

**HIV-1 & -2 Co-Infections with Multi-Drug Resistant (MDR) Uropathogens in Port Harcourt, Nigeria**

Frank-Peterside N, Chukwugozim-Umejuru R, Okerentugba PO, Okonko IO

Medical Microbiology Unit, Department of Microbiology, University of Port Harcourt,  
P.M.B. 5323, Choba, East-West Road, Port Harcourt, Rivers State, Nigeria;Tel: +2348035380891; E-Mail: [mac2finney@yahoo.com](mailto:mac2finney@yahoo.com), [iheanyi.okonko@uniport.edu.ng](mailto:iheanyi.okonko@uniport.edu.ng)

**ABSTRACT:** This study was carried out to detect the presence of HIV-1 & -2 coinfections with MDR uropathogens among patients in Port Harcourt, Nigeria. Determine® HIV-1/2, HIV -1/2 Stat- Pak® Assay and Bi-Spot HIV-1 & 2 ImmunoConfirm test kits were used for the detection of HIV-1 and HIV-2 in serum samples. Urine samples were analyzed using standard techniques. Overall prevalence rate of HIV was 61.0%, HIV-1 (94.0%), HIV-2 (6.0%) and HIV-1/ HIV-2 coinfection (0.0%), urinary tract infection [UTI] (85.4%), and UTI/HIV (60.0%). It showed that of the HIV-negatives subjects, 28(43.7%) had UTI while of the 50 HIV-positive subjects, 42(84.0%) had UTI. Females had the highest prevalence of HIV (78.0%) and UTI (70.0%) compared to their male counterparts ( $p < 0.05$ ). Only females (100.0%) were infected with HIV-2 and 36(76.6%) of the HIV-positive females were infected with HIV-1. HIV positive males were only infected by HIV-1. UTI was higher in HIV-positive subjects [48(70.6%)] than in HIV-negative subjects [20 (29.4%)]. Age groups (45-68 years) had highest prevalence of HIV (62.0%) and UTI (68.6%) at  $p < 0.05$ . Married individuals had highest prevalence of HIV (80.0%) and UTI (54.7%) than the singles ( $p < 0.05$ ). *Escherichia coli* (39.3%) was most predominant in HIV-negative subjects while *Staphylococcus aureus* (66.7%) was most predominant among HIV-positive subjects (31.5%). Generally, the study showed that *Staphylococcus aureus* [37(54.3%)] was the most predominant uropathogen, followed by *Escherichia coli* (24.3%), *Klebsiella pneumoniae* (8.6%), *Proteus* sp (4.3%), *Enterococcus faecalis* (2.9%), *Streptococcus pyogenes* (1.4%) and a mixed infection of *S. aureus* and *E. coli* (4.3%). The antibiotic susceptibility screening and antibiograms of isolates showed presence of multi-drug resistance (MDR) uropathogens. Nalidixic acid, septrin, ampicillin, penicillin and augumentin are not likely a choice antibiotic for HIV and non-HIV patients while ciproflox, ofloxacin and streptomycin are good choice antibiotic for isolates from HIV, non-HIV and HIV/UTI infected patients. However, it was observed that antibiotic synergy is what makes these drugs effective. This study however, further confirmed the presence of HIV/UTI coinfections in Port Harcourt, Nigeria. It also showed that sex, age and marital status was significantly associated with prevalence of HIV, UTI and HIV/UTI. General surveillance and public health education to stop the spread of the infection from this group is advocated.

[Frank-Peterside N, Chukwugozim-Umejuru R, Okerentugba PO, Okonko IO. **HIV-1 & -2 Co-Infections with Multi-Drug Resistant (MDR) Uropathogens in Port Harcourt, Nigeria.** *Nat Sci* 2013;11(11):11-20]. (ISSN: 1545-0740). <http://www.sciencepub.net/nature>. 3

**Keywords:** Antibiograms, Antibiotic susceptibility screening, Co-infections, HIV, UTI, HIV/UTI, Multi-drug resistance (MDR), Uropathogens

**INTRODUCTION**

Urinary tract infection is one of the significant illnesses that cause burden on national exchequer (Jai et al., 2012). Due to widespread and injudicious use of antibiotics at community level we are encountered more and more resistance pattern of micro-organisms to common antibiotics (Jai et al., 2012). Urinary tract infection is not only common nosocomial infection but an important source of morbidity in community as well (Sharma, 1997; Acharya et al., 2011; Jai et al., 2012). It is the most frequent cause of illness in humans after respiratory tract infection (Liperky, 1989; Jai et al., 2012). It has been observed that *E. coli* is the sole causative agent in more than 80% of uncomplicated UTI (Rao-Bhau et al., 1987; Jai et al., 2012). Main cause of urinary tract infection is obstruction of urinary tract including stone disease, pelvi-ureteric junction obstruction, benign

prostate hyperplasia, vesico-ureteric reflux, urethral strictures and neuropathic bladder (Measly and Levison, 1991; Jai et al., 2012).

Urinalysis only indicates presence of bacteria and leucocytes in the urine, which is indirect evidence of UTI but it can only be confirmed on the basis of microscopy and microbial culture (Van Nostrand et al., 2000; Jai et al., 2012). To ensure appropriate therapy, current knowledge of the organisms that cause UTI and their antibiotic susceptibility is mandatory (Gupta et al., 2002; Jai et al., 2012). There are various reports available about changing pattern of pathogen and their susceptibility to routinely used antibiotics in last two decades due to extra chromosomal genetic elements, which simultaneously carry gene for resistance to number of antibiotics and this has made the situation miserable, especially in

gram negative bacteria (Ram et al., 2000; Jai et al., 2012).

HIV/AIDS is a major public health problem in Cameroon which had a prevalence of 5.1% in 2010 with 141 new infections per day (Nsagha et al., 2012). The fear of voluntary counseling and testing (VCT) is an obstacle to HIV prevention. The pandemic is dominated by HIV-1, which was discovered in 1983 (Nsagha et al., 2012). In 1987, HIV-2 was discovered which is very common in West Africa and has not shown any significant spread from there (Nsagha et al., 2012). HIV-2 is less easily transmitted than HIV-1 and the period between initial infection and illness is longer than with HIV-1 (Cheesbrough, 2006; Nsagha et al., 2012).

