Frequency and Antibiogram of uropathogens isolated from Urine Samples of HIV Infected Patients on Antiretroviral Therapy

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Abstract: Opportunistic infections including urinary tract infections (UTIs) are among the predominant cause of morbidity and mortality among HIV infected patients. Persons living with HIV/AIDS (PLWHA) are prone to infection from non-pathogenic microbes in the environment than normal individuals; and this development has been greatly attributed to the weakened immune system of HIV infected patients which makes it difficult to protect the body against invading commensal organisms. In this study, midstream urine (MSU) samples from HIV infected patients who attended the Federal Teaching Hospital Abakaliki (FETHA), Ebonyi State, Nigeria for routine antiretroviral therapy were evaluated by microbiological analysis for uropathogens. Antibiogram was also investigated on all isolated uropathogens by the Kirby-Bauer disk diffusion method. The uropathogens isolated from the MSU of HIV infected patients in this study were identified as: *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Proteus mirabilis*. *S. aureus* was the most prevalent isolated organism (n=34). This was followed by *K. pneumoniae* (n=17), *E. coli* (16) and *P. mirabilis* (n=8). All uropathogens produced varying rates of susceptibility and resistance patterns to the tested antibiotics. High sensitivity was observed with gentamicin, ciprofloxacin, ampicillin and amoxycillin-clavulanic acid. Cefotaxime, ceftazidime, cefazolin, ceftriaxone, cefepime, and cefoxitin (which are all 3rd-generation β-lactams) showed less efficacy against the uropathogens. This study draws attention to the increasing rate of UTIs amongst HIV infected patients in Abakaliki metropolis, Ebonyi State of Nigeria, and the resistance of uropathogens to some available antibiotics. Therefore, there is need to checkmate the menace through proper detection and treatment of affected individuals in order to improve the health status of PLWHA in this environment.

Keywords: HIV/AIDS, Opportunistic Infections, Uropathogens, Resistance, Nigeria

1. Introduction

Urinary tract infections (UTIs) involves bacterial invasion and multiplication in the organs of the urinary tract system including the kidney, bladder, ureters, urethra and other associated appendages, and it is manifested as at least 100,000 organisms per millimeter of urine in asymptomatic patient or as more than 100 organisms per millimeter of urine with accompanying pyuria (white blood cell >7/ml) in a symptomatic patient [1,2,3]. UTIs which accounts for a significant proportion of patients daily hospital visits is majorly caused by pathogenic bacteria, and their frequency are gradually increasing amongst HIV infected patients as an opportunistic infection. The emergence of human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) has greatly increased the incidences of opportunistic infections (especially UTIs) amongst infected patients. HIV/AIDS is one of the greatest public health crisis faced by the global community still without a total cure. More than 40 million people are living with HIV and to this pool additional 14,000 people are estimated to be added everyday [4]. Epidemiologically, AIDS occurs worldwide, and two strains (HIV-1 and HIV-2) are majorly responsible for the disease, but the HIV-2 strain is predominant in the West of Africa [5]. AIDS is a viral

disease caused by HIV, and it is an infection that debilitates the host's immune system, making it prone to opportunistic microbes including uropathogens [4,5]. The infection can be passed on when an individual's broken skin or mucous membrane comes in contact with infected body fluids including blood, semen, and vaginal secretions. Microbes found in urine samples of humans are as a result of acute urinary UTIs, and this remains one of the most common problems for which patients seek medical treatment in the community [6,7,8]. More than 90 % of UTIs are due to enteric Gram positive and Gram negative bacteria including *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis* and *Klebsiella pneumoniae* [5,9]. These microbes have been implicated in some opportunistic infections caused by pathogenic bacteria in HIV/AIDS patients [10]. Antiretroviral therapy is usually recommended for all HIV infected patients regardless of the results of their CD^4 count. And once treatment with these drugs has begun, it is recommended that it is continued without stopping in order to improve the health status of the affected patients [11]. There has been concern about the prevalence of UTIs amongst HIV infected patients in recent times, thus we investigated the occurrence and antibiogram of uropathogens amongst some selected HIV infected patients who attended the Federal Teaching Hospital Abakaliki (FETHA), Ebonyi State, Nigeria for routine antiretroviral therapy.

2. Materials and Method

Sample collection: A total of 80 midstream urine (MSU) samples from HIV infected patients on antiretroviral therapy (ART) at the Federal Teaching Hospital Abakaliki (FETHA), Ebonyi State, Nigeria were aseptically collected into sterile collection containers. The study period lasted for a period of two months (June-July, 2013). The samples were transported to the Microbiology laboratory of the Ebonyi State University, Abakaliki, Nigeria where they were analyzed.

Processing and identification: All MSU samples were aseptically inoculated on MacConkey agar, blood agar, eosin methylene blue (EMB) agar, and cysteine lactose electrolyte deficient (CLED) agar. Plates were incubated for 24 hrs at 37°C. Suspected colonies on growth media were subcultured onto MacConkey agar, blood agar, EMB, and CLED media, and incubated for 24 hrs at 37°C. These were purified by subculturing onto nutrient agar plates for further identification. Isolated organisms were identified by standard microbiology identification techniques including Gram staining, catalase test, Voges Proskauer, motility test, urease, oxidase test, coagulase test and indole test [12].

