Prevalence of Bacterial Uropathogens in a cohort of HIV-Positive Males in Port Harcourt, Nigeria

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ABSTRACT: A total of one hundred samples from male subjects were analyzed, of which 46 were HIV-positive males attending the anti–retroviral clinic (ARV) of the University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Nigeria. They were all within the age range of 35 -69 years. The urine samples were analysed bacterologically using standard methods. Of the 96 samples analysed, 38 (39.6%) had urinary tract infections (UTIs). UTIs and HIV were both present in 23.9% of 46 HIV-seropositive subjects. The specific prevalence was 54.0% among HIV seronegative subjects (controls). The study found significant differences (54.0 vs. 23.9, $P<0.05$) in bacterial uropathogens prevalence of HIV seropositive and HIV seronegative males. The study also found significant age differences (31.6 vs. 18.5, $P<0.05$) in bacterial uropathogens prevalence. Age group 35-45 years had higher prevalence 6(31.6%) than age group 46-69 years 5(18.5%). UTI symptoms were discret. UTI infection was primarily evidenced by a bacteriuria > 10^5 colony-forming units/ml of mid-stream urine. Generally, the main pathogens were *Staphylococcus aureus* had 13(34.2%), *Escherichia coli* 10(26.3%), *Klebsiella species* 7(18.4%) and *Proteus mirabilis* 4(10.5%) and *Pseudomonas aeruginosa* 4(10.5%). *Staphylococcus aureus* 7(63.6%) was the most predominant among the HIV seropositive males, followed by *Escherichia coli* 2(18.2%) while *Proteus mirabilis* 0(0.0%) was absent. Among the HIV seronegative males, *Escherichia coli* 8(29.6%) was the most predominant, followed by *Staphylococcus aureus* 6(22.2%) and *Proteus mirabilis* was present. All isolated bacterial uropathogens were susceptible to Ceftaxidime (a Cephalosporin), Chloramphenicol, Cloxacillin, Nitrofurantoin, Gentamicin, Ciprofloxacin and Nalidixic acid. It also showed that Ampicillin, Penicillin, Erythromycin, Tetracycline, Cotrimoxazole, Augmentin, Amoxicillin and Streptomycin were less effective or resistant. In conclusion, this study showed a higher prevalence of bacterial uropathogens among the HIV negative patients as compared to other findings on HIV/AIDS patients. The antibiogram showed that Ceftaxidime, Cloxacillin, Nitrofurantoin, Gentamicin, Chloramphenicol, Nalidixic acid and Ciprofloxacin remains the effective drug of choice. Cephalosporin of 3rd generation, aminoglycosides, and fluoroquinolone can be used like treatment of first line in urinary tract infection suspicion case in Port Harcourt, Nigeria. Judicious use of these drugs is essential to preserve their efficacies. The findings of this study is hoped will be very useful when considering the management of HIV/AIDS patients from bacterial opportunistic urinary tract infections in Port Harcourt, Nigeria.
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1. Introduction

Among the opportunistic infection, urinary tract infection (UTI) accounts for 60.0% the AIDS defining illness (Deokar and Bodhankar, 2009). UTI represents a considerable health problem amongst HIV infected patients (Samuel et al., 2012). UTIs are an important health problem in HIV-infected persons, where the incidence is between 5.0% and 20.0% (Foxman, 2002; Bigwan and Wakjissa, 2013). Fabian et al. (2009) in South Africa found 29.1% of cases of UTI, to have predominantly harboured *Escherichia coli* (70.0%).

Recent reports suggest that the incidence of urinary tract infection is increased in HIV positive patients (Bigwan and Wakjissa, 2013). There is evidence that bacteriuria is more common as HIV disease progresses (Bigwan and Wakjissa, 2013). Studies have shown that the incidence of UTIs is greater among men and women infected with HIV than among men and women who are sero-negative for HIV (Foxman, 2002; Bigwan and Wakjissa, 2013).

Some studies have indicated that the risk of bacteriuria and UTI may be increased in HIV-infected patients and is inversely related to CD4+ lymphocyte counts (Heyns and Fisher, 2005; Bigwan and Wakjissa, 2013). UTI in HIV-positive patients tends to recur, requiring longer treatment and it is suggested that treatment should be culture-specific (Heyns and Fisher, 2005; Bigwan and Wakjissa, 2013).