HIV-1 and HIV-2 co-infection in West Africa show 0.4% HIV-1 and 0.2% HIV-2 in northern Benin (Zanchette et al., 1990; Nsagha et al., 2012), 6.6% HIV-1 and 0.9% HIV-2 in central Benin (Chippaux et al., 1990; Nsagha et al., 2012), 25% HIV-2 and 5% HIV-1/HIV-2 in Mauritania (Baidy et al., 1993; Nsagha et al., 2012), 65% HIV-1, 24% HIV-2 and 11% HIV-1/HIV-2 in Senegal (Ndiaye et al., 2008; Nsagha et al., 2012) and 12.1% HIV-1, 0.5% HIV-2 and 1.6% co-infection in north western Nigeria (Abdulazeez et al., 2008; Nsagha et al., 2012).

HIV-1/HIV-2 co-infection studies revealed a difference in the progression to AIDS among HIV-1, HIV-2 or co-infection and their response to antiretrovirals (Cheesbrough, 2006; Nsagha et al., 2012). As the HIV/AIDS pandemic advances, VCT should be provided along with sensitization (UNICEF, 2009; Nsagha et al., 2012). VCT is important in improving care and support (UNAIDS, 2001; Nsagha et al., 2012) and many developing countries are instituting it as part of the primary health care package (Coovadia, 2000; Nsagha et al., 2012). Developments in cotrimoxazole prophylaxis (Wiktor et al., 1999; Anglaret et al., 1999; Nsagha et al., 2012) and tuberculosis preventive therapies (Mwingwa et al., 1998; Nsagha et al., 2012) for HIV people, antiretroviral (ARV) therapy and PMTCT, have expanded to VCT (Shaffer et al., 1999; Carpenter et al., 2000; Nsagha et al., 2012).

In 2008 WHO suggested that the use of presumptive clinical diagnosis in accordance with nationally defined algorithms would be required in settings with limited access to HIV virological testing (WHO, 2008; Nathoo et al., 2012).

This study is aimed to determine the prevalence of HIV-1 and HIV-2 among patients in Port Harcourt, Nigeria and to determine pattern of uropathogens isolated from HIV positive and HIV negative subjects and their resistance and sensitivity to different antibiotics so that the clinicians may suggest

more appropriate treatment regime at initial stage in benefit of patient as well.

## 2. MATERIALS AND METHODS

### 2.1. Study population

Samples were collected from the HIV clinic at University of Port Harcourt Teaching Hospital (UPTH), Choba Port Harcourt and Buguma General Hospital, Port Harcourt, Nigeria. Subjects were randomly selected for the study. Eighty-two subjects were used in this study. Fifty of the subjects were HIV infected patients while 32 were HIV negative. About 2ml of the subject blood were collected using a sterile syringe into a plain bottle. Suspicion of UTI was made on the basis of urinary symptoms, fever, purulent urine or hematuria. Their urine samples were sent to Medical Microbiology Laboratory working under supervision of qualified microbiologist. Specimen was collected by standard "clean catch" mid stream method in patients who had no catheter in place. Before collecting the sample, male subjects were asked to clean the genital part with soap and water while female patients were told to do the genital toilet using soap and water and the vulva was washed and the labia was carefully separated prior to voiding the urine in sterile bottle. Samples were tested for presence of white blood cells and cast.

### 2.2. Isolation and Identification of isolates

Samples were examined and processed on the blood agar, CLED and Mckonkey's medium by standard loop method and incubated for at least 24 hours at 37°C. Plates were observed for bacterial growth. Culture results were interpreted as significant and insignificant according to standard i.e. a growth of  $\geq 10^5$  CFU/ml was labelled as significant bacteriuria.

### 2.3. Antibiotics sensitivity test

Antibiotic susceptibility test of antibiotics and their interpretation was carried out for bacterial isolates by Kirby-Baur technique as recommended by National Committee for Clinical Laboratory Standards (2000). Uropathogens were identified on the basis of Gram's reaction, colony morphology and standard biochemical tests. Antibiotic susceptibility was tested by disc diffusion method for all 1<sup>st</sup> and 2<sup>nd</sup> line antibiotics. First line antibiotics tested were Ampicillin, Augumentin, Co-trimoxazole (Septrin), Gentamicin, Nalidixic acid, Penicillin and Streptomycin. Second line antibiotics tested were Ceporex, Ofloxacin, Peflacin and Ciproflox.

### 2.4. Screening for HIV-1 and HIV-2 Antibodies

The Abbott Determine® HIV-1/2 Test cards (manufactured by Inverness Medical, Japan) and Chembio HIV -1/2 Stat- Pak® Assay (manufactured

by Chembio Diagnostic Systems, USA) which are single-use immune chromatographic, rapid screening test for the detection of HIV-1 and-2 antibodies. All tests were carried out according to the manufacturer's specification.

### 2.5. Confirmation and Differentiation of HIV- 1 from HIV-2

Bi-Spot HIV-1 & 2 ImmunoConfirm kits (manufactured by Orgenics Ltd., Israel) was employed to differentiate HIV-1 from HIV-2. The immunocomb II HIV 1 & 2 Bispot is an indirect solid-phase enzyme immuno-assay (EIA). This was also carried out according to the manufacturer's specification.

### 2.6. Data Analysis

SPSS 20.0 for Windows statistical package was employed in the analysis of the data generated. The normal distribution was tested by the kolmogororsmirnov test. The distribution was grouped into age, percentage detection, educational status and parity. The chi-square was used to test for goodness of fit between the percentage detection and percentage screened independency / dependence between parity and percentage detection and dependence between percentage positivity and educational status. An error probability (P value) <0.05 was considered significant.

## 3. Results Analysis

From the bio data of the subjects, ages ranged from 20-68 years. It also showed that 37(45.1%) were singles while 45(54.9%) were married. Forty-nine (59.8%) of the subjects were females while 33(40.2%) were males.

### 3.1. Overall HIV and UTI prevalence in the study population

Of the 82 samples tested, 50(61.0%) were positive with DETERMINE HIV-1/2. Among the 50 positive cases, there were 39(78.0%) and 11(22.0%) males ( $P \leq 0.05$ ) (Table 1). Of the 50 positive cases tested with Biospot IMMUNOCONFIRM, 47(94.0%) were HIV-1, 3(6.0%) HIV-2 and 0(0.0%) HIV-1/HIV-2 coinfection (Table 2). No data on profession, religion, educational level, health area and the tribe of the participants was collected in this study.