Antimicrobial susceptibility test: Susceptibility studies was carried out by the Kirby-Bauer disk diffusion technique using some selected antibiotics including gentamicin, ampicillin, cefazolin, cefepime, cefotaxime, amoxicillin/clavulanic acid, cefoxitin, ceftazidime, ceftriaxone and ciprofloxacin on Mueller-Hinton (MH) agar (Oxoid, UK) plates in line with the guidelines of the Clinical Laboratory Standards Institute, CLSI [13]. All plates were incubated for 24 hrs at 37°C, and the resulting zones of inhibition recorded and interpreted as per the CLSI criteria [13].

3. Results

The socioeconomic demographic characteristics of patients included in this study are shown in Table 1. Demographic features of patients included in this study (i.e. persons living with HIV/AIDS, PLWHA) indicated that more samples was recovered from females than males, and from those with primary or no education than those with some level of secondary and tertiary education. There were more PLWHA that were singles than those that were married (Table 1). Table 2 shows the prevalence of uropathogens isolated from the MSU patients of the HIV infected patients. A total of seventy-five (75) uropathogens including *Escherichia coli* (n=16), *Klebsiella pneumoniae* (n=17), *Staphylococcus aureus* (n=34) and *Proteus mirabilis* (n=8) were recovered from the MSU samples of the HIV infected patients who attended the Federal Teaching Hospital Abakaliki (FETHA), Ebonyi State, Nigeria. Out of the 80 MSU samples employed in this study, only 75 samples produced uropathogens which comprised of 51 (68 %) isolates in females and 24 (32 %) isolates in males. The results of the antimicrobial susceptibility of all uropathogens to some selected antibiotics are shown in Table 3.

Table 1. Demographic features of patients

Characteristics	Occurrence	Percentage (%)
Sex		
Male	26	32.5
Female	54	67.5
Total	80	100
Age		
<25	33	41.25
25-30	25	31.25
30-35	12	15.0
35-40	10	12.5
Total	80	100
Marital status		
Single	65	81.25
Married	15	18.75
Total	80	100
Educational status		
No formal education	30	37.50
Primary	20	25.0
Secondary	27	33.75
Tertiary	03	3.75
Total	80	100

Table 2. *Frequency of isolated uropathogens*

S/No.	Organism	Number (n)	Percentage (%)
1.	*Escherichia coli*	16	21.33
2.	*Klebsiella pneumoniae*	17	22.67
3.	*Staphylococcus aureus*	34	45.33
4.	*Proteus mirabilis*	8	10.67

4. Discussion

Bacterial infections including urinary tract infections (UTIs) caused by opportunistic pathogens in persons living with HIV/AIDS (PLWHA) has been on the increase, and a source of concern to health practitioners across the world. Infection with human immunodeficiency virus (HIV) that eventually results to acquired immunodeficiency syndrome (AIDS) incapacitates and weakens the natural defense mechanisms of the body, thus making the affected individual to be easily susceptible to invasion by non-pathogenic microorganisms in the environment. Several reports have elucidated the growing level of opportunistic infections amongst PLWHA [4,6,10,14,15,16]. Uropathogens were detected in higher numbers in females (67.5 %) than in males (32.5 %) in this study (Table 1). UTIs is usually more prevalent in women than in males due to the anatomic built and shortness of the female's urethra which keeps it in constant contact with microorganisms in the environment and even from the vagina [1,2,3]. The finding in this study is however noteworthy because the shortness of the female urethra and its closeness to the external genitalia (i.e. the vagina) makes contamination of the female's urinary tract system from the external genitalia almost unavoidable, and this makes UTIs to be more frequent in females than in males. Predisposing factors including HIV infection also increases the chances of a patient acquiring a UTI due to the repression of the immune system as is been reported elsewhere [6,14,15,16]. Opportunistic infections in HIV infected patients have also been reported as important co-morbidities in PLWHA [6]. The frequency of distribution of uropathogens isolated in this study shows that *staphylococcus aureus* was the most prevalent isolated organism (45.33 %), and this was followed by *Klebsiella pneumoniae, Escherichia coli* and *Proteus mirabilis* with the percentage prevalence of 22.67 %, 21.33 % and 10.67 % respectively (Table 2). These uropathogens (*S. aureus, K. pneumoniae, E. coli,* and *P. mirabilis*) are notorious as the major causative agents implicated in both complicated and uncomplicated urinary tract infection [2,3]. In Limpopo, South Africa, *E. coli* and *K. pneumoniae* have been reported as the predominant uropathogens isolated from urine samples of HIV infected patients [16].

