

Antibiotic Resistance of Bacterial Isolates from HIV Positive Patients with Urinary Tract Infection (UTI) in Portharcourt, Nigeria

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Abstract

This study was carried out to evaluate the prevalence of HIV associated Urinary tract infections (UTI) in Portharcourt Metropolis. 286 urine samples were collected from 246 HIV seropositive and 40 HIV seronegative individuals. Mid stream urine samples collected from these individuals were processed by standard protocols to examine for bacterial opportunistic pathogens. Antibiotic susceptibility testing was performed using Kirby-Bauer's disc diffusion method. Out of 246 HIV individuals examined, 141 (57.3%) urine samples yielded growth of bacterial isolates, with age group 24-30 years recording the highest number of isolates 45 (32.9%), while those above 44 years old had the least with 11 (7.8%). About 24 (60%) samples out of 40 seronegative individuals yielded growth of bacteria and age group 17-23 years recorded the highest number of isolates 7 (29.1%), while those above 44 years had the least 1 (4.1%). Female individuals recorded the highest number of bacterial isolates than males. A total of 165 bacteria were identified and grouped into four genera out of which *Staphylococcus aureus* had the highest percentage of occurrence 49 (29.7%), followed by *Escherichia coli* 47 (28.5%), *Pseudomonas aeruginosa* 46 (27.9%) and *Klebsiella pneumoniae* 23 (13.9%). Out of 111 bacterial isolates that exhibited multidrug resistance, HIV Seropositive individuals had 103 (92.8%) and HIV seronegative individuals 8 (7.2%). Over all, *Staphylococcus aureus* recorded the highest number of multidrug resistant bacteria 36 (32.4%), followed by *Pseudomonas aeruginosa* 34 (30.6%). The high levels of multidrug resistance in HIV seropositive individuals are a serious public Health concern. Therefore appropriate health education to reduce self-medication and drug abuse is very imperative and desirous.

Keywords: HIV; UTI; MDR; Drug abuse; Portharcourt

Introduction

Human Immunodeficiency Virus (HIV) is a lentivirus that causes immunodeficiency syndrome [1,2] a condition in humans in which progressive failure of the immune system allows life threatening opportunistic infections to thrive. Most untreated people infected with HIV-1 eventually develop AIDS [3]. These individuals usually die of opportunistic infections or malignancies associated with progressive failure of the immune system. HIV infects vital cells in the human immune system such as helper T cells (specifically CD4⁺ T cells), macrophages and dendritic cells [4]. HIV infection leads to low levels of CD4⁺ T cells through three main mechanisms. First, direct viral killing of the infected cells; second, increased rate of apoptosis in infected cells; and third, killing of infected CD4⁺ cells by CD8 cytotoxic lymphocytes that recognize infected cells. When CD4⁺ cells number decline below a critical level, cell mediated immunity is lost and the body becomes progressively more susceptible to opportunistic infections. Urinary tract infection (UTI) is one of the significant illnesses that cause burden. It is the most common nosocomial infection, but an important source of morbidity as well [5]. UTI is one of the most common bacterial infection and cause of morbidity and hospitalization in HIV positive individuals [6]. HIV disease is associated with a variety of renal syndromes in patients with low CD4⁺ cell counts, causing neurologic complications which lead to urinary stasis and ultimately infection [7,8]. Once a patient's CD4⁺ T cell count falls below 200cells/mm³, the individual is then at risk of a variety of opportunistic infections. The infectious organisms may include fungi, protozoa, viruses and bacteria. The most predominant causative organisms are encapsulated bacteria notably: *Streptococcus pneumoniae* and *Haemophilus influenzae*, but non-typhoidal Salmonella, *Staphylococcus aureus* and *Pseudomonas aeruginosa* have also been implicated [9]. Among opportunistic

infections, UTI accounts for 60% of AIDS defining illnesses [10]. The prevalence of data on the frequency of UTIs in HIV infected patients is scanty and not updated in Port Harcourt, Rivers State. Urinary tract infections account for a significant proportion of patient's daily hospital visits in HIV patients [11]. UTIs also account for a large proportion of antibacterial drug consumption [6]. Resistance to commonly prescribed antibiotics for UTI is an expanding global problem both in developed as well as developing countries [12]. Due to the wide spread and injudicious use of antibiotic, we are encountered with more resistance patterns to common antibiotics [13]. UTI became quite alarming as isolated uropathogens exhibited high percentage resistance to almost all antibiotics [14]. These multidrug resistant (MDR) pathogens are relentlessly multiplying in HIV patients and thus, becoming an important circulating source of infection especially in Port Harcourt Metropolis.

