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Prevalence and antibiotic resistance pattern of blood culture isolates from human immuno-deficiency virus (HIV) patients on highly active anti-retroviral therapy (HAART) in Nigeria

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Bloodstream infections remain public health problem and have resulted to morbidity and mortality in HIV/AIDS patients. This study was therefore aimed at determining the prevalence of bacteremia in HIV infected patients on highly active anti-retroviral therapy (HAART) attending Federal Medical Centre, Ido-Ekiti, Ekiti State. A total of 105 blood samples were cultured using brain heart infusion and subcultured on bismuth sulphite agar, MacConkey agar and chocolate agar. Isolates were identified using cultural and biochemical characterization. Antibiotic susceptibility test was done using disc diffusion method. The prevalence of bloodstream infection in HIV infected patients on HAART was found to be 22.9% and they were having the $CD4^+$ T-cell counts of <300 cells/µl. There were five males and 19 females that had bloodstream bacterial infection. A total number of 24 isolates were recovered with *Escherichia coli* being the most prevalent bacterial isolates 12(50.0%), followed by *Staphylococcus aureus*, 8 (33.3%) and *Streptococcus pneumoniae*, 4 (16.7%). The antibiotic susceptibility test revealed a high level of resistance with highest resistance seen in Cotrimoxazole (87.5%), followed by ciprofloaxcin (83.3%), ampicillin (79.2%), levofloxacin (62.5%) while ceftazidime, ceftriaxone and gentamycin (54.2% each) showed the least resistance. The overall percentage of antibiotics resistance of the 7 selected antibiotics tested against isolated bacterial had 71.4, 67.9 and 66.1% resistance for *S. pneumonia*, *Escherichia coli* and *S. aureus*, respectively. However, the prevalence of bloodstream infection among HIV patients on HAART in Ekiti was relatively high and resistance to cephalosporins drugs was lower as compared to fluoroquinolones. Therefore, cephalosporin group of antibiotics is recommended for the treatment of bacteria bloodstream infection in HIV/AIDS patients.

Key words: Bacteremia, human immunodeficiency virus (HIV), antibiotics resistance, Nigeria.

INTRODUCTION

Bacteria bloodstream infections constitute a significant public health problem (Adeleye et al., 2010) and it occurs more frequently in HIV infected patients than in HIV-negative patients and is associated with higher rates of morbidity and mortality (Ojo-Bola and Oluyege, 2014). Mortality rates in HIV patients as compared to those without HIV differ between reports. It has been reported that bacterial infections are the leading cause of deaths in patients with the acquired immune deficiency syndrome (AIDS) (Gordon, 2008).

Bloodstream infections have long been one of the hallmarks of the acquired immune deficiency syndrome since the beginning of the epidemic (Whimbey et al., 1986; Bekele et al., 2003) and it can be defined as the presence of viable bacteria in the blood with a documented positive blood culture and the presence of chemical symptoms of systemic infection (Garner et al., 1998). However, it was responsible for 8% of all AIDS deaths (Stein et al., 1992). Bekele et al. (2003) reported that of 1,225 hospitalized patients at the university of health science centre, 88 (7%) had blood stream infection and 73 of the infections were community acquired. Consequently, the presence of bloodstream infections is associated with an increased mortality rate, length of hospital stay and intensive care unit admission rate. Also, a high percentage of bloodstream infections, ranging from 10 to 63% were observed in hospitalized HIV infected individuals presenting with fever in a number of studies conducted in sub-Saharan Africa (Archibald and Den Dulk, 1998; McDonalds and Archibald, 1999; Peters et al., 2004). Another study showed that bloodstream infections were associated with recent HIV diagnosis. Lower CD_4^+ T-cell counts were also strongly associated with the patients having blood stream infections (Verma and McCarthy, 2010).

Symptoms of abdominal illness such as nausea, vomiting and loss of appetite were also associated with blood stream infections caused by bacterial pathogens (Verma and McCarthy, 2010). HIV-infected patients may be predisposed to blood stream infections due to several conditions such as defective cell-mediated immunity, altered B-cell function and qualitative and quantitative deficits of neutrophils leading to an increase in the susceptibility of the patient to bacterial infections (Mertins and Ortona, 1990; Zurlo and Lane, 1997).