While the spectrum of opportunistic infections due to HIV infection has been widely studied and discussed in some areas (Zouiten et al.,

2003; Samuel et al., 2012), there are still very limited data available in Nigeria on urinary tract infections (UTI) in HIV-infected subjects (Samuel et al., 2012). This study therefore aimed to determine the prevalence of bacterial uropathogens in a cohort of HIV-positive males attending antiretroviral (ARV) clinic of University of Port Harcourt Teaching Hospital (UPTH) and their antibiotic sensitivity pattern.

2. Materials and Methods

Ninety-six male subjects attending the University of Port Harcourt Teaching Hospital (UPTH) health facilities were recruited for this study. This study was carried out from March 2011 to December 2011. Ages of these subjects ranged from 35-69 years. Mid stream urine (MSU) samples were collected from these subjects consisting of 46 HIV-positive and 50 HIV-negative males. These urine samples were transported in a commercially available collection and transport system for urine specimens to the Medical Microbiology Laboratory, Department of Microbiology, University of Port Harcourt, Port Harcourt, Nigeria for analysis using standard laboratory procedures. A UTI was defined as a pure culture of $> 10^5$ colony forming units (CFU)/ml in a MSU from a patient with symptoms attributable to UTI. Specimens were inoculated onto cystine lactose electrolyte deficient (CLED) and MacConkey agar plates which were designed to differentiate between lactose fermenters and non-lactose fermenters and also to select for enteric organisms that might be present. The inoculated plates were then incubated at 37°c for 24 hours. The chocolate agar plates were also inoculated and incubated in a carbon dioxide jar at 37°c for 18 to 24 hours in order for anaerobic organisms to grow, after which the plates were then examined for reddish, mucoid, pale or tiny colonies. Suspect colonies were subcultured onto chocolate agar plates to obtain pure colonies. They were then gram stained, and inoculated slants and motility tests carried out. Species identification was carried out using gram staining reaction, catalase, coagulase, motility, urease, sugar fermentation and indole tests. Cultures from cystine lactose electrolyte deficient (CLED) and MacConkey agar were subcultured onto nutrient agar for the appropriate biochemical test to be carried out. Antibiotics sensitivity was performed using the disc diffusion (modified Kirby-Bauer) technique. Antibiotics used in this study based on local availability included Ampicillin (10µg), Chloramphenicol (10µg), Cloxacillin (5µg), Erythromycin (5µg), Gentamicin (10µg), Penicillin (10µg), Streptomycin (10µg) and Ceftaxidime (10 µg) for Gram positive bactaria. For Gram negative bacteria, it included Tetracycline (30 µg), Cotrimoxazole (25 µg), Gentamycin (10 µg), Nitroforantion (30 µg), Nalidixic acid (30 µg), Ciprofloxacin (30 µg) and Amoxicillin/Clavanate (Augmentin, 25 µg). Data was analysed mostly using percentages. The f-test, t- test and chi- square (x^2) tests of significance were employed where appropriate for statistical analysis. Differences were considered significant at $P \leq 0.05$.

3. Results

Ninety-six (96) midstream urine samples were collected; of which 46 samples were from HIV-seropositive males attending the anti-retroviral (ARV) clinic of the UPTH while 50 from apparently healthy HIV–seronegative males. Of the 96 samples analysed, 38 (39.6%) had urinary tract infections of which 11(23.9%) were HIV seropositives and 27(54.0%) were HIV seronegatives (controls). It also showed that 44 (45.8%) samples had no significant bacteria growth (i.e. $< 10^5$ colony forming units (CFU)/ml) and 14 (14.6%) samples had no bacteria growth (Table 1).

Table 1 shows the prevalence of UTI in relation to HIV serostatus of subjects. There was significant difference (54.0 vs. 23.9, $P<0.05$) in the prevalence of bacterial uropathogens between HIV seropositive and HIV seronegative males. Bacterial uropathogens were more prevalent among HIV seronegative males (controls) than HIV seropositive males (Table 1). The study found significant age differences (31.6 vs. 18.5, $P<0.05$) in bacterial uropathogens prevalence of HIV seropositive and HIV seronegative males (controls). Table 2 shows the prevalence of UTI in relation to ages of HIV seropositive males. It indicates that age group 35-45 years had higher prevalence 6(31.6%) than age group 46-69 years 5(18.5%).