The present study was carried out to evaluate the prevalence of various bacterial isolates from HIV seropositive and HIV seronegative urinary tract infected individuals attending University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt Rivers State.

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Materials and Methods

Sample collection and processing

A total of 286 individuals attending Clinic at University of Port Harcourt Teaching Hospital (UPTH) during the period of February 2015 to November 2015 were used for this study. They consisted of 246 seropositive individuals and 40 seronegative individuals. The HIV seropositive individuals included: 111 males and 135 females; while the HIV seronegative individuals included: 25 females and 15 males. Ethical clearance was obtained from the Ethical Review Board.

Mid stream urine samples were collected in sterile containers by taking all precautions to avoid contamination. The urine samples were immediately taken to the laboratory and cultured within 30 min of collection on MacConkey and Cystein lactose electrolyte deficient (CLED) agar. The plates were incubated at 37°C for 24 h, while microscopy was also carried out following standard protocols. In case of delay, the samples were kept at 4°C in the refrigerator and cultured within 6 h. The isolates were identified using standard biochemical reactions [15].

Antibiotic sensitivity test

The isolates were tested for their susceptibility to commonly prescribed antibiotics using Kirby Bauer disc diffusion method [16,17]. Antibiotics used for the isolates were Cotrimoxazole (23.75 µg), Ciprofloxacin (5 µg), gentamicin (10 µg), Ceftazidime (30 µg), Erythromycin (15 µg), Ofloxacin (5 µg), Oxacillin (1 µg), Ceftriaxone (30 µg), Streptomycin (10 µg), Tetracycline (25 µg), Amoxicillin/Clavulanic acid (20 µg), Chloramphenicol (30 µg), Nitrofurantoin (30 µg) [18]. Isolates showing resistance to three or more categories of antibiotics were considered as multidrug resistant bacteria [19].

Results

Out of the 246 HIV seropositive individuals who were analyzed during this study, 135 (54.9%) were females, while 111 (45.1%) were males. Out of 40 HIV seronegative individuals, 25 (62.5%) were females, and 15 (37.5%) were male. The age group ranged 17 years to 44 years and above as shown in Table 1. About 141 (57.3%) urine sample collected from HIV seropositive individuals yielded growth of bacterial isolates. Age group 24–30 years had the highest number of bacterial isolates 45 (32.9%), followed by age group 17–35 years 37(26.2%), while those above 44 years recorded the least 11 (7.8%). Female individuals were more affected than male as shown in Table 2. About 24 (60%) urine sample from HIV seronegative individuals yielded growth of bacterial, with age group 17–23 years recording the highest frequency of occurrence 7 (29.1%), followed by age group 24–30 years and 31–37 years 6 (25%) each. Those above 44 years old had the least 1 (4.1%) as shown in Table 3. A total of 165 bacteria isolates were identified and grouped into 4 genera out of which *Staphylococcus aureus* had the highest percentage of occurrence 49 (29.7%) followed by *Escherichia coli* 47 (28.5%), *Pseudomonas aeruginosa* 46 (27.9%) and *Klebsiella pneumoniae* 23(13.9%) as shown in Table 4. Identified isolates were tested against commonly prescribed antibiotics, and isolates from HIV seropositive individuals exhibited high levels of multidrug resistance ability with *S. aureus* 32 (31.1%), *E. coli* 31 (30.1%), *P. aeruginosa* 30 (29.1%) and *K. pneumoniae* 10 (9.7%); while from HIV seronegative individuals, *E. coli* and *K. pneumoniae* did not show any MDR ability as shown in Tables 5 and 6.

Discussion

It was observed that patients with immunosuppression have very high chances of developing bacteria. UTI in HIV-positive patients tends to recur, requiring longer treatment and it is suggested that treatment should be culture-specific [20].