However, Kiertiburanakul et al. (2012) reported that opportunistic infections are still the leading causes of blood stream infection (BSI) among HIV infected patients in the HAART era in Thailand. Out of 140 patients tested 91 (65%) who had CD4+ T cell median of 32 cells/µl had blood stream infection. Common bacterial associated with bloodstream infection in HIV infected individuals include: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae, klebsiella pneumoniae, Pseudomonas aeruginosa* (Verma and McCarthy, 2010). The frequent presence of bacteria in blood stream of HIV/AIDS patients compare with the HIV negative patients was said to be due to the debilitated immune system which can leave individual vulnerable to bacterial infections (Ojo-Bola and Oluyege, 2014).

The clinical manifestation of HIV secondary infections in developing countries, including Nigeria, shows a high prevalence of infections of blood, skin, gut, respiratory tract, tuberculosis and malnutrition. The virus has a specific capacity to infect the CD4+ T-cells, resulting in progressive decline in their CD4 + T-cells number. This has serious health consequences, since CD4+ T-cell counts may almost be absent (Adeleye et al., 2010).

However, antibiotics is fundamental to illness control in these patients; unfortunately the multiple antibiotic resistance among bacterial isolates from various study groups are frightening (Adeleye et al., 2010; Ojo-Bola and Oluyege, 2014). This is because such organisms can become endemic within the environment and pose serious public health threats to the treatment and management of HIV/AIDS patients (Ojo-Bola and Oluyege, 2014).

Although, HIV patients are at higher risk of BSI, nevertheless, the introduction of HAART was said to have reduced the rate of bacteria associated blood stream infection; yet HIV/AIDS patients who are on HAART still has BSI, it then becomes imperative to know in this study area, the prevalence and antibiotics resistance pattern of bacterial isolated from HIV/AIDS on HAART. This will provide a more recent update on the treatment regimes for effective management of BSI.

METHODOLOGY

Study area

The study was conducted among HIV infected individuals attending Federal Medical Centre (FMC), Ido Ekiti, Ekiti State, Nigeria. FMC is located in Ido Ekiti, the principal town in Ido/Osi Local Government of Ekiti State with an estimated population of 107,000. The hospital is serving five contiguous states (Ekiti, Osun, Ondo, Kogi and Kwara State).

Study subject

A total of 105 blood samples were collected from the HIV/AIDS patients on HAART presenting with fever (axillary temperature ≥ 37.5°C) and seek medical attention in the above-named hospital between March, 2013 to December, 2013.

Ethical consideration, questionnaire and informed consent

The study was approved by the Medical and Research Ethics Committee of the Federal Medical Centre, Ido Ekiti, Ekiti State. The consent of the patients was sought prior to collection of blood samples. The samples were collected by medical laboratory scientist and dispensed unto brain heart infusion broth under aseptic condition.

Isolation and identification of bacterial isolates from culture of HIV infected patients

Five milliliters of blood samples were collected from the HIV

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Table 1. Demographic data of the HIV infected patients on highly active antiretroviral therapy (HAART) attending Federal Medical Centre, Ido-Ekiti.

Age group (years)	Male	Female	Total (%)
15- 25	6	10	16 (15.2)
26-35	10	19	29 (27.6)
36-45	5	19	24 (22.9)
46-55	6	14	20 (19.0)
56-65	6	5	11 (10.5)
66-75	5	0	5 (4.8)
Total	38	67	105 (100.0)

infected individuals by venous puncture from the anticubal foci on the arm after disinfecting the area with 70% of alcohol. The blood samples were inoculated into pre warmed brain heart infusion broth and incubated at 37°C for seven days. Subcultures were made on Bismuth Sulphite agar, MacConkey agar and Chocolate agar respectively from cultures showing signs of bacterial growth. The suspected colonies from Bismuth Sulfite Agar plates, MacConkey agar plates and Chocolates agar paltes were isolated using their morphology and biochemical characteristics using standard methods as described by Cheesbrough (2000). The following biochemical tests were used for the identification; catalase, coagulase, indole, motility, substrate utilization test while optochin test for *Streptococcus pneumoniae*. All the reagents were purchased from reputable company (LAB M, Lancashire, UK).

