

Assessment of Blood CD4 Count and Antibiogram Profile of Bacteria Isolated from HIV Patients

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Abstract

In this study, the blood CD4 count and the bacterial profile in the stool of Human Immunodeficiency Virus (HIV) positive individuals attending Antiretroviral therapy (ART) clinic in a tertiary health institution in Ekiti State, Nigeria was investigated. In addition, the antibiogram of the bacterial isolates was also investigated. A total of 150 HIV patients was recruited for the study. Samples of their blood and stool were collected for the investigation. Their blood was used to determine their CD4 count using cytometry method while their stools were cultured on microbiological media and pure isolates were identified using standard microbiological techniques. The antibiogram of the isolates was determined using disk diffusion method. HIV negative individuals were used as control. The results showed that the CD4 count of HIV patients ranged from 5 to 1278 cells/mm³, while the most frequently encountered bacteria in their stool are *Pseudomonas aeruginosa*, *Morganella morganii*, *Aeromonas* sp, *Enterococcus* sp. and *Lactobacillus* sp. All these bacterial spp however are absent from the stool of the control subjects. Pathogenic bacteria such as *Salmonella typhi* [6 (40% and 4 (26%)], *Shigella* species [7 (41%) and 5 (28%)], *Pseudomonas aeruginosa* [3(37.5%) and 4(50%)] were prevalent among patients with CD4 count level 200-350 cell/mm³ and 200 cell/mm³ respectively but are statistically insignificant ($p>0.05$). The isolated bacterial spp., were resistant to most of the conventional antibiotics tested and the resistance was plasmid mediated in 95.2% of the isolates. This study shows the importance of investigating associated bacterial pathogens in HIV patients and evaluating the antibiogram profile of such pathogens before prescribing antibiotics to such patients in order to checkmate bacterial infections that may complicate the infection.

Keywords: Assessment; CD4 count; Antibiogram profile; HIV patients

Introduction

In 2004, the World Health Organization (WHO) identified HIV/AIDS as the world's most urgent public health challenge, as AIDS represents the greatest lethal epidemic in recent history [1] and as at the end of 2014, Nigeria accounted for 9% of the of the global HIV infection, with 3.2 million people living with HIV, 220,000 newly infected and 210,000 AIDS-related death [2]. HIV patients are at frequent risk of opportunistic infections caused by different microorganisms including bacteria, fungi, viruses, protozoa and helminthes [3]. The most frequently encounter complication in HIV infection is gastrointestinal disease mostly caused by enterobacteriaceae. Bacteria opportunistic infection accounted for about 90% cases of the complication, though; they are treatable with broad or narrow spectrum antibiotics [4,5] and the persistent of some bacteria among HIV patients with low CD4 count level are reported in many literatures [6].

Also, bacterial resistance to several classes of antibiotics in HIV-infected individuals in Sub-Saharan countries is on the increase, ranging between 0.25 and 21% in some instances [7].

But the control of these infections constitutes a challenge because of the emergence of multiple antibiotic resistances.

To know the associated opportunistic bacterial among patient with different CD4 count level and to guide appropriate antibiotic use, the prevalence of opportunistic bacterial infections and the antibiogram profile of bacterial isolates in HIV-infected and HIV negatives individuals were studied.

Materials and Methods

Collection

A total of 150 HIV patients attending Ekiti State University Teaching Hospital Ado Ekiti and 50 suspected HIV negative individuals as control were recruited for the investigation. Blood and stool samples were collect from the participants. The blood sample collected from

collected from HIV patients was used to determined their CD4 count level while blood sample from suspected HIV negative individual to determine their HIV status.

Sample Analysis

Assessment of blood sample from apparently healthy individuals for HIV status

Screening for HIV sero-status was performed using Alere Determine™ HIV-1/2 Ag/Ab Combo (Determine Combo). This is a rapid test capable of detecting HIV-1 p24 antigen and HIV-1 and HIV-2 antibodies. The p24 antigen is a part of the HIV virus and can be detected before antibodies develop. If the test is reactive, the Determine Combo indicates whether the reaction is caused by antigen, antibody, or both. Whole blood was used and it was collected by finger pricking. A drop of blood was placed on the developer and was observed for 15 minutes as it migrated along the strip. Appearance of only the control line (zone C) indicates negative result (North Carolina HIV Prevention Program, 2015) [8].