In this study, 246 HIV seropositive and 40 HIV seronegative individuals were analysed. 141 (85.5%) bacterial isolates were isolated from HIV seropositive and 24 (14.5%) from HIV seronegative individuals. These findings are in accordance with another study where bacteria were isolated from HIV reactive patients and HIV non-reactive patients. Four bacterial genera including *Staphylococcus aureus* 49 (29.7%), *Escherichia coli* 47 (28.7%), *Pseudomonas aeruginosa* 46 (27.9%) and *Klebsiella pneumoniae* 23 (13.9%) were isolated. This observation correlates with earlier findings [21], and in contrast with *Enterococcus species* reported as the most common urinary isolates [22]. *Staphylococcus aureus* had the highest percentage of occurrence, and it had earlier been identified as the most common uropathogen [23], though in contrast to a study where *E. coli* had the highest percentage of occurrence 27 (36.4%) [5]. Colonization with *Staphylococcus aureus* has been reported to be a risk factor for subsequent clinical infection in HIV positive patients [24,25]. Globally, *Staphylococcus aureus* infections have been reported to be an important cause of morbidity and mortality [26]. HIV positive individuals are at increased risk of opportunistic and common bacterial infections [27,28]. Results also revealed that that HIV seropositive individuals exhibited significant levels of bacterial colonization with multiple drug resistant *S. aureus* 32 (31.1%), *E. coli* 31 (30.1%), *P. aeruginosa* 30 (29.1%) and *K. pneumoniae* 10 (9.7%) which is similar with earlier findings [21]. This further confirms that immunocompromised status like HIV is a hot spot for multiple drug resistant pathogens to multiply relentlessly and become source of infection to other healthy population and this situation raises serious health concern. Most of the isolates were resistant to oxacillin, tetracycline, chloramphenicol and ampicillin. This emergence of multiple drug resistance in the management of UTI among HIV individuals is a serious public health problem particularly in the developing world where aside from high level of poverty, ignorance and poor hygiene practices, there is a high prevalence of sub-standard and spurious drugs of questionable quality in circulation [29]. This high MDR antibiotic level also suggests a very high resistance gene pool due perhaps to gross misuse and inappropriate usage of antibacterial agents. The upsurge in antibiotic resistance noticed in this study is in agreement with earlier work [30], where antibiotic abuse and high prevalence of self medication with antibiotics were identified as being responsible for the selection of antibiotic resistant bacterial strains. The presence of multiple drug resistant bacteria in the urine of HIV seropositive individuals will significantly increase the risk of super imposed opportunistic infections which may be less susceptible to antibiotic treatments. Urinary tract infection in HIV

Year	HIV Seropositive group		HIV Seronegative group		Total numbers of cases analyzed	
	Female	Male	Female	Male	Female	Male
17-23	15	10	5	1	20	11
24-30	29	21	7	5	36	26
31-37	48	39	6	4	54	43
38-44	35	30	4	3	39	33
>44	8	11	3	2	11	13
Total	135	111	25	15	160	126

Table 1: Age and sex distribution of HIV seropositive and HIV seronegative patients with UTI.

Age (yrs)	<i>Escherichia coli</i>		<i>Klebsiella pneumoniae</i>		<i>Pseudomonas aeruginosa</i>		<i>Staphylococcus aureus</i>		Total		
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Total
17–23	7	2	4	1	9	2	7	5	27	10	37
24–30	9	4	6	-	11	4	8	3	34	11	45
31–37	5	5	3	-	7	1	4	4	19	10	29
38–44	5	2	2	2	3	-	3	2	13	6	19
>44	2	1	1	1	2	-	2	2	7	4	11
Total	28	14	16	4	32	7	24	16	100	41	141

Table 2: Age and sex distribution of HIV seropositive urinary tract infected patients.

Age	<i>Escherichia coli</i>		<i>Klebsiella pneumoniae</i>		<i>Pseudomonas aeruginosa</i>		<i>Staphylococcus aureus</i>		Total		
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Total
17–23	-	1	1	-	1	-	2	2	4	3	7
24–30	2	-	1	-	1	-	2	-	6	-	6
31–37	1	-	1	-	2	1	1	-	5	1	6
38–44	1	-	-	-	1	-	1	1	3	1	4
>44	-	-	-	-	1	-	-	-	1	-	1
Total	4	1	3	-	6	1	6	3	19	5	24

Table 3: Age and sex distribution of HIV seronegative urinary tract infected patients.

Bacterial Isolates	HIV Seropositive (n=246) With culture growth	HIV seronegative (n=40) With culture growth
<i>Escherichia coli</i>	42	5
<i>Klebsiella Pneumoniae</i>	20	3
<i>P. aeruginosa</i>	39	7
<i>S. aureus</i>	40	9
Total	141	24

Table 4: Distribution of bacterial isolates among HIV seropositive and HIV seronegative UTI patients.