Table 3 shows the frequency of occurrence of bacterial uropathogen. Generally, *Staphylococcus aureus* had 13(34.2%), *Escherichia coli* 10(26.3%), *Klebsiella species* 7(18.4%) and *Proteus mirabilis* 4(10.5%) and *Pseudomonas aeruginosa* 4(10.5%). Among the HIV seropositive males, *Staphylococcus aureus* 7(63.6%) was the most predominant. This was followed by *Escherichia coli* 2(18.2%), *Klebsiella species* 1(9.1%) and *Pseudomonas aeruginosa* 1(9.1%) while *Proteus mirabilis* 0(0.0%) was absent. Among the HIV seronegative males, *Escherichia coli* 8(29.6%) was the most predominant. This was followed by *Staphylococcus aureus* 6(22.2%) and *Klebsiella species* 6(22.2%), *Proteus mirabilis* 4(14.8%) while *Pseudomonas aeruginosa* 3(11.1%) was least predominant. However, *Proteus mirabilis* was only present among the HIV seronegatives (Table 3).

Table 4 indicates that the bacteria isolates are most susceptible to Ceftaxidime, Chloramphenicol,

Cloxacillin, Nitrofurantoin, Gentamicin, Nalidixic acid and Ciprofloxacin were most effective against most of the uropthogens isolated in this study while Ampicillin, Penicillin, Erythromycin, Tetracycline, Cotrimoxazole, Augmentin, Amoxicillin and Streptomycin were less effective or resistant (Table 4).

Table 1: Prevalence of UTI in relation to HIV serostatus of subjects

HIV Status	No. Tested (%)	No. Positive for UTI (%)	No significant bacteriuria (%)	No bacterial growth (%)
Seropostives	46(47.9)	11(23.9)	23(50.0)	12(26.1)
Seronegatives	50(52.1)	27(54.0)	21(42.0)	2(4.0)
Total	**96(100.0)**	**38(39.6)**	**44(45.8)**	**14(14.6)**

Table 2: Prevalence of UTI in relation to ages of HIV seropositive males

Age groups (years)	No. Tested (%)	No. Positive for UTI (%)
35-45	19(41.3)	6(31.6)
46-69	27(58.7)	5(18.5)
Total	**46(100.0)**	**11(23.9)**

Table 3: Frequency of occurrence of bacterial uropathogen

Isolates	Total No. (%)	HIV seropositives (%)	HIV negatives (%)
Staphylococcus aureus	13(34.2)	7(63.6)	6(22.2)
Klebsiella species	7(18.4)	1(9.1)	6(22.2)
Escherichia coli	10(26.3)	2(18.2)	8(29.6)
Pseudomonas aeruginosa	4(10.5)	1(9.1)	3(11.1)
Proteus mirabilis	4(10.5)	0(0.0)	4(14.8)
Total	**38(100.0)**	**11(28.9)**	**27(44.3)**

Table 4: Susceptibility and resistance of all bacteria isolated to different antibiotics

Antibiotics	No. Tested	Number of isolate sensitive (%)	Number of isolate resistant (%)
Ampicillin	38	0(0.0)	38(100.0)
Amoxicillin	38	0(0.0)	38(100.0)
Augmentin	38	0(0.0)	38(100.0)
Ceftaxidime	38	38(100.0)	0(0.0)
Chloramphenicol	38	38(100.0)	0(0.0)
Ciprofloxacin	38	38(100.0)	0(0.0)
Cloxacillin	38	38(100.0)	0(0.0)
Erythromycin	38	0(0.0)	38(100.0)
Gentamicin	38	38(100.0)	0(0.0)
Nitrofurantoin	38	38(100.0)	0(0.0)
Nalidixic acid	38	23(60.5)	15(39.5)
Penicillin	38	0(0.0)	38(100.0)
Septrin	38	0(0.0)	38(100.0)
Streptomycin	38	0(0.0)	38(100.0)
Tetracycline	38	0(0.0)	38(100.0)

4. Discussion

Generally, the study showed that of the 96 midstream urine samples of HIV positive and HIV negative male patients screened, 38(39.6%) had urinary tract infections. The finding of this study is higher than the incidence of 11.9% reported by Aiyegoro et al. (2007) among children and adolescents in Ile-Ife, Nigeria. This figure is also higher than the prevalence of 22.0% reported by Ekweozor and Onyemenen (1996) in Ibadan and 25.6% by Nedolisa (1998) in Jos, Nigeria. The finding of this study is also higher than the incidence of 28.1% reported by Olowu and Oyetunji (2003) in Lagos, Nigeria. It is also higher than the prevalence of 30.0% reported by Anochie et al. (2001) in a rural community in Enugu, Nigeria. However, it is lower than the 50.0% reported by Obiogbolu et al. (2009) and the 47.5% reported by Okonko et al. (2009a).