Determination of the CD4 count of the blood collected from HIV patients

CD4 count test was carried out on whole blood samples collected from HIV positive patients using flow cytometry. The flow cytometric

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Received June 07, 2016; Accepted June 29, 2016; Published July 06, 2016

Citation: Ojo BA, Adebolu TT, Odinayo MS (2016) Assessment of Blood CD4 Count and Antibiogram Profile of Bacteria Isolated from HIV Patients.. HIV Curr Res 1: 107. doi: [10.4172/2572-0805.1000107](https://doi.org/10.4172/2572-0805.1000107)

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assays work on the principle of scattering of light due to different sizes, granularity of the cells passing through the laser beam, and also by the fluorescence emitted by the cells after staining with the specific monoclonal antibodies to cell surface markers that are tagged with different fluorescence dyes. The population of interest can be thus identified and gated.

The single-platform approach was employed for absolute CD4 T lymphocytes counts to be derived directly without the need for a haematological analyzer. This can be assessed either by counting CD4 T lymphocytes populations in a precisely determined blood volume or by using the known numbers of fluorescent microbeads admixed to a known volume of CD4-stained blood.

When whole blood is added to the reagents and incubated for 15 minutes, fluorochrome-labeled antibodies in the reagents bind specifically to lymphocyte surface antigens. After a fixative solution is added, the sample is run on the instrument. Here the stained cells come in contact with the green HeNe laser light, which causes the cell to fluoresce. This fluorescent light provides the information necessary for the instrument to count the cells. The calculation of absolute CD3+, CD4+ and CD8+ T-cells is determined automatically by using the built-in Attractions software programme [9].

Bacteriological Investigation

Culture

Using calibrated wire loop (0.001 mL), samples were inoculated in to blood agar and MacConkey agar (Rapid Labs Ltd. and Deben diagnostics ltd) after been homogenized in pepton water. It was then incubated for 24 hours at 37°C. Identification of bacteria was done using colony characteristics, gram reaction of the organisms and biochemical tests following standard procedure [10].

Antibiotic susceptibility testing

The bacterial isolates were tested on commercially prepared antibiotic disk impregnated with Gentamicin (10µg), ofloxacin (5 µg), agumentin (30 µg), nitrofurantoin (300 µg), ceftanidime (30 µg), cefuroxime (30 µg), cefprofuxacin (5 µg), ampicillin (10 µg), tetracycline (50 µg), erythromycin (10 µg), using the agar disc-diffusion technique

developed by Bauer et al. [11]. The zones of inhibition were measured using a transparent ruler and compared with standard set by [12].

Plasmid extraction

Selected isolates with multiple resistance were subjected to plasmid detection as described by A 1.5 ml of overnight broth culture was spanned for 1 minute in a micro-centrifuge to pellet cells. The supernatant was gently decanted leaving 50-100 µL together with cell and vortex at speed to resuspend cells completely. TENS (300 µL) was added and mixed by inverting tubes 3-5times until the mixture become tricky and the tubes were set on ice to prevent degradation of chromosomal DNA which may be co-precipitated with plasmid DNA. Sodium acetate (3.0 M) was then added at pH 5.2. Then vortexed to mix completely. The mixture was then spinned for 5 minutes in micro-centrifuge to pellet cell debris and chromosomal DNA. The supernatant was then transferred into a fresh tube and mixed it well with 900 µL of ice-cold absolute ethanol. This was then Spinned for 10 minutes to pellet plasmid DNA (White pellet is observed) [13,14].