Table 1 also shows the overall prevalence of urinary tract infection (UTI). It also showed that 70(85.4%) had urinary tract infection (UTI) while 12(14.6%) had no UTI (Tables 1). Of the 70 subjects who had UTI, 42(60.0%) tested positive for HIV. Of the 12 subjects who had no UTI, 8(66.7%) tested positive for HIV (Table 1). It showed that of the HIV-negatives subjects, 28(43.7%) had UTI while of the 50 HIV-positive subjects, 42(84.0%) had UTI.

**Table 1: Overall HIV and UTI prevalence in the study population**

Status	No. (%)	UTI coinfection (%)
<b>HIV serostatus</b>		
Reactive	50(61.0)	42(84.0)
Non-reactive	32(39.0)	28(87.5)
<b>Total</b>	<b>82(100.0)</b>	<b>70(85.4)</b>
<b>HIV serostatus</b>		
No. of HIV-1 positive cases	47(94.0)	40(85.1)
No. of HIV-2 positive cases	3(6.0)	2(66.7)
No. of HIV-1/HIV-1 coinfection cases	0(0.0)	0(0.0)
<b>Total</b>	<b>50(100.0)</b>	<b>42(84.0)</b>
<b>UTI</b>		<b>HIV coinfection (%)</b>
Positive	70(85.4)	42(60.0)
Negative	12(14.6)	8(66.7)
<b>Total</b>	<b>82(100.0)</b>	<b>50(61.0)</b>

### 3.2. HIV seropositivity and UTI coinfection according to gender among the seroprevalence cases

Table 2 shows the prevalence of HIV and UTI coinfection in relation to sex. It showed that females had higher prevalence of HIV [39(78.0%)] compared to their male counterparts [11(22.0%)]. Only females (100.0%) were infected with HIV-2 and 36(76.6%) of the HIV-positive females were infected with HIV-1. HIV positive males were only infected by HIV-1. It also showed that females [49(70.0%)] had higher prevalence of urinary tract infection (UTI) than their male counterparts [21(32.8%)]. There was significant difference in the distribution of HIV and UTI in the different sex group ( $p < 0.05$ ).

**Table 2: HIV seropositivity and UTI coinfection according to gender among the seroprevalence cases**

Status	Total No. (%)	Males No. (%)	Females No. (%)
<b>HIV</b>			
Reactive	50(61.0)	11 (22.0)	39(78.0)
Non-reactive	32(39.0)	22(68.7)	10(31.3)
<b>Total</b>	<b>82(100.0)</b>	<b>33(40.2)</b>	<b>49(59.8)</b>
<b>HIV serostatus</b>			
No. of HIV-1 positive cases	47(94.0)	11(23.4)	36(76.6)
No. of HIV-2 positive cases	3(6.0)	0(0.0)	3(100.0)
No. of HIV-1/HIV-1 coinfection cases	0(0.0)	0(0.0)	0(0.0)
<b>Total</b>	<b>50(100.0)</b>	<b>11(22.0)</b>	<b>39(78.0)</b>
<b>UTI</b>			
Positive	70(85.4)	21(30.0)	49(70.0)
Negative	12(14.6)	12(100.0)	0(0.0)
<b>Total</b>	<b>82(100.0)</b>	<b>33(40.2)</b>	<b>49(59.8)</b>

**3.3. HIV seropositivity and UTI coinfection according to age and gender among the seroprevalence cases**

Table 3 shows the prevalence of HIV-1 and HIV-2 antibodies with and without UTI in relation to age. It showed that subjects within age groups 45-68 years [31(62.0%)] had higher prevalence of HIV than those in age groups 20-44 years [19(38.0%)]. Also, age groups 45-68 years had higher prevalence of HIV-1 [29(61.7%)] and HIV-2 [2(66.7%)] than those in age groups 20-44 years who had prevalence of 38.3% for HIV-1 and 33.3% for HIV-2 antibodies. It also showed that age groups 45-68 years had higher prevalence of UTI [48(68.6%)] than those in age groups 20-44 years [22(31.4%)]. There was significant difference in the distribution of HIV and UTI in the different age groups ( $p < 0.05$ ).

**Table 3: HIV seropositivity and UTI coinfection according to age and gender among the seroprevalence cases**

Status	No. (%)	20-44years (%)	45-68 years (%)
<b>HIV</b>			
Reactive	50(61.0)	19 (38.0)	31(62.0)
Non-reactive	32(39.0)	11(34.4)	21(65.6)
<b>Total</b>	<b>82(100.0)</b>	<b>33(40.2)</b>	<b>49(59.8)</b>
<b>Gender</b>			
No. of positive males	11(22.0)	7(63.6)	4(36.4)
No. of positive females	39(78.0)	12(30.8)	27(69.2)
<b>Total</b>	<b>50(100.0)</b>	<b>19(38.0)</b>	<b>31(62.0)</b>
<b>HIV serostatus</b>			
No. of HIV-1 positive cases	47(94.0)	18(38.3)	29(61.7)
No. of HIV-2 positive cases	3(6.0)	1(33.3)	2(66.7)
No. of HIV-1/HIV-1 coinfection cases	0(0.0)	0(0.0)	0(0.0)
<b>Total</b>	<b>50(100.0)</b>	<b>19(38.0)</b>	<b>31(62.0)</b>
<b>UTI</b>			
Positive	70(85.4)	22(31.4)	48(68.6)
Negative	12(14.6)	11(91.7)	1(8.3)
<b>Total</b>	<b>82(100.0)</b>	<b>33(40.2)</b>	<b>49(59.8)</b>

**3.4. HIV seropositivity and UTI coinfection according to marital status and gender among the seroprevalence cases**

Table 4 shows the prevalence of HIV-1 and HIV-2 antibodies with and without UTI in relation to marital status. It showed that subjects who were married had higher prevalence of HIV [40(80.0%)], HIV-1 [37(78.7%)], HIV-2 [3(100.0%)] and UTI [35(54.7%)] than those who were singles. There was significant difference in the distribution of HIV and UTI in the different marital status ( $p < 0.05$ ).