Of the 46 midstream urine samples of HIV positive male patients analyzed, 11(23.9%) had UTI while of the 50 urine samples of HIV negative males analyzed, 27(54.0%) also had UTI. The study found significant differences (54.0 vs. 23.9, P<0.05) in bacterial uropathogens prevalence of HIV seropositive and HIV seronegative males (controls). Bacterial uropathogens were more prevalent among HIV seronegative males than HIV seropositive males. This present finding disagreed that uropathogens causing UTIs are higher in HIV positive individuals than HIV negative individuals (Bigwan and Wakjissa, 2013). The higher prevalence of the bacterial pathogens in all the HIV seronegative males (controls) may be attributed to their exposure to HIV/AIDS being the major predisposing risk factor and probably other risk factors such as diabetes, increased sexual activity and contamination from anus after defecation (Sheffield and Cunningham, 2005; Obiogbolu et al., 2009; Bigwan and Wakjissa, 2013).

In this study, the prevalence of bacterial uropathogens among HIV seropositive male subjects was found to be 23.9%. This is in consonance with similar studies carried out in Nigeria and outside Nigeria. Bigwan and Wakjissa (2013) found a prevalence of 23.5% in a similar study in Jos, Nigeria. A study by Samuel et al. (2012) in Calabar, Nigeria recorded a prevalence of 25.3%. The prevalence reported in this study is much lower than the prevalence of 48.7% recorded by Iweriebor et al. (2012) in South Africa and higher than the 29.1% reported Fabian et al. (2009) in South Africa.

The study found significant age differences (31.6 vs. 18.5, P<0.05) in bacterial uropathogens prevalence of HIV seropositive and HIV seronegative males (controls). The prevalence of UTI in relation to age groups indicates that age group 35-45 years had higher prevalence 6(31.6%) while the least was recorded within the age group 46-69 years. This disagrees with Bigwan and Wakjissa (2013) who reported higher prevalence of UTI in age group ≥ 46 years. This indicates that UTI is distributed in the two age groups with a significant relationship ($p < 0.05$).

The study also showed that 45.8% samples had no significant bacteria growth (i.e. $< 10^5$ colony forming units (CFU)/ml) and 14.6% samples had no bacteria growth. Presence of insignificant growth or sterile urine may be due to prior use of antibiotics or improper method of collecting samples (Jai et al., 2012; Frank-Peterside et al., 2013). Although definitive diagnosis is based on culture results but looking at the significant bacteriuria in 38.9% of samples shows good clinical co-relation between clinical and microbiological diagnosis (Das et al., 2006; Jai et al., 2012; Frank-Peterside et al., 2013).

The distribution of the bacterial isolates in this study indicates that *Staphylococcus aureus* had the highest prevalence with 13(34.2%), followed by *Escherichia coli* with 10(26.3%). Also, *Staphylococcus aureus* was the highest prevalence with 7(63.6%) among the HIV seropositive males. This observation agrees favourably with our previous study in Port Harcourt, Nigeria (Frank-Peterside et al., 2013). However, this present study disagrees with most previous studies on community acquired UTI (Cheesbrough, 2000; Allan, 2001; Fabian et al., 2009; Obiogbolu et al., 2009; Okonko et al., 2009a; Jai et al., 2012; Samuel et al., 2012; Bigwan and Wakjissa, 2013). *Staphylococcus aureus* which is the highest isolate has a high propensity for causing infections especially in young sexually actives (Mims et al., 2004; Bigwan and Wakjissa, 2013).