Curing of plasmid by treatment using acridine orange

This was done by growing the bacteria cell in broth overnight. Nutrient broth (5 mls) supplemented with 0.1 mg/mL acridine orange was prepared and the bacteria were now subculture in it. The organism was then subcultured on nutrient agar [15].

Ethical approval

Ethical clearance was obtained from the Ethical Committee of Ekiti state University Teaching Hospital, Ado Ekiti and patients consent were seek before collecting samples.

Result

Out of 150 HIV patients attending the ART clinic at EKSUTH, Ado-Ekiti that were recruited for this investigation, 112 (74.7%) were found to be females while 38 (25.3%) were found to be males (Table 1). Also based on the WHO class of CD4 level before initiation of Antiretroviral Therapy (ART), patients with CD4 count level between 200-350 cells/mm³ had the percentage 60 (40%) followed by patients with CD4 count \geq 350 cells/mm³ 48 (32%) (Table 2).

Bacterial isolates

A total of 335 bacterial isolates were obtained from both the stool of HIV positive and HIV negative participant (261(77.9%) and 74(22.1%) respectively). The most frequent of the isolate was *Escherichia coli* (33.5%) followed by *Klebsiell apneumoniae* (10.7%). The bacterial isolate from HIV positive individuals were similar to those isolated from HIV negative individuals except *Aeromonas* spp, *Pseudomonas aeruginosa*, *Morganella morganii*, and *Enterococcus* spp that were only present in HIV positive individuals (Table 3).

Frequency of concurrency of bacterial isolated from the stool of HIV patients investigated based on their CD4 count group

The following bacterial isolates were found to be prevalent among patients with CD4 count below 200 cells/mm³. These are *Serratia marcescens*, *Pseudomonas aeruginosa* and *Aeromonas* species that were found only among patients with CD4 count below 200 cells/mm³. *Salmonella paratyphi A*, *Klebsiella pneumoniae*, *S. aureus*, and *Lactobacilli* sp were also frequently isolated among the people with CD4 count between 200-350 cells/mm³, while *Enterobacter* species *Salmonellae* sp., *Morganella morganii* and coagulase negative *Staphylococcus* were prevalent among patients with CD4 count 350 cells/mm³ and above. The percentage representation of the frequency of occurrence of these bacterial isolates are presented in Table 4.

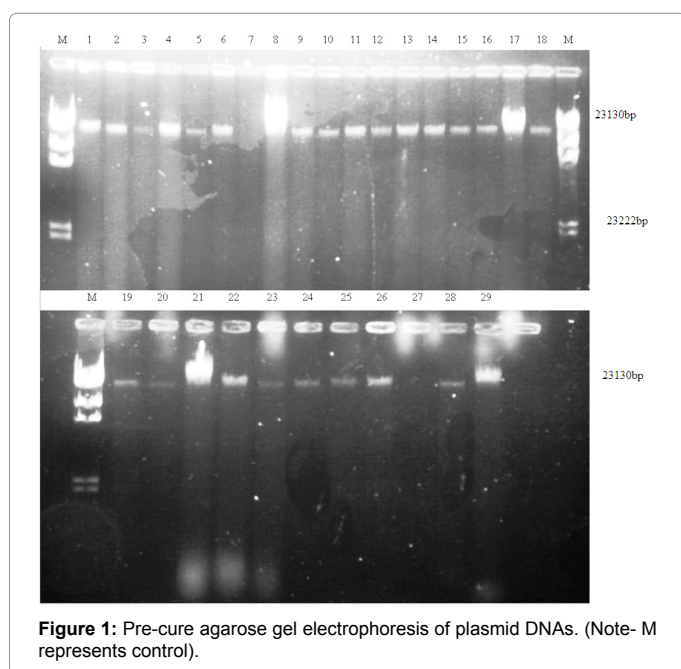


Figure 1: Pre-cure agarose gel electrophoresis of plasmid DNAs. (Note- M represents control).