Antibiotic susceptibility test

The antibiotics susceptibility test was performed using Kirby-bauer disc diffusion method as described by CLSI (2011). Standardized inoculum was inoculated onto sterile Mueller Hinton agar plates using standard techniques for all the isolates except *S. pneumoniae* that was done on chocolate agar. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab. The dried surface of the Mueller-Hinton agar plates were inoculated by streaking the swab over the entire sterile agar surface to ensure even distribution of inoculums. The concentration of the antibiotics in the test discs were as follows: levofloxacin (5 µg/ml), ciprofloxacin (5 µg/ml), ceftazidime (30 µg/ml), ceftriaxone (30 µg/ml), ampicillin (25 µg/ml), cotrimoxazole (1.25+23.75 µg/ml) and gentamycin (10 µg/ml). The discs were dispensed onto the surface of the surface of the inoculated agar plate with a sterile forcep. Each disc were pressed down to ensure complete contact with the agar surface and distributed evenly so that they were no closer than 24 mm from centre to centre. The plates were inverted and placed in an incubator set to 37°C for 24 h. The resulting zones of inhibition were measured to the nearest whole millimeter using a meter rule and were interpreted using interpretative standards of CLSI M100-S12. Performance standards for antimicrobial susceptibility testing and the organisms were reported as susceptible, intermediate or resistant to the antibiotic being tested. The antibiotics discs were purchased from Oxoid (Basingstoke, Hampshire, UK).

Statistical analysis

Statistical procedure for social science (SPSS) version 16.0 (SPSS Inc. Chigago IL) using frequency tables was used to analyse the data generated from this study.

RESULTS

The study population comprised of one hundred and five HIV infected patients on HAART of which sixty seven were females and thirty eight (38) males. They were aged 15-75 years with a mean age of 45.4 years. Distribution according to age range showed that the majority of the HIV infected patients were in the age group of 26-35 years followed by those whose age ranged between 36-45 years and the least number was between the age group of 66-75 years (Table 1). Twenty four (24) of the HIV infected patients had a $CD4^+$ T-cell counts of less than or equal to 299 cells/µl and had positive bacterial growth while the remaining eighty-one (81) HIV infected patients had no bacterial growth with the $CD4^+$ T-cell of ≥ 500 cells/µl (Table 2).

The bacterial profile of isolates from blood culture of HIV infected patients on HAART revealed that *E. coli* had the highest percentage of 50% followed by the *S. aureus* with 33.3% while *S. pneumoniae* had the lowest percentage of 16.67% (Table 3).

S. pneumoniae exhibited the highest percentage resistance of 71.4% to the seven antibiotics tested followed by *E. coli* with a percentage resistance of 67.9%, *S. aureus* had the least percentage resistance of 66.1%. Cotrimoxazole had the highest percentage of 87.5 followed by ciprofloxacin with 83.3% and ampicillin with 79.2% and the least was ceftazidime. Both ceftriaxone and gentamycin had the same percentage resistance of 54%.

The antibiotic resistance pattern of bacterial isolates from blood culture showed that *E. coli* had ten different antibiotic resistance patterns (Table 4). *S. pneumoniae* had the least antibiotic resistance pattern of 4 (Table 5).

DISCUSSION

Blood stream infection is more common among HIV-infected individuals and remains World Health Organization (WHO) stage 4 defining condition (Gordon, 2008). Bloodstream infections constitute a significant public health problem and represent an important cause of morbidity and mortality in HIV/AIDS patients (Adeleye et al., 2008). However, from this study, the prevalence of bacteria in the blood samples of HIV infected patient on HAART attending Federal Medical Center Ido-Ekiti was 22.9%. The prevalence observed in this study is higher as compared to the study conducted in University Teaching Hospital during October 2005-2006 Lagos State, Nigeria which was 12.9% (Adeleye et al., 2010). Also, study conducted in Malawi showed 30% prevalent rate of HIV infected patients with blood stream infections (Bonadio et al., 1998).

