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Bacteriology and Antimicrobial Profile of Urinary Tract Infection in Adult Patients with Human Immunodeficiency Virus in a Nigerian Teaching Hospital

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Abstract

Introduction: There has been a rising incidence of treatment failures of Urinary Tract Infection (UTI) in patients with human Immunodeficiency Virus Infection (HIV). This study was carried out to determine the bacteriology of urinary tract infections in patients with HIV infections in University of Nigeria Teaching Hospital, Enugu.

Methods: The study was a cross sectional study, involving 300 adult HIV positive patients, matched for age and sex with apparently healthy HIV negative subjects as control. Mid-stream urine samples from both groups were cultured on MacConkey, blood agar plates and incubated aerobically at 37°C. Antimicrobial susceptibility testing was done with agar diffusion method and interpreted according to CSLI guidelines. Extended spectrum beta-lactamases production was confirmed phenotypically using double disc synergy test.

Results: The mean age of the subjects was 22.0 ± 3 years. There was a preponderance of females 76.0%. (P=0.493). The prevalence of UTI in the study group was 5.7% as against 2.7% in the control group. The most predominant bacteria isolated from study group were *Escherichia coli* (55.0%) with the least being *Staphyloccocus saprophyticus* (5.0%). In the control group only *Escherichia coli* (62.5%) and *Klebsiella pneumoniae* (37.5%) were isolated (r=0.973, P=0.005). Most of the organisms isolated were resistant to the commonly used antibiotics. Twenty percent (20.0%) of the *Klebsiella pneumoniae* and 14.0% of *Escherichia coli* isolated were ESBL producing.

Conclusion: UTI has a higher prevalence among HIV patients. Most of the organisms isolated were resistant to the commonly used antibiotics. Routine urine culture should be done for each patient for appropriate and judicious antibiotic therapy.

Keywords: Antimicrobials; Bacteriology; ESBLs; HIV; Patients; Profile; Resistance; UTI; Treatment

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Introduction

Urinary Tract Infection (UTI) is an important health challenge in people living with Human Immunodeficiency Virus (HIV). Almost every part of their genitourinary system is affected with different diseases, mainly due to suppression of immune system [1]. By its nature, HIV predisposes to multi-system/organ infections [2] and bacterial infections are common among them. The virus decreases the $CD4^+$ cells, and as such, the individual's immune system can no longer fight against invading normal flora [3.4]. A study that was done in Tanzania reported significant association between UTI and $CD4^+$ cell count less than 200 per micro liter among people living with HIV [5]. The adolescent/young adult with HIV infection has enhanced risk of having UTI.

UTI in HIV patients has been associated with a rising incidence of nephropathies with treatment failures due to antimicrobial resistance mediated by production of Extended Spectrum Beta Lactamases (ESBLs) among bacteria. HIV nephropathies have been extensively documented both in adults and children [2,6]. The complications of the nephropathies is worsen by untreated, poorly treated and chronic UTI [7] and presence of these portends multiple morbidities.

Previous studies done in Nigeria were carried out in other geographic zones and centers but no such study had been carried out in our center. In Benin [2] south-south Nigeria, Ile-Ife [8] south-west, and Jos [7] North-central, Nigeria, the rates reported were 6.3%, 5.8% and 24.0% respectively. In Kumasi, Ghana [9] Zagreb, Croatia [10] and Rio de Janeiro [11] the rates were 7.3%, 18.0% and 13.0% respectively. It has been known that *Escherichia coli* (75%-80%) is the predominant organism implicated in UTI in normal population however, accounted for 25% of bacteria cause of UTI in HIV patients [12]. In addition, *K. pneumoniae, Enterobacter, Staphyloccocus spp and Pseudomonas aeruginosa among others* have also been isolated with variable rates in different studies [13,14]. These infections are usually poly-microbial, and the organisms are often multi-antibiotics resistant [15].

The emergence of multidrug resistant uropathogenic organisms are posing a serious public health problem globally and is associated with poor patient outcomes, increased length of hospital stay and increased costs [16]. This public health problem is particularly immense in developing countries such as Nigeria, where quality laboratory facilities are scare with high fake drugs in circulation, abuse and inappropriate use of antibiotics [17]. Development of antimicrobial resistance is a natural phenomenon but is being driven by the in-appropriate use of antimicrobials. Effective management of UTI in adults with HIV infection entails accurate identification of the uropathogens, adequate knowledge of the local sensitivity and resistant pattern of the isolates. These will also be associated with significant clinical and financial benefits, through the reduction of mortality rates, length of hospitalization and overall costs [18]. There is a need for antibiotic surveillance of urinary pathogens so that therapy can be based on judicious use of antibiotics arising from guidelines and policies.