Age group (years)	Gender		Total (%)
	Male (%)	Female (%)	
<25	2 (20.0)	8 (80.0)	10 (6.7)
26-35	13 (24.5)	40 (75.5)	53 (35.3)
36-45	11 (21.2)	41 (78.8)	52 (34.7)
46-56	6 (33.3)	12 (66.7)	18 (12.7)
56-65	3 (27.3)	8 (72.7)	11 (7.3)
66-75	3 (50.0)	3 (50.0)	6 (4.0)
Total	38 (25.3)	112 (74.7)	150 (100.0)

Table 1: Distribution of HIV infection among patients used for the investigation based on gender and age.

Risk level	Frequency	Percentage%
Healthy (above 350 cells/mm ³)	48	32.0
low risk (200-350 cells/mm ³)	60	40.0
high risk (below 200 cell/mm ³)	42	28.0
Total	150	100.0

Table 2: Distribution of CD4 count level based on AIDS risk level. Percentages are represented in the parentheses.

Bacteria isolates	HIV patient (%)	Control (%)	Total isolate	Total %
<i>Serratia marcescens</i>	8 (72.7)	3 (27.3)	11	3.3
<i>Escherichia coli</i>	93 (78.2)	26 (21.8)	119	35.5
<i>Salmonella typhi</i>	15 (78.9)	4 (21.1)	19	5.7
<i>Citrobacter freundii</i>	25 (89.3)	3 (10.7)	28	8.4
<i>Shigella</i> spp	17 (85.0)	3 (15.0)	20	6.0
<i>Salmonella paratyphi A</i>	3 (75.0)	1 (25.0)	4	1.2
<i>Klebsiell apneumoniae</i>	30 (83.3)	6 (16.7)	36	10.7
<i>Enterobacter</i> spp	8 (57.1)	6 (42.9)	14	4.2
<i>Proteus vulgaris</i>	0 (0.0)	1 (100.0)	1	0.3
<i>Yersinia enterocolitica</i>	3 (75.0)	1 (25.0)	4	1.2
Other Salmonellae	3 (30.0)	7 (70.0)	10	3.0
<i>Proteus mirabilis</i>	13 (72.2)	5 (27.8)	18	5.4
<i>Providencia</i> spp	9 (81.8)	2 (18.2)	11	3.3
<i>Pseudomonas aeruginosa</i>	8 (100.0)	0 (0.0)	8	2.4
<i>Morganella morganii</i>	7 (100.0)	0 (0.0)	7	2.1
<i>Aeromonas</i> spp	1 (100.0)	0 (0.0)	1	0.3
<i>Staphylococcus aureus</i>	4 (66.7)	2 (33.3)	6	1.8
Coagulase negative <i>Staphylococcus</i>	1 (50.0)	1 (50.0)	2	0.6
<i>Streptococcus</i> spp	5 (62.5)	3 (37.5)	8	2.4
<i>Lactobacillus</i> spp	3 (100.0)	0 (0.0)	3	0.9
<i>Enterococcus</i> spp	5 (100.0)	0 (0.0)	5	1.5
Total	261 (77.9)	74 (22.1)	335	100.0

Table 3: Frequency of occurrence of the bacteria isolated from the stool of HIV patients and control individual. Percentages are represented in the parentheses.

Antibiogram profile

All the gram negative isolates from both HIV positive and HIV negative individuals were highly resistance to ampicillin (213 (87.7%) and 66 (97.1%) respectively) and augmentin (174 (71.7%) and 46 (67.6%) respectively) while the least resistance was observed in Cefprofuxacin (18 (14.8%) and 13 (10.1%) respectively) (Table 5). Also the Gram positive isolates from HIV negative individuals had higher resistance to majority of the antibiotics used; ampicillin 6 (100%), agumentin 5 (83.3%), tetracycline and 5 (83.3%), erythromycin 4 (66.7%) as compared to isolates from HIV negative individuals. However, isolates from HIV negative individuals had higher resistance to ofloxacin 14 (77.8%) and gentamicin 7 (72.2%) (Table 6).