However, *Escherichia coli* had the highest prevalence with 8(29.6%) among the HIV seronegative males. *Proteus mirabilis* was present among the HIV seronegatives only. This is in agreement with most previous studies on community acquired UTI (Cheesbrough, 2000; Allan, 2001; Fabian et al., 2009; Obiogbolu et al., 2009; Okonko et al., 2009a; Samuel et al., 2012; Bigwan and Wakjissa, 2013). UTI due to *Escherichia coli* is a common finding (Obiogbolu et al., 2009; Fabian et al., 2009; Okonko et al., 2009a; Mwaka et al., 2011; Bigwan and Wakjissa, 2013).

This study revealed that Ceftaxidime (a Cephalosporin), Chloramphenicol, Cloxacillin, Nitrofurantoin, Gentamicin, Nalidixic acid and Ciprofloxacin were most effective against of the uropthogens isolated in this study while Ampicillin, Penicillin, Erythromycin, Tetracycline, Cotrimoxazole, Augmentin, Amoxicillin and Streptomycin were less effective or resistant. This also agrees with the findings of Bigwan and Wakjissa (2013) who reported Gentamicin, Nitrofurantoin and Augmentin more effective against most of the urinary isolates. It also agrees with the findings of Okonko et al. (2009b) who reported that Nitrofurantoin and Nalidixic acid remains the effective drug of choice against uropathogens.

Sensitivity to fluoroquinolones alongside other antibiotics was reported in this study. However, in recent years, use of fluoroquinolones has increased in many countries and emergence of resistance of bacterial isolates to fluoroquinolones has also been observed (Umolu et al., 2006; Frank-Peterside et al., 2013). There are number of studies in which mentioned about resistance of micro-organisms to conventional antibiotics like ciprofloxacin (Ehinmidu, 2003; Umolu et al., 2006; Frank-Peterside et al., 2013). This observed resistance to these drugs is a probable indication of earlier exposure of the isolates

to these drugs, which may have enhanced resistant development (Ehinmidu, 2003; Frank-Peterside et al., 2013).

Resistance to Ampicillin, Amoxicllin, Augmentin and Penicillin was reported in this study. This is similar to what was observed by Aibinu *et al.* (2004), Jai et al. (2012) and in our previous study (Frank-Peterside et al., 2013) who reported 100.0% resistance of their *E. coli* isolates to ampicillin.

It has been widely reported that Co-trimoxazole is active against most common urinary pathogens and has been widely used as prophylaxis against *Pneumocystis carinii* pneumonia (PCP) in immunocompromised individuals (Evans et al., 1995). Resistance to Cotrimoxazole (septrin) was reported for all bacterial uropathogens isolated in this study. This also agrees with previous findings (Densenclos *et al*., 1988; Jai et al., 2012; Frank-Peterside et al., 2013). Densenclos *et al*. (1988) reported 53.0% of their *E. coli* isolates were resistant cotrimoxazole (septrin). Jai et al. (2012) reported 69.0% of their *E. coli* isolates were resistant cotrimoxazole. A 52.9% resistance to Cotrimoxazole was reported in our previous study (Frank-Peterside et al., 2013). These previous findings are in harmony with the report of this present study, showing 100.0%.

This study also finds an increasing trend of resistance by common uropathogens to routine antibiotics used in this study (Bigwan and Wakjissa, 2013). The reason for this high resistance to commonly used antibiotics may be due to widespread and indiscriminate use in our environment (Jai et al., 2012; Frank-Peterside et al., 2013). The common practice of self medication, use of fake, adulterated and substandard drugs and drug abuse could also explain this unfortunate trend (Bigwan and Wakjissa, 2013).

5. Conclusion

This study reveals a high prevalence of bacterial uropathogens in a cohort of HIV seropositive male patients in Port Harcourt, Nigeria. These uropathogens include *Staphylococcus aureus, Escherichia coli*, *Klebsiella species*, *Proteus mirabilis* and *Pseudomonas aeruginosa.* The study showed a high sensitivity of most of the pathogens to the some of the antibiotics used. It also indicates that Ceftaxidime, Cloxacillin, Nitrofurantoin, Gentamicin, Chloramphenicol, Nalidixic acid and Ciprofloxacin remains the effective drug of choice, judicious use of these drugs is essential to preserve their efficacies. The findings of this study is hoped will be very useful when considering the management of HIV/AIDS patients from bacterial opportunistic urinary tract infections in Port Harcourt, Nigeria.

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