. A colony of yeast was picked and emulsified aseptically in 0.5 ml of human serum in a test tube and then incubated at 37°C for 2-4 hours. A drop of the mixture was examined microscopically under low and high power objectives. A short hyphal extension arising laterally from yeast cells with no constriction at the point of origin was considered positive for *Candida albicans* (Yasin *et al.* 1986).

#### Antibiotic Susceptibility Testing

The agar disk diffusion technique (Kirby-Bauer) was used for antibiotic susceptibility testing. Standardized inoculum (0.5 McFarland turbidity standard equivalents to  $1.5 \times 10^8$  CFU/ml) each of the test bacteria were spread on agar plates using a sterile glass rod and allowed to dry. The appropriate multi disk specific for Gram positive and for Gram negative bacteria (Gentamycin (10 µg) Cotrimoxazole (25 µg) Ciprofloxacin (5 µg) Cephalosporin (30 µg) Septrin (25 µg) Ofloxacin (5 µg) Chloramphenicol (30 µg) Tetracycline (30 µg) were then placed onto the surface of the dried inoculated plates using sterile forceps. The plates were left at room temperature for

1 hour to allow diffusion of the antibiotics from the disk into the medium (Iram, *et al* 2012). The plates were further incubated for 24 hours at 37°C. Interpretation of the results was based on diameter of zones of inhibitions which were measured in millimeter using transparent ruler (Iram, *et al* 2012)

#### Results

Out of the 240 urine samples examined, 39 (16.25%) bacterial species were recovered. The bacterial isolates included *Escherichia coli* 12 (30.77%), *Staphylococcus aureus* 10 (25.64%), *Klebsiella pneumoniae* 4 (10.26%), *Enterobacter* sp 4 (10.26%), *Staphylococcus saprophyticus* 3 (7.69%), *Citrobacter freundii* 2 (5.13%), *Pseudomonas aeruginosa* 2 (5.13%), *Proteus mirabilis* 1 (2.56%) and *Enterococcus* sp 1 (2.56%) (Table 1).

The distribution of bacterial UTI in relation to age showed that age group 20-24 had the highest prevalence of (33.30%) followed by age group 40-44 with (25.0%). Age groups <19, 55-59 and ≥60 had the least prevalence of (0.0%) each. A statistically non-significant relationship was observed among the age groups ( $p > 0.617$ ) (Table 2).

In relation to gender, the distribution of bacterial UTI revealed that the females had higher prevalence of 36 (17.60%) compared to the males 3 (8.33%). Statistically, no significant relationship was observed  $P > 0.05$  (Table 2).

A total of 33 yeast isolates were recovered. Of this number, *Candida albicans* had the highest frequency of 21 (63.64%) while the non-albicans yeast species collectively had 12 (36.36%) (Table 3). In relation to gender, the distribution of yeast infection revealed a prevalence of 33 (100.0%) for females while no male had yeast infection with prevalence of 0 (0.00%). A statistically significant relationship was observed in the prevalence of yeast infection in relation to gender ( $p < 0.05$ ) (Table 3). The antimicrobial susceptibility profile of the Gram positive and Gram negative bacterial isolates from the HIV/AIDS patients was depicted in Table 4.

Among the drugs tested, gentamycin (10µg) was found to be more active, 25 (64.1%) of the 39 isolates show sensitivity to the drug. All the Gram negative bacteria tested were found to be resistant to erythromycin (0.0%),

and all the Gram positive bacterial isolates were resistant to tetracycline and nalidixic acid (0.0%), while only *Enterococcus* sp was resistant to gentamycin (0.00%) but showed 100% sensitivity to augmentin.

**Table 1:** Occurrence of Bacteria in the Urine of Patients Attending ART Clinic at JUTH, Jos.

Organism	Number (%) isolated
<i>Escherichia coli</i>	12 (30.77)
<i>Staphylococcus aureus</i>	10 (25.64)
<i>Staphylococcus saprophyticus</i>	3 (7.69)
<i>Proteus mirabilis</i>	1 (2.56)
<i>Klebsiella pneumonia</i>	4 (10.26)
<i>Enterococcus</i> sp	1 (2.56)
<i>Enterobacter</i> sp	4 (10.26)
<i>Citrobacter freundii</i>	2 (5.13)
<i>Pseudomonas aeruginosa</i>	2 (5.13)
<b>Total</b>	<b>39 (16.25)</b>

Key: ART= Anti-retroviral therapy, JUTH= Jos University Teaching Hospital.

**Table 2:** Prevalence of Urinary Tract Pathogenic Bacteria in Relation to Age and Gender among HIV/AIDS Patients Attending ART Clinic at JUTH, Jos.