Plasmid detection and effect of curing

A total of 29 bacterial isolates with multiple antibiotic resistance

were selected from the bacteria isolated from both seropositive patients and seronegative individuals and were tested for the presence of resistance plasmid. Out of 29 isolates subjected to plasmid determination, 27 (93.1%) were observed to carry heavy plasmid. Plasmid DNAs are represented in Figure 1 while the post Agarose Gel Electrophoresis of Plasmid DNAs determination is presented. Tables 7 and 8 represent the pre-cured antibiogram profile of selected multiple antibiotic resistant bacteria isolates.

Effect of plasmid curing on antibiotic resistance of the selected bacterial isolates

The effect of plasmid curing on the antibiotic sensitivity profile of the selected bacterial isolates revealed that majorities of the resistance were not plasmid base, as many of the bacterial selected still showed resistance to the same antibiotic. All the isolates were observed to be resistance to augmentin and ampicillin after curing. It was also

Organism	Healthy (≥ 350 cells/mm ³) (%)	Low (200-350 cells/mm ³) (%)	High risk (≤ 200 cells/mm ³) (%)	X ² Values	P Values
<i>Serratia marcescens</i>	1 (12.5)	3 (37.5)	4 (50.0)	6.0	0.1
<i>Escherichia coli</i>	41 (44.1)	26 (28.0)	26 (28.0)	3.0	0.2
<i>Salmonella typhi</i>	5 (33.3)	6 (40.0)	4 (26.7)	6.0	0.1
<i>Citrobacter freundii</i>	6 (24.0)	12 (48.0)	7 (28.0)	6.0	0.1
<i>Shigella species</i>	5 (29.4)	7 (41.2)	5 (29.4)	3.0	0.2
<i>Salmonella paratyphi A</i>	1 (33.3)	2 (66.7)	0 (0.0)	6.0	2
<i>Klebsiella pneumonia</i>	7 (23.3)	19 (63.3)	4 (13.3)	6.0	0.2
<i>Enterobacter species</i>	4 (50.0)	2 (25.0)	2 (25.0)	3.0	0.2
<i>Proteus vulgaris</i>	0 (0.0)	0 (0.0)	0 (0.0)		NS
<i>Yersinia enterocolitica</i>	1 (33.3)	1 (33.3)	1 (33.3)		NS
<i>Salmonellae spp.</i>	3 (100.0)	0 (0.0)	0 (0.0)	3.0	0.2
<i>Proteus mirabilis</i>	4 (30.8)	5 (38.5)	4 (30.8)	3.0	0.2
<i>Providencia species</i>	1 (11.1)	4 (44.4)	4 (44.4)	3.0	0.2
<i>Pseudomonas aeruginosa</i>	1 (12.5)	3 (37.5)	4 (50.0)	6.0	0.1
<i>Morganella morganii</i>	5 (71.4)	1 (14.3)	1 (14.3)	3.0	0.2
<i>Aeromonas species</i>	0 (0.0)	0 (0.0)	1 (100.0)	3.0	0.2
<i>Staphylococcus aureus</i>	2 (50.0)	2 (50.0)	0 (0.0)	3.0	0.2
Coag negative <i>Staphylococcus</i>	1 (100.0)	0 (0.0)	0 (0.0)	3.0	0.2
<i>Streptococcus spp.</i>	1 (20.0)	2 (40.0)	2 (40.0)	3.0	0.2
<i>Lactobacillus spp.</i>	1 (33.3)	2 (66.7)	0 (0.0)	6.0	0.1
<i>Enterococcus spp.</i>	2 (40.0)	2 (40.0)	1 (20.0)	3.0	0.2
Total	92 (35.2)	99 (37.9)	70 (26.8)		

Table 4: Frequency of occurrence of bacterial isolates among different groups of CD4 count level of HIV positive patients. **Note:** Coag= Coagulase, Figures in parentheses represent parentheses.