**Table 4: HIV seropositivity and UTI coinfection according to marital status and gender among the seroprevalence cases**

Parameters	No. (%)	Singles (%)	Married (%)
<b>HIV</b>			
Reactive	50(61.0)	10 (20.0)	40(80.0)
Non-reactive	32(39.0)	27(84.4)	5(15.6)
<b>Total</b>	<b>82(100.0)</b>	<b>37(45.1)</b>	<b>45(54.9)</b>
<b>HIV serostatus</b>			
No. of HIV-1 positive cases	47(94.0)	10(21.3)	37(78.7)
No. of HIV-2 positive cases	3(6.0)	0(0.0)	3(100.0)
No. of HIV-1/HIV-1 coinfection cases	0(0.0)	0(0.0)	0(0.0)
<b>Gender</b>			
No. of positive males	11(22.0)	2(18.2)	9(81.8)
No. of positive females	39(78.0)	17(43.6)	22(56.4)
<b>Total</b>	<b>50(100.0)</b>	<b>19(38.0)</b>	<b>31(62.0)</b>
<b>UTI</b>			
Positive	70(85.4)	29(41.4)	41(58.6)
Negative	12(14.6)	8(66.7)	4(33.3)
<b>Total</b>	<b>82(100.0)</b>	<b>37(45.1)</b>	<b>45(54.9)</b>

**Table 5: Frequency of Occurrence of urine isolates among subjects**

Isolates	No. (%)	HIV-Negative	HIV-Positive
<i>Klebisella pneumoniae</i>	6(8.6)	6(100.0)	0(0.0)
<i>Escherichia coli</i>	17(24.3)	11(64.7)	6(35.3)
<i>Proteus sp.</i>	3(4.3)	1(33.3)	2(66.7)
<i>Streptococcus pyogenes</i>	1(1.4)	0(0.0)	1(100.0)
<i>Enterococcus faecalis</i>	2(2.9)	0(0.0)	2(100.0)
<i>Staphylococcus aureus</i>	38(54.3)	10(26.3)	28(73.7)
<i>S. aureus &amp; E. coli</i>	3(4.3)	0(0.0)	3(100.0)
<b>Total</b>	<b>70(100.0)</b>	<b>28(40.0)</b>	<b>42(60.0)</b>
<b>Distribution of urine isolates among HIV-Negative Subjects</b>			
Isolates	No. (%)		
<i>Klebisella pneumoniae</i>	6(21.4)		
<i>Escherichia coli</i>	11(39.3)		
<i>Proteus sp.</i>	1(3.6)		
<i>Staphylococcus aureus</i>	10(35.7)		
<b>Total</b>	<b>28(100.0)</b>		
<b>Distribution of urine isolates among HIV-Positive Subjects</b>			
Isolates	No. (%)	HIV-1	HIV-2
<i>Escherichia coli</i>	6(14.3)	6(100.0)	0(0.0)
<i>Proteus sp.</i>	2(4.8)	2(100.0)	0(0.0)
<i>Streptococcus pyogenes</i>	1(2.4)	1(50.0)	1(50.0)
<i>Enterococcus faecalis</i>	2(4.8)	1(50.0)	1(50.0)
<i>Staphylococcus aureus</i>	28(66.7)	28(100.0)	0(0.0)
<i>S. aureus &amp; E. coli</i>	3(7.1)	1(50.0)	1(50.0)
<b>Total</b>	<b>42(100.0)</b>	<b>39(92.9)</b>	<b>3(7.1)</b>

**3.5. Frequency of occurrence of uropathogens from isolated HIV-positive and HIV-negative Subjects**

Table 5 shows frequency of occurrence of uropathogens isolated from HIV-positive and HIV-negative subjects. It showed that *Staphylococcus aureus* [38(54.3%)] was the most predominant bacteria. This was followed by *Escherichia coli* [17(24.3%)], *Klebsiella pneumoniae* [6(8.6%)], *Proteus sp* [3(4.3%)], *Enterococcus faecalis* [2(2.9%)], *Streptococcus pyogenes* [1(1.4%)] and a mixed infection of *Staphylococcus aureus* and *Escherichia coli* [3(4.3%)].

It showed that of the HIV-negative subjects, 28(40.0%) yielded growth of bacteria pathogens as follows: *Escherichia coli* [11(39.3%)], *Staphylococcus aureus* [10(35.7%)], *Klebsiella pneumoniae* [6(21.4%)] and *Proteus* sp [1(3.6%)]. Among the 50 HIV-positive subjects, 42(60.0%) yielded growth of bacteria pathogens as follows: *Staphylococcus aureus* [28(66.7%)], *Escherichia coli* [6(14.3%)], *Proteus* sp. [2(4.8%)], *Enterococcus faecalis* [2(4.8%)] and *Streptococcus pyogenes* [1(2.4%)].

### 3.6 Antibiotic Sensitivity and Resistance profile of urine isolates from HIV-positive and HIV-negative subjects

Table 6 shows antibiotic sensitivity and resistance profile of urine isolates from HIV-positive and HIV-negative subjects. Detailed results of the antibiotic resistance screening tests and the summary of the antibiogram profiles obtained are presented in tables 6 and 7 respectively. The results show that all isolates from HIV-negative subjects and *E. coli* isolates from HIV-positive subjects are multidrug resistant, i.e. are resistant to four or more antibiotics. All isolates are resistant to nalidixic acid (Table 6).

**Table 6: Antibiotic Sensitivity and Resistance profile of urine isolates from HIV-positive and HIV-negative subjects**

Isolates	PEN	AMP	CEP	OFL	NAL	PEF	GEN	AUG	GPR	SEP	STR
<b>HIV Subjects</b>											
<i>E. coli</i>	S	S	S	S	R	S	R	R	S	R	S
<i>S. pyogenes</i>	S	S	S	S	R	S	S	S	S	S	S
<i>E. faecalis</i>	S	S	S	S	R	S	S	R	S	R	S
<i>S. aureus</i>	S	S	S	S	R	S	S	S	S	S	S
<i>S. aureus</i> & <i>E. coli</i>	S	S	S	S	R	S	S	S	S	R	S
<b>HIV-Negatives Subjects</b>											
<i>K. pneumoniae</i>	R	R	S	S	R	S	S	S	S	R	S
<i>E. coli</i>	R	S	S	S	R	S	R	R	R	S	S
<i>P. mirabilis</i>	R	R	R	S	R	S	R	R	S	R	S
<i>S. aureus</i>	R	S	R	S	R	R	R	R	S	S	S

**Key:** R – Resistance; S – Sensitive; PEN-Penicillin; AMP-Ampicillin; CEP-Ceporex; OFL-Ofloxacin; NAL-Nalidixic acid; PEF-Peflacin; GEN-Gentamycin; AUG-Augmentin; CIP-Ciproflox SEP-Septrin; STR-Streptomycin

Table 7 shows the results of the percentage antibiotic sensitive and resistance of urine isolates. High sensitivity to ofloxacin (66.7-100.0%), peflacin (50.0-100.0%), streptomycin (66.7-100.0%), gentamycin (50.0-100.0%) and ciproflox (86.8-100.0%) was recorded. High resistance to penicillin (50.0-100.0%), ampicillin (58.8-100.0%), nalidixic acid (50.0-100.0%) and augmentin (50.0-100.0%) were observed.