Age group (Yrs)	Total Examined	Total Positive (%)	Male		Female	
			No Examined	No. Positive (%)	No Examined	Total Positive (%)
≤19	1	0 (0.00)	0	0 (0.00)	1	0 (0.00)
20-24	3	1 (33.3)	1	0 (00.0)	2	1 (50.0)
25-29	20	3 (15.0)	4	1 (25.0)	16	2 (12.5)
30-34	41	8 (19.5)	7	0 (0.00)	34	8 (23.5)
35-39	70	11 (15.7)	11	1 (9.10)	59	10 (16.9)
40-44	36	9 (25.0)	5	1 (20.0)	31	8 (25.8)
45-49	28	4 (14.3)	2	0 (0.00)	26	4 (15.4)
50-54	18	3 (16.7)	2	0 (0.00)	16	3 (18.8)
55-59	16	0 (0.00)	3	0 (0.00)	13	0 (0.00)
≥60	07	0 (0.00)	1	0 (0.00)	06	0 (0.00)

$\chi^2 = 1.905$ ,  $p = 0.163$ , ART= Anti-retroviral therapy, JUTH= Jos University Teaching Hospital.

**Table 3:** Distribution of Yeast Species on Relation to Gender among HIV/AIDS Patients attending ART clinic at JUTH, Jos.

Isolate	Male (%) (n=36)	Female (%) (n=204)	Total (%)
<b>Non candida yeast</b>	0 (0.0)	12 (5.8)	12 (5.0)
<i>Candida albicans</i>	0 (0.0)	21 (10.3)	21 (8.8)

ART= Anti-retroviral therapy, JUTH= Jos University Teaching Hospital.

**Table 4:** Antimicrobial Susceptibility of Gram Negative and Positive Bacteria Isolated from UTI among HIV/AIDS Patients Attending ART Clinic at JUTH, Jos.

Organism	No. Tested	GEN (%)	AUG (%)	ERY (%)	CPX (%)	CEP (%)	SXT (%)	OFX (%)	CHL (%)	TET (%)	NXA (%)
<i>E. coli</i>	12	8(66.6)	2(16.7)	0(0.00)	6(50.0)	2(16.7)	0(0.00)	6(50.0)	NA	NA	NA
<i>P. mirabilis</i>	1	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	NA	NA	NA
<i>K. pneumonia</i>	4	2(50.0)	1(25.0)	0(0.00)	4(100)	1(25.0)	1(25.0)	4(100)	NA	NA	NA
<i>Entobacter sp.</i>	4	2(50.0)	2(50.0)	0(0.00)	2(50.0)	1(25.0)	0(0.00)	3(75.0)	NA	NA	NA
<i>Citrobacter freundii</i>	2	2(100)	1(50.0)	0(0.00)	2(100)	0(0.00)	0(0.00)	2(100)	NA	NA	NA
<i>P. aeruginosa</i>	2	1(50.0)	1(50.0)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(100)	NA	NA	NA
<i>S. aureus</i>	10	7(70.0)	4(40.0)	3(30.0)	NA	NA	NA	NA	4(40.0)	0(0.00)	0(0.00)
<i>S. saprophyticus</i>	3	2(66.7)	3(100)	1(33.3)	NA	NA	NA	NA	2(66.7)	0(0.00)	0(0.00)
<i>Enterococcus sp.</i>	1	0(0.00)	1(100)	1(100)	NA	NA	NA	NA	1(100)	0(0.00)	0(0.00)
Total (%)	<b>39</b>	<b>25(64.1)</b>	<b>15(38.5)</b>	<b>5(12.8)</b>	<b>15(38.5)</b>	<b>4(10.3)</b>	<b>1(2.6)</b>	<b>18(46.2)</b>	<b>7(17.9)</b>	<b>0(0.00)</b>	<b>0(0.00)</b>

Key: NA= Not Applicable, GEN= Gentamycin(10µg), AUG= Augmentin(20µg), ERY= Erythromycin(15µg) CPX= Ciprofloxacin(5µg), NXA=Nalidixicacid(30µg) CEP=Cephalosporin30(µg), SXT=Septrin(25µg), OFX=Ofloxacin(5µg), CHL=Chloramphenicol(30µg) TET=Tetracycline(30µg)Resistance= <12mm, Susceptibility= >15mm, ART= Anti-retroviral therapy, JUTH= Jos University Teaching Hospital.

## Discussion

In this study, 39 (16.25%) out of the 240 midstream urine samples of HIV positive patients screened had urinary tract infections. Our result, though lower has corroborated the earlier findings of Essien *et al.* (2015) and Bigwan and Wakjissa (2013) in studies similarly carried out in Jos, Plateau state among people living with HIV/AIDS with prevalence of 19.3% and 23.5% respectively. Our result is also lower than the prevalence of 25.3% (Samuel *et al.*, 2012) and 48.7% (Iweriebor *et al.*, 2012) for UTI reported for similar studies carried out in Calabar, Nigeria and South Africa respectively. While all the findings including our own underscore the public health importance of UTI to people living with HIV/AIDS, the lower prevalence observed in our study may be attributed to the antiretroviral treatment being received by these patients at the APIN Centre, JUTH.