Microorganisms	CAZ (clinical)	CAZ (control)	CXR (clinical) (%)	CRX (control)	GEN (clinical)	GEN (control)	CPR (clinical)	CRP (control)	Total Clinical	Total control
<i>Serratia marcescens</i>	4 (50.0)	0 (0.0)	1 (12.5)	0 (0.0)	2 (25.0)	0 (0.0)	2 (25.0)	0 (0.0)	8	3
<i>Escherichia coli</i>	22 (23.7)	16 (61.5)	19 (20.4)	15 (57.7)	19 (20.4)	11 (42.3)	14 (15.1)	10 (38.5)	93	26
<i>Salmonella typhi</i>	1 (6.73)	1 (25.0)	3 (20.0)	2 (50.0)	3 (00.0)	1 (25.0)	3 (20.0)	0 (0.0)	15	4
<i>Citrobacter freundii</i>	8 (32.0)	0 (0.0)	10 (40.0)	0 (10.0)	11 (44.0)	2 (66.7)	5 (20.0)	0 (0.0)	25	3
<i>Shigella spp</i>	7 (41.2)	0 (0.0)	4 (23.5)	0 (0.0)	5 (29.4)	0 (0.0)	3 (17.6)	0 (0.0)	17	3
<i>Salmonella Paratyphi A</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3	1
<i>Klebsiella pneumonia</i>	2 (6.7)	1 (16.7)	8 (26.7)	1 (16.7)	8 (26.6)	1 (16.7)	0 (0.0)	0 (0.0)	30	6
<i>Enterobacter species</i>	1 (12.5)	0 (0.0)	0 (0.0)	3 (50.0)	1 (12.5)	0 (0.0)	1 (12.5)	0 (0.0)	8	6
<i>Yersinia enterocolitica</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3	1
<i>Salmonellae spp.</i>	0 (0.0)	1 (14.3)	0 (0.0)	2 (28.6)	0 (0.0)	1 (14.3)	0 (0.0)	1 (14.3)	3	7
<i>Proteus vulgaris</i>	0 (0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	1 (100.0)	0	1
<i>Proteus mirabilis</i>	2 (15.4)	2 (40.0)	0 (0.0)	1 (20.0)	0 (0.0)	1 (20.0)	0 (0.0)	1 (20.0)	13	5
<i>Providencia spp.</i>	0 (0.0)	1 (50.0)	5 (55.6)	1 (50.0)	6 (66.7)	0 (0.0)	6 (66.7)	0 (0.0)	9	2
<i>Pseudomonas aeruginosa</i>	6 (75.0)	NI	2 (25.0)	NI	2 (25.0)	NI	2 (25.0)	NI	8	0