**Table 7: Percentage Sensitivity and Resistance to antibiotics**

Isolates	No. (%)		PEN	AMP	CEP	OFL	NAL	PEF	GEN	AUG	CIP	SEP	STR
<i>K. pneumoniae</i>	6(8.6)	S	0(0.0)	0(0.0)	6(100.0)	5(83.3)	0(0.0)	6(100.0)	6(100.0)	0(0.0)	6(100.0)	0(0.0)	6(100.0)
		R	6(100.0)	6(100.0)	0(0.0)	1(16.7)	6(100.0)	0(0.0)	0(0.0)	6(100.0)	0(0.0)	6(100.0)	0(0.0)
<i>E. coli</i>	17(24.3)	S	6(35.3)	7(41.2)	14(82.4)	13(76.5)	5(29.4)	17(100.0)	17(100.0)	0(0.0)	17(100.0)	8(47.1)	17(100.0)
		R	11(64.7)	10(58.8)	3(17.6)	4(23.5)	12(70.6)	0(0.0)	0(0.0)	18(100.0)	0(0.0)	9(52.9)	0(0.0)
<i>S. pyogenes</i>	1(1.4)	S	1(100.0)	1(100.0)	1(100.0)	1(100.0)	0(0.0)	1(100.0)	1(100.0)	1(100.0)	1(100.0)	1(100.0)	1(100.0)
		R	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
<i>E. faecalis</i>	2(2.9)	S	1(50.0)	0(0.0)	2(100.0)	2(100.0)	1(50.0)	1(50.0)	1(50.0)	1(50.0)	2(100.0)	1(50.0)	2(100.0)
		R	1(50.0)	2(100.0)	0(0.0)	0(0.0)	1(50.0)	1(50.0)	1(50.0)	1(50.0)	0(0.0)	1(50.0)	0(0.0)
<i>S. aureus</i>	38(54.3)	S	14(36.8)	14(36.8)	13(34.2)	33(86.8)	2(5.3)	22(57.9)	20(52.6)	14(36.8)	33(86.8)	7(18.4)	30(78.9)
		R	24(63.2)	24(63.2)	25(65.8)	5(13.2)	36(94.7)	16(42.1)	18(47.4)	24(63.2)	5(13.2)	31(81.6)	8(21.1)
<i>S. aureus</i> & <i>E. coli</i>	3(4.3)	S	3(100.0)	3(100.0)	3(100.0)	3(100.0)	0(0.0)	3(100.0)	3(100.0)	0(0.0)	3(100.0)	0(0.0)	3(100.0)
		R	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(100.0)	0(0.0)	0(0.0)	3(100.0)	0(0.0)	3(100.0)	0(0.0)
<i>Proteus</i> sp.	3(4.3)	S	0(0.0)	0(0.0)	3(100.0)	2(66.7)	0(0.0)	3(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(33.3)
		R	3(100.0)	3(100.0)	0(0.0)	1(33.3)	3(100.0)	0(0.0)	3(100.0)	3(100.0)	3(100.0)	3(100.0)	2(66.7)

**Key:** R – Resistance; S – Sensitive; PEN-Penicillin; AMP-Ampicillin; CEP-Ceporex; OFL-Ofloxacin; NAL-Nalidixic acid; PEF-Peflacin; GEN-Gentamycin; AUG-Augmentin; CIP-Ciproflox SEP-Septrin; STR-Streptomycin

## DISCUSSION

This study is examined the prevalence of HIV-1 and HIV-2 among patients in Port Harcourt,

Nigeria. It also aimed to determined pattern of uropathogens isolated from HIV positive and HIV negative subjects and their resistance and sensitivity to

different antibiotics. HIV/AIDS is a pandemic affecting the rich and the poor, the educated and illiterate, the married and single. It has its tentacles in all groups of people. However, the age range during this pandemic are the youths i.e. the sexually active group, this group range from 18–45 years. UTI is an infection that affects women as well as men but women are known to be more prone to it.

In course of this study, it was helpful finding out that sex, age and marital status of the subjects made a significant difference in the prevalence of HIV and UTI. This study confirmed that UTI is more prevalent in females than males and that HIV cuts across all age groups. It showed that females are more prone to HIV and UTI than males (UNAIDS, 2010).

In this study, there were more HIV-1 than HIV-2 which agrees with the findings of Nsagha et al. (2012) where there were more HIV-1 than HIV-2. It also agrees with the study of Abdulazeez et al. (2008), who found 12.1% and 0.5% for HIV-1 and HIV-2 respectively. Our finding contrasts the findings of a Malian study by Maiga et al. (1993) where there were more HIV-2 than HIV-1 and that of Ndiaye et al. (2008). This study also disagrees with that of Abdulazeez et al. (2008) who found 1.6% for HIV-1/HIV-2 coinfection. The absence of HIV-1/HIV-2 coinfection in our study disagrees with that of Abdulazeez et al. (2008) and Nsagha et al. (2012). Our finding however, does not rule out the possibility of more co-infection with the two virus strains. HIV-1/HIV-2 co-infection may lead to anti-retroviral resistance (Nsagha et al., 2012).

The 61.0% overall HIV seroprevalence was significantly high in the population as compared to the 4.1% prevalence in the Nigerian. The presence of many higher institutions of learning in Port Harcourt increases the youth population who are more vulnerable because of their sexual habits (Nsagha et al., 2012).