The driving force for this study emanates from the paucity of such study in this environment and non-existent of local documented data on the aetiology, prevalence, antibiotic susceptibility and resistant patterns of uropathogenic organisms among the adult HIV patients in UNTH. This study will improve paucity of data and help the clinicians to manage the patients effectively.

Study area and design

This was a hospital based cross-sectional study. The study was conducted in PEPFAR clinic at the University of Nigeria Teaching Hospital (UNTH) Ituku-Ozalla, Enugu from January 2016 to May 2017. UNTH Ituku-Ozalla, Enugu is situated in Enugu state which is one of the five states in the south-east geopolitical zone of Nigeria. Approval for this study was obtained from Health Research and Ethics committee of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu. Informed and written consent were obtained from the participants or parents of the children less than 18 years.

Materials and Methods

A total of 300 urine samples were collected from HIV positive adult patients aged 15-49 years at the UNTH Enugu. HIV diagnosis was based on the western blot method. Adult HIV positive patients on recent antibiotic therapy (two weeks) from the time of this study were excluded. A control group consisting of 300 adult patients, (15-49 yrs.) who were HIV negative, matched for age and sex were used as controls. Both the study population and the control groups were randomly selected. Midstream urine (MSU) samples were collected with sterile wide-mouthed urine container, examined and inoculated onto blood agar and MacConkey agar (all from Titan Biotech Limited, Bhiwadi-301019 Rajasthan, India) using a calibrated wire loop with a capacity of 1 µl. All inoculated plates were incubated at 37°C for 18-24 hours aerobically and the number of colonies was counted. A positive culture was defined as one having more than 100,000 bacteria per ml of a single type of bacterium. Pure isolates of bacterial pathogen were preliminarily characterized by colony morphology and standard bacteriological tests. Further identification of the Gram-negative organisms was done with API 20E identification System (BioMerieux, Marcy-IEtoile, France).

Determination of antimicrobial susceptibility

Antibiotic susceptibility testing was done on Mueller-Hinton agar (Oxoid, England) according to the guidelines by the CLSI [19]. The drugs/antibiotics disks (Oxoid, England) used for Gram-negative rods were cotrimoxazole (25 µg), amoxicillin (30 µg) plus clavulanic acid (10 µg), gentamicin (10 µg), nitrofurantion (200 µg) amoxicillin (25 µg). Others were Ofloxacin (5 µg), meropenem (10 µg) cefepime (30 µg), ceftazidime (30 µg), ciprofloxacin (30 µg), ceftriaxone (30 µg), while for Gram-positive isolates, the antibiotics used were ampicillin, nitrofurantoin, ciprofloxacin, ofloxacin, cefotaxime, ceftazidime, ceftriaxone and meropenem and for *P. aeuroginosa*, cefepime, ofloxacin, ciprofloxacin, gentamicin and meropenem.

Extended spectrum beta-lactamase screening and confirmatory tests were carried out using the CSLI recommended indicator and confirmatory drugs. Double Disk Synergy Test (DDST) was used for the confirmation of ESBL enzyme production by the isolates.

Inoculum size determination and quality control

Quality control bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923 *and Klebsiella pneumoniae* ATCC 700063) were used. Pure cultures of bacterial isolates were suspended in 5 ml of sterile saline in a clear plastic (polystyrene) test tube to achieve a turbidity equivalent to 0.5 McFarland standard.

Statistical analysis

Statistical analyses were performed using SPSS, version 25. Descriptive and inferential statistics were used Mann-Whitney tests were used to assess the correlation of the organisms isolated in the two groups and differences in the gender. A P value <0.05 was considered statistically significant.