Organisms	OFL (clinical) (%)	OFL (control) (%)	AUG (clinical) (%)	AUG (control) (%)	NIT (clinical) (%)	NIT (control) (%)	AMP (clinical) (%)	AMP (control) (%)	Total (control)	Total Clinical
<i>Morganella morganii</i>	0 (0.0)	NI	0 (0.0)	NI	0 (0.0)	NI	0 (0.0)	NI	7	0
<i>Aeromonas</i> spp.	0 (0.0)	NI	0 (0.0)	NI	(0.0)	NI	0 (0.0)	NI	1	0
Total	53 (21.8)	23 (33.8)	52 (21.4)	25 (36.8)	49 (23.5)	18 (26.6)	36 (14.8)	13 (10.1)	243	68
<i>Serratia marcescens</i>	2 (25.0)	0 (0.0)	8 (100.0)	3 (100.0)	0 (0.0)	0 (0.0)	8 (100.0)	3 (100.0)	3	8
<i>Escherichia coli</i>	14 (15.1)	11 (42.3)	61 (65.6)	18 (69.2)	6 (6.5)	11 (42.3)	77 (82.8)	26 (100.0)	26	93
<i>Salmonella typhi</i>	3 (20.0)	0 (0.0)	15 (100.0)	3 (75.0)	0 (0.0)	0 (0.0)	15 (100.0)	4 (100.0)	4	15
<i>Citrobacter freundii</i>	5 (20.0)	0 (0.0)	22 (88.0)	0 (0.0)	2 (8.0)	1 (33.3)	25 (100.0)	3 (100.0)	3	25
<i>Shigella</i> spp.	3 (17.6)	0 (0.0)	11 (64.7)	3 (100.0)	5 (29.4)	0 (0.0)	15 (88.2)	3 (100.0)	3	17
<i>Salmonella Paratyphi A</i>	0 (0.0)	0 (0.0)	3 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	3 (100.0)	1 (100.0)	1	3
<i>Klebsiella pneumonia</i>	0 (0.0)	0 (0.0)	18 (60.0)	5 (83.3)	0 (0.0)	0 (0.0)	30 (100.0)	6 (100.0)	6	30
<i>Enterobacter</i> spp.	1 (12.5)	0 (0.0)	4 (50.0)	2 (33.3)	0 (0.0)	6 (100.0)	8 (100.0)	6 (100.0)	6	8
<i>Yersinia enterocolitica</i>	0 (0.0)	0 (0.0)	1 (33.3)	1 (100.0)	0 (0.0)	0 (0.0)	3 (100.0)	1 (100.0)	1	3
<i>Salmonellae</i> spp	0 (0.0)	1 (14.3)	1 (100.0)	6 (85.7)	0 (0.0)	0 (0.0)	3 (100.0)	7 (100.0)	7	3
<i>Proteus vulgaris</i>	NI	1 (100.0)	NI	1 (100.0)	NI	0 (0.0)	0 (0.0)	1 (100.0)	1	0
<i>Proteus mirabilis</i>	0 (0.0)	1 (20.0)	7 (53.8)	1 (20.0)	0 (0.0)	1 (20.0)	9 (69.2)	3 (60.0)	5	13
<i>Providencia</i> spp.	6 (66.7)	0 (00.0)	6 (66.7)	2 (100.0)	0 (0.0)	0 (0.0)	8 (88.9)	2 (100.0)	2	9
<i>Pseudomonas aeruginosa</i>	2 (25.0)	NI	8 (100.0)	NI	5 (62.5)	NI	5 (62.5)	NI	0	8
<i>Morganella morganii</i>	0 (0.0)	NI	7 (100.0)	NI	0 (0.0)	NI	3 (42.9)	NI	0	7
<i>Aeromonas</i> spp.	0 (0.0)	NI	0 (0.0)	NI	0 (0.0)	NI	1 (100.0)	NI	0	1
Total	36 (14.8)	15 (20.6)	174 (71.6)	46 (67.6)	18 (7.4)	19 (27.9)	213 (87.7)	66 (97.1)	68	243

Table 5: Antibigram profile of the Gram negative bacteria isolated from HIV patients and the HIV negative individuals investigated. Teams: Gentamicin (GEN), Ofloxacin (OFL), Augmentin (AUG), Nitrofurantoin (NIT), Cefandime (CAZ), Cefuroxime (CRX), Cefprofuxacin (CPR), and Ampicillin (AMP). NI= No Isolated. Note: percentages are compared across the row.