In this study, there was significant difference in HIV infection with age and sex ( $p < 0.05$ ); hence females and older age groups have the likelihood of being infected with HIV. Our findings showed that the most infected were in the 45-68 age brackets which disagrees with results of UNAIDS (2009), that the HIV prevalence in Nigeria is more amongst people aged 15-49 years. Our findings disagree with that of the Nsagha et al. (2012) who reported that most infected were in the 20-29 and 30-39 age brackets. According to Nsagha et al. (2012), the HIV prevalence in Cameroon is also more amongst people aged 15-49 years. In line with other reports, during the early years of HIV infection in Nigeria, social stigma was very high and treatment was not easily available (Eboko, 2008; Nsagha et al., 2012). With the availability of

free HIV/AIDS drugs (Baggaley, 2001), many people are willing to go for VCT and treatment.

In this study, the overall prevalence of urinary tract infection (UTI) was 85.4%, UTI (66.7% and HIV/UTI (60.0%). Looking at our findings, significant numbers of middle aged (45-68 years) patients had highest incidence of UTI. Females had highest incidence of UTI may be due to the proximity of their bladder outlet to the vagina. Married individuals had highest incidence of UTI. Although definitive diagnosis is based on culture results but looking at the significant bacteriuria in 85.4% of samples shows good clinical co-relation between clinical and microbiological diagnosis (Das et al., 2006; Jai et al., 2012).

In this study, the frequency of occurrence of uropathogens showed that *Staphylococcus aureus* [37(52.9%)] was the most predominant microorganism. This was followed by *Escherichia coli* [18(25.7%)], *Klebsiella pneumoniae* [6(8.6%)], *Proteus* sp [3(4.3%)], *Enterococcus faecalis* [2(2.9%)], *Streptococcus pyogenes* [1(1.4%)] and a mixed infection of *Staphylococcus aureus* and *Escherichia coli* [3(4.3%)]. 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).

Interestingly in the present study, most of the pathogens isolated were Gram negative rods. This disagrees with the findings of Akram et al. (2007), Yoon et al. (2011) and Jai et al. (2012) in which all detected organisms were gram negative. Gram negative bacteria have several properties to attach and invade urothelium in comparison to gram positive pathogens (Jai et al., 2012).

This study showed that *Escherichia coli* [11(39.3%)] was the most predominant organisms isolated from HIV-negative individuals. This was closely followed by *Staphylococcus aureus* [10(35.7%)], *Klebsiella pneumoniae* [6(21.4%)] and *Proteus* sp [1(3.6%)]. This is in agreement with Jai et al. (2012) who reported that *E. coli* (64.5%) was the most common isolate found in urine samples. High resistance (50.0-100.0%) of *E. coli* to antimicrobial agents tested was observed in this study. This is similar to what was observed by Aibinu et al. (2004) and Jai et al. (2012) who reported 100.0% resistance of their *E. coli* isolates to ampicillin. 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. Their finding is in harmony with the report of this study, showing 50.0%. 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).

In our study, *Staphylococcus aureus* [27(64.3%)] was the most predominant organisms isolated from HIV-positive individuals. This was followed by *Escherichia coli* [7(16.7%)], *Proteus* sp. [2(4.8%)], *Enterococcus faecalis* [2(4.8%)] and *Streptococcus pyogenes* [1(2.4%)].

From the results, high sensitivity to ofloxacin (66.7-100.0%), peflacin (50.0-100.0%), streptomycin (66.7-100.0%), gentamycin (50.0-100.0%) and ciproflox (86.8-100.0%) was recorded. It also showed that high resistance to high resistance to penicillin (50.0-100.0%), ampicillin (58.8-100.0%), nalidixic acid (50.0-100.0%) and augumentin (50.0-100.0%) were observed. There are number of studies in which mentioned about resistance of micro-organisms to conventional antibiotics like ciprofloxacin (Srinivasa et al., 1999; Ehinmidu, 2003; Umolu et al., 2006). 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 (Krumpermann, 1983; Ehinmidu, 2003).

In recent years, use of fluoroquinolones has increased in many countries and emergence of resistance of bacterial isolates to fluoroquinolones has been observed (Umolu et al., 2006). In this study, 100.0% of the isolates were sensitive to ofloxacin and peflacin. In previous years, *E. coli* was 100.0% susceptible to the fluoroquinolones. In 1996, Egri-Okwaji (1996) reported 100.0% susceptibility of *E. coli* isolates to ofloxacin. Umolu et al. (2006) reported 22.1% of ofloxacin resistance in their study, which is on the high side. Similar high resistance of *E. coli* to ofloxacin has also been documented by Alex et al. (2001); they observed that 24% of 189 *E. coli* isolates were resistant to ofloxacin.

In line with Umolu et al. (2006), the reason for the high resistance to most of the commonly used antibiotics observed in this study may be due to increasing an irrational consumption rate, transmission of resistant isolates between people and consumption of food from animals that have received antibiotics (Umolu et al., 2006). Self-medication and non-compliance with medication and sales of substandard drug may account for the rise in antibiotic resistance observed in this community (Umolu et al., 2006).

Detailed results of the antibiotic resistance screening tests in this study showed that all isolates from HIV-negative subjects and *E. coli* isolates from HIV-positive subjects are multidrug resistant (MDR), i.e. are resistant to four or more antibiotics. All isolates are resistant to nalidixic acid. Multiple drug resistance (MDR) among UTI isolates in USA was reported to be 7.1% in 2000 (Sahm et al., 2001; Umolu et al., 2006). Such multi drug resistance has serious implications for the empiric therapy of infections caused by *E. coli* and for the possible co-

selection of antimicrobial resistance mediated by multi drug resistance plasmids (Sherley et al., 2004; Umolu et al., 2006).

From the results of this study, multidrug resistant isolates i.e. isolates resistant to three or more antibiotics, were observed to be very common in the study area as 50.0% and 100.0% of isolates from HIV-positive and HIV-negative individuals respectively showed multidrug resistance. This is similar to what was observed by Umolu et al. (2006) who reported that 67.0% of the *E. coli* isolated from human clinical isolates in Lagos showed multidrug resistance. One of the limitations of this study is that we could not obtain some demographic information from participants because of logistic reasons. The fact that few numbers of subjects was used is another limitation to this study.