The distribution of the bacterial isolates in this study showed that *Escherichia coli* had the highest frequency of occurrence (30.77%) followed by *Staphylococcus aureus* with (25.64%). Our result is in agreement with the finding of Samuel *et al.*, (2012) in a study on community acquired UTI conducted in Calabar, Nigeria. Urinary tract infections due to *Escherichia coli* which is a normal flora of the gastrointestinal tract is a common finding in women as a result of microorganisms ascending from the peri-urethral areas contaminated by faecal flora due to warm and moist environment therein in addition to their close proximity to the anus and *Staphylococcus aureus* which came second in the frequency of occurrence is a normal flora of the skin and has a high propensity for causing infections especially in sexually active young women (Mwaka *et al.*, 2011).

The prevalence of urinary tract pathogenic bacteria in relation to age groups indicated that age group 20-24 had the highest prevalence of (33.3%). Age groups <19, 55-59 and ≥60 had the least prevalence of (0.0%) each but the difference was statistically non-significant. However, Essien, *et al.*, 2015 reported that, age group

<1 had the highest prevalence 50% which was in contrast to the outcome of this study. The variation in the rate of infection observed among the age groups may be attributed to the mode of exposure to HIV/AIDS being the major predisposing risk factor and probably sample size.

The distribution of UTI in relation to gender revealed that the female patients had a higher prevalence of (17.60%) compared to the males (8.33%). The finding that females had higher prevalence of UTI than males agrees with the earlier reports of (Njunda *et al.*, 2009; Aiyegoro *et al.*, 2007; Anochie *et al.*, 2001 and Inyang-Etoh *et al.*, 2009). A statistically non-significant association was observed in the prevalence of UTI between the males and the females ( $p > 0.05$ ). Close proximity of the female urethral meatus to the anus, shorter urethra, and sexual intercourse, incontinence, bad toilet habits have all been reported as predisposing factors that encourage the higher prevalence of UTI in females (Bigwan and Wakjissa, 2013; Adabara *et al.*, 2012 and Orret and Davis, 2005).

The prevalence of urinary tract pathogenic bacteria in relation to marital status reveals that the widow had the highest prevalence of UTI with 9 (31%), followed by the singles and married with 8 (19.50%) and 22 (12.90%) respectively. The variation observed in the prevalence among the groups may be due to the sexual behaviour of the individual groups. Married women are more likely to be sexually disciplined as a matter of obligation than their single and widowed counterparts. Widows and singles on the other hand are more likely to give in to socioeconomic pressures thereby engaging in immorality.

The prevalence of yeast infection in relation to gender shows that only females had yeast infection with prevalence of 33 (16.2%) while no male had yeast infection. A statistically significant relationship was observed ( $p < 0.05$ ). The reason for this observation may be as a result of yeast being normal flora/commensal of the gut as a result of which they may easily establish opportunistic infections when the immune

system is weakened or due to the practice of poor hygiene among these individuals. The prevalence of different yeast isolated shows that *Candida albicans* had the highest prevalence with 21 (63.6%), followed by Yeast not *Candida albicans* with 12 (36.4%). The reason for the high observation of *Candida albicans* occurrence may be due to hygiene practices among the individuals, weakened immune system and *C. albicans* being a commensal and a constituent of the normal gut flora and gastrointestinal tract. This study revealed that gentamicin, augmentin, chloramphenicol, ciprofloxacin, and Ofloxacin were more active against most of the urinary isolates while Tetracycline, erythromycin, septrin, and nalidixic acid were inactive. This agrees with similar studies carried out by Bigwan and Wakjissa (2013) but they reported resistance of isolates to Cotrimoxazole. Essien *et al.*, (2015) reported susceptibility of urinary tract bacterial isolates to ciprofloxacin but high resistance to ampicillin. The upsurge in antibiotic resistance pattern seen in this study could be due to antibiotic abuse and self-medication being practiced in many developing countries including Nigeria. Also low cost and availability of these drugs could be

another contributing factor for antibacterial resistance in this locality (Imade and Eghafona, 2010). There is an increasing trend of resistance by common uropathogens to routine antibiotics as being noted in this study. The common practice of self-medication, use of fake, adulterated and substandard drugs and drug abuse could explain this unfortunate trend.

### CONCLUSION

This study reveals that some uropathogens have been associated with HIV/AIDS patients in Jos area. The uropathogens identified in this study were *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterococcus sp.*, *Enterobacter sp.*, *Citrobacter freundii*, *Pseudomonas aeruginosa* and *Candida albicans*. The antibiotic susceptibility screening shows that, gentamicin, Augmentin, chloramphenicol, ciprofloxacin, and ofloxacin were active against most of the urinary isolates while tetracycline, and nalidixic acid were inactive. It is hoped that these findings will be useful when considering the management of HIV/AIDS patients especially individuals receiving care at HIV/AIDS care centres nationwide.

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