The prevalence of bacteria in relation to sex of the HIV/AIDS patients revealed that there were more females than males that had bacteria in their blood and they are in ratio 13:11 in the study subject and this is a reflection of

Table 2. The $CD4^+$ T-cells count in relation to sex of the HIV infected patients on HAART Attending Federal Medical Centre, Ido-Ekiti.

$CD4^+$ T-cells count (cell/ ml)	Sex distribution		Total (%)	Bacteria growth
	Male	Female		
100-199	6	7	13 (12.3)	Positive
200-299	5	6	11(10.5)	Positive
300-399	0	0	0 (0.0)	-
400-499	0	0	0 (0.0)	-
500-599	15	21	36 (34.3)	Negative
600-699	7	18	25 (23.8)	Negative
700-799	5	13	18 (17.1)	Negative
800-899	0	2	2 (1.9)	Negative
Total	38	67	105 (100.0)	

Table 3. Bacteria profile of isolates from blood culture of HIV patients on highly active anti-retroviral therapy (HAART).

Organism	No. of isolates (%)
Staphylococcus aureus	8 (33.3)
Escherichia coli	12 (50.0)
*Streptococcus pneumonia*e	4 (16.7)
Total	24 (22.9)

Table 4. Antibiotic resistance in bacterial isolates from blood culture of HIV infected patients on highly active anti-retroviral therapy (HAART).

Organism	lev	cip	cefta	ceftr	amp	cot	gen	Total average
Escherichia coli (*n*=12) (%)	8(66.6)	10(83.3)	5(41.7)	5(41.7)	10(83.3)	11(91.6)	8(66.6)	8.1 (67.9)
Staphylococcus aureus (n= 8) (%)	5(62.5)	6(75.0)	5(62.5)	5(62.5)	5(62.5)	7(87.5)	4(50.0)	5.3 (66.1)
Streptococcus pneumoniae (n=4) (%)	2(50.0)	4(100.0)	3(75.0)	3(75.0)	4(100.0)	3(75.0)	1(25.0)	2.9 (71.4)
Total Average (n =24) (%)	15(62.5)	20(83.3)	13(54.2)	13(54.2)	19(79.2)	21(87.5)	13(54.2)	(54.2)

Cip = ciprofloxacin, lev = levofloxacin, cefta = ceftazidime, cot = cotrimoxazole, ceftr = ceftriaxone, gen = gentamicin, amp = ampicillin.

disproportionate impact of HIV on women and girls than on men. Obi et al. (2007) established that there were more HIV positive females than males in the studies in South Africa, indicating a gender bias. The vulnerability of women to HIV in sub-Saharan African stem from their greater physiological susceptibility and the severe social, legal and economic disadvantage that confront them. In sub-Saharan African region, as worldwide, female population is a key factor in the epidemiology of HIV and AIDS because 50% of all adults with HIV infection are predominantly women infected via heterosexual transmission. Furthermore, females are the most severely affected (Mitchell et al., 2003; Hickey and Shanson, 1993).

There are twenty four (UNAIDS /WHO, 2009) bacterial isolates of three different generals from one hundred and five samples examined with total prevalence rate of 22.9%. There were 12 *E. coli* with prevalent rate of 50% followed by *S. aureus*, 8 (33.3%) and *S. pneumoniae*, 4 (16.67%). This agreed with the study conducted by Kiertburanakul et al. (2012) that reported *E. coli* and *S. pneumonia* as the most common Gram negative and positive organisms isolated from the bloodstream of hospitalized HIV-infected patients in Thailand. Other studies have suggested an increased risk of bloodstream infections due to aerobic Gram negative bacilli such as *E. coli* in HIV/AIDS patients particularly in those having invasive devices (Zurlo and Lane, 1997; WHO, 2005; Tumbarello and Tacconelli, 1998). Also, the most recent study conducted by Ojo-Bola and Oluyege (2014) reported that *E. coli* had the highest prevalence of bacterial associated with pneumonia in HIV/AIDS patients and this can be disseminated to bacterial bloodstream infection.

Table 5. Resistance pattern of bacteria isolates from blood culture of HIV patients on highly active anti-retroviral therapy.