Antibiotics	<i>Escherichia coli</i> HIV+n=42 (%) HIV-n=5 (%)		<i>Klebsiella pneumoniae</i> HIV+n=20 (%) HIV-n=3 (%)		<i>Pseudomonas pneumoniae</i> HIV+n=39 (%) HIV-n=7 (%)		<i>Staphylococcus aureus</i> HIV+n=40 (%) HIV-n=9 (%)	
	OXA	39 (92.81)	2 (40.0)	13 (65.0)	1 (33.3)	37 (94.9)	5 (71.4)	38 (95.0)
GEN	3 (7.1)	0 (0.0)	4 (20.0)	0 (0.0)	10 (25.6)	3 (42.9)	13 (32.5)	3 (33.3)
CAZ	40 (95.2)	1 (20.0)	10 (50.0)	2 (66.6)	31 (79.5)	4 (57.1)	32 (80.0)	4 (44.4)
AMC	15 (35.7)	1 (20.0)	8 (40.0)	1 (33.3)	29 (74.3)	4 (57.1)	25 (62.5)	3 (33.3)
STR	10 (23.8)	2 (40.0)	12 (60.0)	1 (33.3)	19 (48.7)	3 (42.9)	27 (67.5)	3(33.3)
CRO.	29 (69.0)	3 (00.0)	12 (60.0)	1 (33.3)	30 (76.9)	3 (42.9)	16 (40.0)	2 (22.2)
OFX	1 (2.4)	1 (20.0)	13 (15.0)	0 (0.0)	8 (20.5)	2 (28.6)	4 (10.0)	1 (11.1)
ERY	18 (42.9)	3 (60.0)	2 (10.0)	1 (33.3)	31 (79.5)	5 (71.4)	25 (62.5)	4 (44.4)
TET	21 (50.0)	1 (20.0)	9 (45.0)	1 (33.3)	38 (97.4)	6 (85.7)	36 (90.0)	4 (44.4)
CHL	31 (73.8)	2 (40.0)	12 (60.0)	2 (66.6)	38 (97.4)	6 (85.7)	28 (70.0)	4 (44.4)
AMP.	37 (88.0)	4 (80.0)	12 (60.0)	1 (33.3)	30 (76.9)	5 (71.4)	26 (65.0)	3 (33.3)
CIP	7 (16.7)	1 (20.0)	5 (25.0)	0 (0.0)	10 (25.6)	3 (42.9)	9 (22.5)	2 (22.2)
NIT	19 (45.2)	2 (40.0)	4 (20.0)	0 (0.0)	30 (76.9)	4 (57.1)	15 (37.5)	2(22.2)
COT	18 (42.9)	2 (40.0)	13 (65.0)	1 (33.3)	37 (94.9)	6 (85.7)	30 (75.0)	4 (44.4)

OXA=Oxacillin, GEN=Gentamicin, CAZ=Ceftazidime, AMC=Amoxycillin, STR=Streptomycin, CRO=Ceftriaxone, OFX=Ofloxacin, ERY=Erythromycin, TET=Tetracycline, CHL=Chloramphenicol, AMP=Ampicillin, CIP=Ciprofloxacin, NIT=Nitrofurantoin, COT=Cotrimoxazole

Table 5: Antibiotic resistance profile of bacterial isolates from HIV seropositive and seronegative urinary tract infected patients.

Isolates	HIV Seropositive Individuals		HIV Seronegative Individuals	
	Total number of Isolates examined	MDR isolates	Total number of Isolates examined	MDR Isolates
<i>Escherichia coli</i>	42	31	5	0
<i>Klebsiella pneumoniae</i>	20	10	3	0
<i>Pseudomonas aeruginosa</i>	39	30	7	4
<i>Staphylococcus aureus</i>	40	32	9	4
Total	141	103	24	8

Table 6: Frequency of occurrence of multi drug resistant (MDR) bacteria isolated from HIV seropositive and seronegative urinary tract infected individuals in portharcourt metropolis.

positive patients tend to recur, requiring longer treatments and it is suggested that treatment should be culture-specific [20]. According to the findings of this study, the need for appropriate health education to reduce self-medication and drug abuse is very imperative and desirous. These results also suggest the need for further investigation into the mechanism of drug resistance among immune compromised individuals.

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