Results

A total of three hundred (300) MSU samples from adult HIV patients were studied consisting of 150 males and 150 females. Another three hundred (300) MSU samples from adult patients, sero-negative for HIV I and II antibodies matched for sex and age

were included as controls. Their ages were between 15-49 years. Three hundred and nine (51.5%) were married, 161 (26.8%) single with the least 49 (0.7%) divorced (**Table 1**). Of the 300 MSU samples from adult HIV patients, 17 (5.7%) yielded significant growth of uropathogenic organisms. The positive isolates were gotten from 5(25%) males and 12 (75%) females, given a ratio of 1:4 while in the control group 8 (2.7%) had significant growth of uro-pathogenic organisms giving a male to female ratio of 1: 3. (P=0.296) The most predominant uro-pathogenic organism isolated from study group was *Escherichia coli* 11(55.0%), followed by *Klebsiella pneumoniae* 5 (25.0%) with the least isolated being *Pseudomonas aeruginosa* 1(5.0%) and *Staphylococcus saprophyticus* 1(5.0%). In the control group only *Escherichia coli* 5(62.5%) and *Klebsiella pneumoniae* 3(37.5%) were isolated. (r=0.973, P=0.005) (**Figure 1**). The antibiotics susceptibility patterns of all the isolates were as illustrated in **Table 2**. All the organisms isolated generally showed high susceptibility to meropenem, ofloxacin, ceftazidime, cefepime and ceftriaxone. Almost all the organisms isolated were resistant to cotrimoxazole, nitrofurantoin and amoxicillin-clavulanic acid. *Escherichia coli* showed highest antibiotic susceptibility rate to meropenem and cefepime (100.0% each), closely followed by ceftazidime (90.0%), ceftriaxone (82.0%), ciprofloxacin and Nitrofurantoin (81.8% each), ofloxacin (73.0%). *Klebsiella pneumoniae* was most susceptible to meropenem (100.0%), ceftazidime and ofloxacin (80.0% each), and ceftriaxone (60.0%) but resistant to cotrimoxazole (40.0%) and amoxicillin-clavulanic acid (20.0%), whereas *Proteus mirabilis* was susceptible to meropenem and cotrimoxazole, ceftazidime and ofloxacin (100.0% each) but completely resistant to amoxicillin-clavulanic acid and ceftriaxone. *Pseudomonas aeruginosa* was susceptible

Table 1: The Socio-demographic characteristics of adult HIV patients with UTI.

Demographic characteristics	Age range (Years)	No of male	No of females	Total	Percentage (%)
Age	15-20	10	6	16	2.6
	21-25	4	36	40	6.7
	26-30	10	32	42	7
	31-35	22	130	152	25.7
	36-40	72	102	174	29
	41-45	40	54	94	15.7
	46-49	44	38	82	13.7
Marital status	Single	60	101	161	26.8
	Married	103	206	309	51.5
	Loss of spouse	17	55	72	18
	Divorced	10	39	49	8.2
	None	30	22	52	8.7
Educational status	Primary education	37	54	91	15.2
	Secondary	90	108	198	33
	Tertiary education	114	145	259	43.2

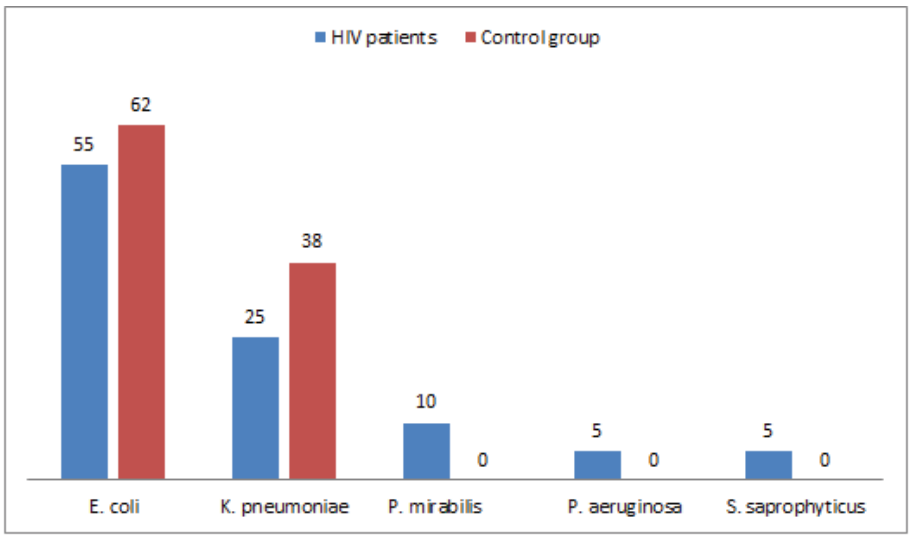

Figure 1: Distribution of the isolates from HIV patients and control group: The red bar denotes the control group and blue bar the HIV patients.