Bacterial species	Resistance pattern	Number of organism showing pattern
	lev, cip, amp, cot, gen,	1
	Amp, Cot, Gen	1
	lev, cip, cefta, ceftri, amp, cot, gen	5
Escherichia coli	cip, amp, cot, gen	1
	cip, ceftri, amp, cot	1
	lev, cip, ceftr, amp, cot	1
		10
	lev, cip cefta, ceftr, amp, cot, gen	2
	cefta, ceftr, cot	1
	lev, cip, ceftr, cot	1
Staphylococcus aureus	cip, cefta, ceftri, amp, cot, gen	1
	lev, cip, amp, cot, gen,	1
	lev, cip, cefta, cot, gen	1
		7
	cip, cefta, ceftr, amp, cot	1
	lev, cip, cefta, ceftr, amp, cot, gen	1
Streptococcus pneumonia	cip, cefta, ceftr, Aamp, cot, gen	1
	lev, cip, amp	1
		4

cip = ciprofloxacin, lev = levofloxacin, cefta = ceftazidime, cot= cotrimoxazole, ceftr = ceftriaxone, gen= gentamicin, amp = ampicillin

However, HIV has a specific capacity to infect the CD4+ T-cells lymphocyte, resulting in progressive decline in their CD4+ T-cells number. This has serious health consequences, since CD4+ T-cells constitute about 10% of the total T-cell pool. In HIV/AIDS patients, the number of CD4+ T-cells may be almost absent. Nevertheless, the era of HAART has greatly reduced the secondary infection in these groups of patients (Adeleye et al., 2008). In this study, the twenty four HIV infected patients with bacterial growth had a CD_4^+ T-cell counts of less than or equal to 299 cells/µl and the remaining 81 (eighty one) patients without bacterial growth had a CD_4^+ T-cell counts ≥ 500 cells/µl. This study agreed with a report that there is a steady decline in the CD_4^+ T-cell counts of patients and by the time opportunistic infections set in, there may be no more CD_4^+ T-cells present in the immune system (UNAIDS/WHO, 2009).

The antibiogram of the isolates were tested with seven antibiotics commonly prescribed in the hospital. *E. coli* showed 66.6% resistance to gentamycin and levofloxacin, 83.3% resistance to ciprofloxacin, 41.7% resistance to ceftazidime and ceftriaxone, 83.3% resistance to ampicillin, 91.6% resistance to cotrimoxazole, *S. aureus* showed 62.5% resistance to levofloxacin, ceftazidime, ceftriaxone and ampicillin, 75% resistance to ciprofloxacin, 87.5% to cotrimoxazole and 50% resistance to gentamycin. *S. pneumoniae* showed 100% resistance to ciprofloxacin and ampicillin, 75% resistance to ceftazidime, ceftriaxone and cotrimoxazole, 50% resistance to levofloxacin and 25% resistance to gentamycin. It has been observed in this study that the resistance of bacteria to some used antibiotics (ciprofloxacin, ceftazidime, cotrimoxazole, levofloxacin and gentamycin) was relatively high. Of a total of seven antibiotics employed in the antibiotic susceptibility test, *E. coli* exhibited a higher percentage of 91.6% resistance to cotrimoxazole, while *S. aureus* also exhibited 81.5% percentage resistance to ciprofloxacin and ampicillin.

The multiple antibiotics resistance is a resistance to more than 3 groups of different antibiotics. *E. coli* showed 10 different pattern of multiple resistance while *S. aureus* and *S. pneumoniae* has 7 and 4 patterns, respectively.

In conclusion, the prevalence of isolation of bacteria from blood culture of HIV infected patients on HAART in this study was found to be 22.9% and was among HIV patients whose CD4+ T-cell falls with ≥ 299 cells/µl; the antibiotic resistance of the bacteria was relatively high to the antibiotics used. This may be as a result of unnecessary use or overuse of antibiotics to treat bacterial infections. However, based on this study, cephalosporins drugs had a lower resistance as compared to fluoroquinolones and it should be used for the treatment

of bacteria associated bloodstream infections.

Conflict of interest

There is no conflict of interest.

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