## 5. CONCLUSION

UTI is no doubt a prevalent infection in women and HIV infected individuals. Thus, attention should be giving to screening for UTI among HIV infected individuals. Eighty-five percent of samples showed significant growth. High yield of positive cultures showed good clinical co-relation in suspected cases of UTI. Organisms isolated in urine cultures were gram negative and gram positive bacteria. Study shows that pathogens causing urinary tract infections are developing resistance against commonly used antibiotics. Since antimicrobial resistant patterns are constantly evolving, and present global public health problem, there is the necessity for constant antimicrobial sensitivity surveillance. This will help clinicians provide safe and effective empiric therapies.

Also, we had a prevalence of 61.0% for HIV-1 and HIV-2, 47(94.0%) for HIV-1, 3(6.0%) for HIV-2 and 0(0.0%) for HIV-1/ HIV-2 coinfection. There were 39(78.0%) females and 11(22.0%) males infected giving a ratio of 3:1. The study further confirms that HIV-1 is the major cause of AIDS. HIV screening in Port Harcourt, Nigeria should test for co-infection; anti-retroviral resistance should also be investigated in Port Harcourt, Nigeria.

The antibiotic susceptibility screening of isolates from both HIV-infected and non-HIV infected individuals in this study showed that nalidixic acid, septrin and augumetin are not choice antibiotics for *E. coli*, *Staphylococcus auerus* and *Proteus* sp infection in HIV infected patients while among non-HIV infected, augumetin, ampicillin, penicillin and nalidixic acid are not choice antibiotics. Also, septrin is not likely a choice antibiotic for HIV and non-HIV patients while ciproflox, ofloxacin are good choice antibiotic for *E. coli*, *Staphylococcus aureus* and *Proteus* sp. This study showed that ceporex, ampicillin, streptomycin, septrin, ciproflex are good drug of choice for *Enterococcus faecalis* and



*Streptococcus pyogenes* infection in UTI/HIV infected patients. However, it was observed that antibiotic synergy is what makes these drugs effective.

## REFERENCES

1. Abdulazeez A, Alo E, and Naphthali R. Concurrent infection of HIV-1 and HIV-2 serotypes in Adamawa State Nigeria. *World Journal of Medical Science* 2008; 3(1): 15-18.
2. Acharya A, Gautam R, Subedee L. Uropathogens and their antimicrobial susceptibility pattern in Bharatpur, Nepal. *Nepal Med Coll J* 2011;13:30-3.
3. Aibinu, I., Adenipekun, E. and Odugbemi. (2004). Emergence of quinolone resistance amongst *Escherichia coli* strains isolated from clinical infections in some Lagos state hospitals, in Nigeria. *Nig. J. Health. Biomed. Sc.* 3 (2):73–78.
4. Akram M, Shahid M, Khan AU. Aetiology and antibiotic resistance pattern of community acquired UTI in JNMC hospital Aligarh India. *Ann Clin Microbiol* 2007;6:4
5. Alex, B., Goesseri, W., Schee, C.V., Margreet, C.V., Cornelissen, J., Hubert, E. (2001). Rapid emergence of Ofloxacin resistant Enterobacteriaceae containing multiple Gentamicin resistant associated integron in a Dutch hospital. *Emerg. Infect. Dis.* 7 (5):862–871.
6. Anglaret X, Chene G, Attia A, Toure S, Lafont S, Combe P, et al. Early chemoprophylaxis with trimethoprim- sulphamethoxazole for HIV-1 infected adults in Abidjan, Côte d'Ivoire: a randomized controlled trial. *The Lancet* 1999; 353(9163): 1463-1468.
7. Baggaley R. Voluntary counselling and testing. Paper for the UNAIDS expert panel on HIV testing in United Nations peacekeeping operations. 17-18th September 2001, New York. Pages 1-23.
8. Baidy Lo B, Adimorty M, Fatimata C, Amadou S. Surveillance of HIV seroprevalence in Mauritania. *Bulletin de Soci t  de Pathologie Exotique* 1993; 86(2):133-135.
9. Carpenter C, Cooper A, Fischl M. Antiretroviral therapy in adults. Updated recommendations of the International AIDS society – USA panel. *Journal of American Medical Association* 2000;283(3): 381-390.
10. Cheesbrough M. Human immunodeficiency virus (HIV). *District Laboratory Practice in Tropical Countries. Part 2, Second Edition.* Cambridge University Press. 2006; 253-265.
11. Chippaux JP, Maillot L, Castel J, Bochet B, Massougbodji A, Zohoun T et al. Prevalence of HIV-1 and HIV-2 antibodies in rural areas of Benin. *Bulletin de Soci t  de Pathologie Exotique* 1990; 83(4): 437-45.
12. Coovadia H. Access to voluntary counseling and testing for HIV in developing countries. *Annals of the New York Academy of Science* 2000;918(2000): 57-63.
13. Das RN, Chandrashekhar TS, Joshi HS, Gurung M, Shreshtha N, Shivananda PG. Frequency and susceptibility profile of pathogens causing urinary tract infections at tertiary care hospital. *Singapore Med J* 2006;47:281
14. Desenclos, J. C., Eergabachew, A., Desmonlins, B., Chouteau, L., Desve, G. and Admassu, N. (1988). Clinical microbiological and antibiotic susceptibility patterns of diarrhoea in Korem, Ethiopia. *J. Trop. Med. Hyg.* 91 (6): 296 – 301.
15. Eboko F. Evaluation of the access to ART and the health care system in Cameroon. Presentation at the WHO meeting on positive synergies between health systems and global health initiatives. 2-3 October 2008, Marseille, France.
16. Egri-Okwaji, M.T.C., Iroha, E.O., Kesah, C.N. and Odugbemi, T. (1996). Bacterial pathogens causing neonatal sepsis in an out-born neonate unit in Lagos, Nigeria. *Nig. Qt. J. Hosp. Med.* 6: 149 – 152.
17. Ehinmidu JO. 2003. Antibiotics susceptibility patterns of urine bacterial isolates in Zaria, Nigeria. *Tropical Journal of Pharmaceutical Research*, December 2003; 2 (2): 223-228
18. Gupta V, Yadav A, Joshi RM. Antibiotic resistance pattern in uropathogens. *Ind J Med Microbiol* 2002;20:96
19. Jai Pal Paryani, Shafique-ur-Rehman Memon, Zakir Hussain Rajpar, Syed Azhar Shah. 2012. Pattern and Sensitivity of Microorganisms Causing Urinary Tract Infection at Teaching Hospital. *JLUMHS* 11(02): 97-100
20. Krumpermann PH. Multiple Antibiotics Resistance Indexing of *E. coli* to Identify High Risks Sources of Faecal Contamination of Foods. *App Environ Microbiol.* 1983; 46:165-170.
21. Liperky BA. Urinary tract infection in men: Epidemiology, pathophysiology, diagnosis and treatment. *Ann Intern Med* 1989;111:138
22. Maiga MY, Diarra B, Guindo A, Maiga YI, Fofano O, Bougoudogo F. Seroprevalence of HIV in Mali on 3496 sera. *Bulletin de la Soci t  de Pathologie Exotique* 1993 ; 86(1): 16-20.
23. Measly RE, Levison ME. Host defence mechanisms in the pathogenesis of UTI. *Med Clin of North America* 1991;75:275
24. Mwingwa A, Hosp M, & Godfrey-Faussett P. Twice weekly tuberculosis preventive therapy in