Table 2: Antibiotic susceptibility pattern of the isolates from HIV patients.

Antibiotic	*E.coli* n=11		*K.pneumo* n=5		*P.aeruginsa* n=1		*P.mirabilis* n=2		*S.sapro* n=1	
	S	R	S	R	S	R	S	R	R	S
COT	3(27.2)	8(72.8)	2(40.0)	3(60.6)	-	-	2(100)	0(100)	0(0)	1(100)
FEP	11(100)	0(0)	5(100)		1(100)	0(0)	2(100)	0(100)	0(0)	1(100)
CRO	9(81.8)	2(18.2)	3(60)	2	-	-	0	2	1(100)	0(0)
CAZ	10(90.9)	1(10.0)	4	1	-	-	2(100)	0(100)	1(100)	0(0)
OFLO	8(72.7)	3(27.3)	4((80)	1	1(100)	0(0)	2(100)	0(100)	1(100)	0(0)
CIP	9(81.8)	2(18.2)	5(100)	0(0)	1(100)	0(0)	2(100)	0	1(100)	0(0)
F	9(81.)	2(1.2)	0	5	-	-	0(0)	2(100)	0(0)	1(100)
MEM	11(100)	0(0)	5(100)	0(0)	1(100)	0(0)	2(100)	0	1(100)	0(0)
GEN	8(72.2)	3(27.)	4	1	0	-	2(100)	0	0	1(100)
AMP	1(9.1)	10(90.9)	0	5	-	-	2(100)	0	1(100)	0(0)
AUG	4(36)	7(64.0)	1	4	-		0(0)	2(100)	1(100)	0(0)

Abbreviations: *E. coli*: *Escherichia coli*; *K. pneumo: Klebsiella pneumonia*; *P. aeruginosa*: *Pseudomonas aeruginosa*; *P. mirabilis*: *Proteus mirabilis*; AUG: Augmetin, COT: Cotrimoxazole; AMP: Ampicillin; GEN: Gentamicin; CIP: Ciprofloxacin; F: Nitrofurantoin; FEP: Cefepime; MEM: Meropenem; CTZ: Ceftazidime; CRP: Ceftriaxone; Oflo: Ofloxacin; S: Sensitive; R: Resistance.

to meropenem, ciprofloxacin, and ofloxacin (100.0% each), but resistant to gentamicin, and ceftriaxone. The *Staphylococcus saprophyticus* isolated was susceptible to meropenem, ampicillin, ofloxacin, ceftazidine, ceftriaxone (100.0% each) but completely resistant to cotrimoxazole and amoxicillin-clavulanic acid. Only one strain of *Klebsiella pnuemoniae* and two strains of *Escherichia coli* of all the isolated organisms tested were phenotypically positive for Extended Spectrum Beta Lactamase (ESBL). The prevalence of ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* isolated were 20.0% (1 out of 5) and 18.1% (2 out of 11) respectively.

Discussion

The prevalence of UTI among the adult HIV patients at the University of Nigeria Teaching Hospital, Enugu in this study was 5.7%. This rate is comparable with the rate of 5.8% obtained from a similar study carried out in Ile-Ife [6]. In a related study at Benin [2] South-South Nigeria; the prevalence rates were 6.3% respectively, slightly higher than the figure obtained in this study. Higher rates of 18.0% and 20.00% were reported in the study done in Zagreb, Croatia [10] and Philadelphia respectively [20]. The high rates reported in the latter studies may be because their work centered on people with acquired immune deficiency syndrome. People with acquired immune deficiency syndrome has profound immune deficiency [10]. In addition, the use of newer and more specific anti-retroviral agents [17] Highly Active Anti-Retroviral Therapy (HAART) [15,21] and increased health awareness [22] in the subjects of this study may account for the lower prevalence rate obtained in this work.

The rate of UTI in the controls in this study was 2.6%, which was higher than an average prevalence of 0.04% in otherwise healthy population of comparable ages according to Travis [23] in Connecticut and 1.8% by the Pinho et al. [11], in Rio de Janeiro and may be due to better socio-economic status, improved living standards of these groups, factors known to minimize the occurrence of urinary tract infections [7,22] got 10.0% among controls in Jos. Different rates were reported in similar studies by some workers in other parts of the globe [3,24]. The higher prevalence rates for UTI in normal subjects obtained by these workers may be as a result of different environmental factors like poor sanitation, peculiar religious and cultural practices (like washing the anus with water after defecation which may introduce faecal matter with endogenous pathogens into the urethra (especially in females), poor health care, low socio-economic standards and poor personal hygiene in each of these locations and countries [25,26].