Table 3. *Antibiogram of uropathogens to some selected antibiotics*

		Percentage Susceptibility and Resistance patterns of uropathogens to some commonly available antibiotics									
		AMC	Ampicillin	Cefazolin	Cefepime	Gentamicin	Cefotaxime	Cefoxitin	Ceftazidime	Ceftriaxone	Ciprofloxacin
S. aureus	Sensitivity	26(72%)	20(55.6%)	34(94.4%)	29(80.6%)	26(72.2%)	34(94.4%)	33(91.6%)	29((80.5%)	30(83.3%)	19(52.8%)
	Resistance	12(33.3)	14(38.9%)	30(83.3%)	18(50%)	17(47.2%)	19(52.8%)	32(88.2%)	12(33.3%)	22(61.1%)	20(55.6%)
E. coli	Sensitivity	10(58.8)	16(94.1)	9(52.9%)	14(82.4%)	13(76.5%)	8(47%)	15(88.2%)	12(70.6%)	14(82.4%)	15(88.2%)
	Resistance	9(52.9)	7(41.2%)	14(82.4%)	11(64.7%)	15(88.2%)	13((76.5%)	6(35.3%)	14(82.4%)	16(94.1%)	12(70.6%)
K. pneumoniae	Sensitivity	15(83.3%)	8(44.4%)	17(94.4%)	16(88.9%)	14(77.7%)	10(55.5%)	13(72.2%)	16(88.9%)	9(50%)	14(77.7%)
	Resistance	12(66.7%)	12(66.7%)	14(77.8%)	9(50%)	6(33.3%)	9(50%)	11(61.1%)	10(55.5%)	16(88.9%)	8(44.4%)
P. mirabilis	Sensitivity	5(55.5%)	7(77.8%)	8(88.9%)	6(66.7%)	5(55.5%)	8(88.9%)	5(55.5%)	4(44.4%)	6(66.7%)	7(77.8%)
	Resistance	1(11.1%)	5(55.5%)	3(33.3%)	7(77.8%)	3(33.3%)	7(77.8%)	8(88.9%)	2(22.2%)	8(88.9%)	4(44.4%)

Key: AMC= Amoxycillin-clavulanic acid

Also in Nigeria, *S. aureus* and *E. coli* in addition to *P. mirabilis* have been reported as the most common bacterial isolates recovered from the microbiological analysis of urine samples of PLWHA [6,14,15]. Bacterial resistance to some commonly available antimicrobial agents (which used to be very effective, though still effective in some cases) has been on the increase and this development has been implicated in the failure of these agents for treating infectious diseases including UTIs [7,8,9]. Drug resistance is a global health phenomenon which is of concern to health practitioners including microbiologists owing to the varying rates and mechanisms with which pathogens that express antimicrobial resistance genes evade the onslaught of a handful of available antibiotics. In this study, the antimicrobial susceptibility pattern of isolated uropathogens including *S. aureus, K. pneumoniae, E. coli,* and *P. mirabilis* was investigated. In the treatment of PLWHA, and who has been diagnosed of a UTI, antibiotic resistance is usually an issue to contain with owing to the less susceptibility of most uropathogens to available antibacterial agents. Isolated uropathogens (*S. aureus, K. pneumoniae, E. coli,* and *P. mirabilis*) in this study showed varying degrees of susceptibility and resistance to some commonly available antibiotics including ciprofloxacin, gentamicin, cefotaxime, ceftazidime, cefoxitin, cefepime, cefazolin, ceftriaxone, ampicillin and amoxicillin-clavulanic acid. The results of

our antibiogram (as envisaged in Table 3 of this study) shows that the uropathogens were mostly resistant to 3rd-generation cephalosporins including cefotaxime, ceftazidime, cefoxitin, cefepime, cefazolin, and ceftriaxone (which are all extended β-lactam agents) than to non-β-lactam agents such as ciprofloxacin and gentamicin. A substantial level of antibacterial activity was also observed on the test pathogens with ampicillin and amoxicillin-clavulanic acid. The acquisition of drugs over-the-counter (OTC) coupled with the use of drugs meant for human use for other non-human purposes such as in livestock rearing and animal husbandry activities may have given force to the growing rate of resistance of uropathogens to available antibiotics as is obtainable in this present day study (Table 3). Similar frequencies of uropathogen resistance to available antibiotics as noted in this study have also been reported in different quarters both within and outside Nigeria [9,10,11,14,15,16,17]. The sustained examination of urine samples of HIV infected patients for uropathogens and their antimicrobial susceptibility profiles to available antibiotics will go a long way in containing drug resistance in such individuals, and this will also help to establish effective therapeutic options which will prevent treatment failures following the use of such agents in the management of opportunistic infections in PLWHA. This study reveals that HIV infected patients (as obtained in our results) are prone to urinary tract infections, and that the uropathogens implicated in the disease are multidrug resistant. In view of the fact that individuals infected with HIV are immunocompromised, UTI in them can lead to other life threatening diseases, and as such, prompt and accurate detection of uropathogens from urine samples of HIV patients is expedient to enhance their health status.

Conclusively, there is high prevalence of uropathogens (including *S. aureus, K. pneumoniae, E. coli,* and *P. mirabilis*) amongst PLWHA on antiretroviral medications in Abakaliki metropolis in Ebonyi State of Nigeria. This development is responsible for the frequency of UTIs, a public health malady in HIV infected patients, thus there is need to contain the situation through advocacy, detection and proper treatment in order to improve the health status of PLWHA in this environment.

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