- HIV infection in Zambia. *AIDS* 1998; 12(18): 2447-2457.
25. Nathoo KJ, Rusakaniko S, Tobaiwa O, Mujuru HA, Ticklay I, Zijenah L. Clinical predictors of HIV infection in hospitalized children aged 2-18 months in Harare, Zimbabwe. *African Health Sciences* 2012; 12(3):259 - 267
  26. National Committee for Clinical Laboratory Standards (2000). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. *NCCLS Approved standard M7-A5 and Informational Supplement M100-S19*. Wayne, PA USA.
  27. Ndiaye P, Diedhiou A, Ly D, Fall C, Tal-Dia A. HIV/AIDS Prevalence among the attendees at the center for voluntary and anonymous detection and support in Pekine/Guediawaye, Senegal. *Medicin Tropicale* 2008; 68(3): 277-82.
  28. Nsagha DS, Njunda AL, Kamga HLF, Assob JCN, Bongkem EA. 2012. HIV-1/HIV-2 co-infection among voluntary counselling and testing subjects at a regional hospital in Cameroon. *African Health Sciences* 2012; 12(3): 276 – 281
  29. Ram S, Gupta R, Gaheer M. Emerging antibiotic resistance among uropathogens. *Ind J Med Sci* 2000;54:388
  30. Rao Bhau LN, Goyal D, Chaturvedi AP, Jayasheela M, Agarwal P. Prevalence of *E. coli* serotype in urinary tract infections. *Indian J Med Microbiol* 1987;7:21
  31. Sahm, D.F., Thornsberry, C., Mayfield, D.C., Jones, M.E., Karlowsky, J.A. (2001). Multidrug resistant urinary tract isolates of *Escherichia coli*: prevalence and patient demographics in the United States. *Antimicrob. Agents Chemother.* 41: 15 – 22.
  32. Shaffer N, Chuachoowong R, Mock PA, Bhadrakom C, Siriwasin W, Young NL et al. Short-course zidovudine for perinatal HIV-1 transmission in Bangkok, Thailand: a randomised controlled trial. *Lancet* 1999; 353(9155): 773-80.
  33. Sharma S. Current understanding of pathogenic mechanisms in UTIs. *Ann Natl Acad Med Sci* 1997;33:31
  34. Sherley, M., Gardon, D.M., Collingnon, P.J. (2004). Evolution of multi-resistance plasmids in Australia clinical isolates of *Escherichia coli*. *Microbiology.* 150: 1539 – 1546.
  35. Srinivasa H, Parija SC, Bhattacharya S, Sehgal R. Incidence of ciprofloxacin resistance in urinary isolates in eastern Nepal. *J Comm Dis* 1999;31:45
  36. Umolu P. Idia, Omigie O., Tاتفeng Y., Omorogbe F.I., Aisabokhale F, Ugboadagah O. P. 2006. Antimicrobial Susceptibility and Plasmid Profiles of *Escherichia coli* Isolates Obtained from Different Human Clinical Specimens in Lagos – Nigeria. *The Journal of American Science.* 2006;2(4):70-75
  37. UNAIDS Report on the Global AIDS Epidemic; 2010  
[www.unaids.org/documents/20101123\\_epislide\\_core\\_en.pdf](http://www.unaids.org/documents/20101123_epislide_core_en.pdf)
  38. UNAIDS. AIDS epidemic update. Available at: <http://www.unaids.org>. 2009. Accessed March 19 2009.
  39. UNAIDS. The impact of Voluntary counseling and testing: A global review of the benefits and challenges. Available at: <http://www.unaids.org>.2001. Accessed May 27 2006.
  40. UNICEF. Cameroon: HIV/AIDS. Available at: [http://www.unicef.org/infobycountry/cameroon\\_statistics.html](http://www.unicef.org/infobycountry/cameroon_statistics.html). 2009. Accessed 23 October 2011.
  41. Van Nostrand JD, Junkins AD, Bartholdi RK. Poor predictive analysis and microscopic examination to detect urinary tract infection. *Am. J. Clin. Pathol.* 2000;113:709
  42. WHO, *Report and recommendations of the WHO guideline review meeting to review recommendations on the diagnosis of HIV infection in infants and children.* 2008, World Health Organization: Geneva
  43. Wiktor SZ, Sassan-Morokro MS, Grant AD, Abouya L, Karon JM, Maurice C, et al. Efficacy of trimethoprim-sulphamethoxazole prophylaxis to decrease morbidity and mortality in HIV-1 infected patients with tuberculosis in Abidjan, Côte d'Ivoire: a randomized controlled trial. *The Lancet* 1999; 353(9163): 1469-1475.
  44. Yoon Ji Eun, Wun Kon Kim, Jin Seok Lee, kyeong-Seob Slim, Tae-Sun Ho. Antibiotic susceptibility and imaging findings of causative microorganisms responsible for acute UTI in children: a five year single centre study. *Korean J Paediatrics* 2011;54:78-85
  45. Zanchette N, Vigano P, Priuli GB, Ferrario MP, Zago M, Pagano A. HIV-1 and HIV-2 prevalence of seropositivity in a population of Western Africa. *European Journal of Epidemiology* 1990; 6(1): 71-75.