The uropathogens isolated from the subjects in this study were predominantly Gram-negative bacilli with *Escherichia coli* (55.0%) being the most common, closely followed by *Klebsiella pneumoniae* (25.0%), *Proteus mirabilis* (10.0%), *S. saprophyticus* and *Pseudomonas aeruginosa* (5.0% each). This compared favourably in terms of the predominant organisms as well as percentage with similar studies in Nigeria [26,27] and other parts of the world [9,20,24] where *E. coli* was the most predominant isolate. In the Benin based study [2], the most predominant urinary isolates were *Escherichia coli* (50.0%), followed by Klebsiella *pneumoniae* (30.0%) and *Staphylococcus aureus (*20.0%), while in the Jos study [7], the most predominant urinary isolates were Escherichia *coli* (43.0%), *Klebsiella species* (14.6%) and *Proteus mirabilis* (10.4%).

In a similar study in south India [28], *E. coli* was the commonest bacterial agent, followed by *K. pneumoniae*. This also contrasts with a study in Croatia [10] where *Enterococcus species* (26.0%) were the most frequent isolate in HIV patients, closely followed by *E. coli*. In a similar study in Durban [12], South Africa, the most common organism isolated was *Pseudomonas aeruginosa* (33.0%) and *Escherichia coli* (25.0%). These variations in the rate of uro-pathogens may be as a result of the very low level

of immune-suppression as well as the socio-cultural differences between the subjects [25,26].

From this study, most of the bacterial isolates were resistant to the common antibiotics but susceptible to the third generation cephalosporins and quinolones, a finding supported by other previous studies. [5,6,7,9] All the isolates in this study were completely susceptible to meropenem, a drug that may be beyond the affordability of most of the populace in Nigeria. Ofloxacin, ciprofloxacin, ceftaxidime, pefloxacin and ceftriaxone showed appreciable coverage against the isolates [2]. There was widespread antibiotic resistance to cotrimoxazole by all the isolates except *Proteus mirabilis*, a drug incorporated into the current drug management of HIV/AIDS [29]. This trend may also be a reflection of the changes in antibiotic sensitivity pattern, recently noted in UTI in association with other morbidities that have been ascribed to widespread self-medication and indiscriminate use of antibiotics that is more likely with patients having HIV/AIDS [5,24].

From this study, only one strain (20.0%) of *K. pneumoniae* and two strains (14.0%) of *Escherichia coli* were ESBLs-producing, using the double disk synergy test (phenotypic method). The prevalence rate of ESBLs for *Klebsiella pneumoniae* and *Escherichia coli* were 20.0% and 14% respectively. In south-west, Nigeria the prevalence rate for multiple beta-lactamases *Klebsiella pneumoniae* was 57% [27]. Recent extensive study by Brink Adrian et al. [30] tendered these rates for ESBLs production for *Klebsiella pneumoniae* and *Escherichia coli* respectively: Globally 23% and 17.0%, South Africa 41.0% and 8.0%, Asia 28.0% and 37%, Europe 17.0% and 9%, Latin America 35.0%, and Middle East 35.0% and 21%.

The incidence of ESBLs producing pathogens are increasing worldwide. The multiple antibiotic-resistance genes encoded on the plasmids of these organisms may allow the maintenance and spread multi-drug resistance among bacterial populations, especially in Nigeria and other developing countries where the use of antibiotics is unregulated [27].

Conclusion

In conclusion, the prevalence of urinary tract infection in adult HIV patients was 5.7%, seen more in subjects aged 20-35 years. The commonest bacterial agents were *Escherichia coli*. Most of the urinary isolates showed reasonably high antibiotic susceptibility to the third generation cephalosporins and the quinolones. Routine antimicrobial susceptibility is highly advocated for effective management of these patients.

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Author's Contribution

Conception and design of study: SOE, INN, MEO, UCO, JCC, CCO. Data collection: SOE, INN. Analysis and/or interpretation of data: SOE, INN, MEO, UCO, JCC, CCO. Drafting the manuscript: SOE, INN, MEO, UCO. Revising the manuscript critically for important intellectual content SOE, INN, MEO, UCO, JCC, CCO. Approval of the version of the manuscript to be published: SOE, INN, MEO, UCO, JCC, CCO. All authors read and approved the final manuscript.

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