Organisms	CAZ (clinical) (%)	CAZ (control) (%)	CRX (clinical) (%)	CRX (control) (%)	Ery (clinical) (%)	Ery (control) (%)	AUG (clinical) (%)	AUG (control) (%)	Total Clinical	Total Control
<i>Staphylococcus aureus</i>	1 (25.0)	2 (100.0)	2 (50.0)	0 (0.0)	1 (25.0)	2 (100.0)	2 (50.0)	2 (100.0)	4	2
Coagulase negative <i>Staphylococcus</i>	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	1	1
<i>Streptococcus</i> spp.	0 (0.0)	3 (60.0)	2 (40.0)	0 (0.0)	2 (40.0)	2 (66.7)	2 (40.0)	2 (66.7)	5	3
<i>Lactobacillus</i> spp.	0 (0.0)	NI	0 (0.0)	NI	0 (0.0)	NI	1 (33.3)	NI	3	0
<i>Enterococcus</i> spp.	1 (20.0)	NI	0 (0.0)	NI	2 (40.0)	NI	3 (60.0)	NI	5	0
Total	2 (11.1)	2 (33.3)	4 (22.2)	0 (0.0)	5 (27.8)	2 (66.7)	12 (66.7)	5 (83.3)	18	6
Microorganisms	OFL (clinical)	OFL (control) (%)	GEN (clinical) (%)	GEN (control) (%)	TET (clinical) (%)	TET (control) (%)	AMP (clinical) (%)	AMP (control) (%)	Total Clinical	Total Control
<i>Staphylococcus aureus</i>	2 (25.0)	0 (0.0)	0 (0.0)	2 (100.0)	3 (75.0)	2 (100.0)	4 (100.0)	2 (100.0)	4	2
Coagulase negative <i>Staphylococcus</i>	1 (100.0)	1 (100.0)	1 (100.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)	1 (100.0)	1	1
<i>Streptococcus</i> spp.	5 (100.0)	3 (100.0)	0 (0.0)	3 (100.0)	1 (20.0)	2 (66.7)	5 (100.0)	3 (100.0)	5	3
<i>Lactobacillus</i> spp.	3 (100.0)	NI	3 (100.0)	NI	2 (66.7)	NI	2 (66.7)	NI	3	0
<i>Enterococcus</i> spp.	2 (40.0)	NI	2 (40.0)	NI	0 (0.0)	NI	3 (60.0)	NI	5	0
Total	14 (77.8)	2 (33.3)	6 (72.2)	2 (33.3)	6 (33.3)	5 (83.3)	3 (83.3)	6 (100.0)	18	6

Table 6: Antibigram profile of the Gram positive bacteria isolates from HIV patients and the HIV negative individual investigated. Teams: Cefandime (CAZ), Cefuroxime (CRX), Erythromycin (10µg), Augmentin (AUG), Ampicillin (AMP), Ofloxacin (OFL), Gentamicin (GEN) and Tetracycline. Note: percentages are compared across the row.

observed that curing had no effect on the resistance pattern of all the isolates from HIV patients except *Escherichia coli* (2B and 114C) and *Citrobacter freundii* (117A) that displayed resistance to antibiotic they had been previously sensitive to. Among the isolates from the control, *Escherichia coli* (8A, 26B and 31A) and *Salmonella typhi* (37A) were

observed to be resistant to augmentin, cefuroxime and ceftandime that had been previously sensitive to. However, *Escherichia coli* (17C), and *Proteus mirabilis* (3B) were observed to have lost their resistance to ceftandime, gentamicin, cefprofuxacin, nitrofurantoin; ceftandime, cefuroxime, cefprofuxacin and ofloxacin respectively (Table 9).

Serial Numbers	Codes	Organisms	Antibiotic	Groups
1	53	<i>Escherichia coli</i>	CAZ, CRX,OFL, AUG, AMP	3
2	62	<i>Escherichia coli</i>	GEN, AUG, AMP	2
3	64	<i>Escherichia coli</i>	CAZ,OFL,AUG,AMP	3
4	102a	<i>Escherichia coli</i>	AUG,CPR,AMP	2
5	2B	<i>Escherichia coli</i>	CAZ,AUG,AMP	2
6	101B	<i>Escherichia coli</i>	AUG,CPR,AMP	2
7	114C	<i>Escherichia coli</i>	OFL,AUG, AMP	2
8	54	<i>Escherichia coli</i>	CAZ, OFL,AUG, AMP	3
9	30	<i>Escherichia coli</i>	CAZ,CRX,AUG,NIT,AMP	3
10	102C	<i>Escherichia coli</i>	CAZ,CRX,GEN,CPR,NIT,AMP	4
11	100b	<i>Escherichia coli</i>	CRX,GEN,AUG,OFL,AMP	4
12	110b	<i>Morganella morganii</i>	AUG,AMP	1
13	70	<i>Citrobacter freundii</i>	CAZ,CRX,OFL,AUG,NIT,AMP	3
14	117A	<i>Citrobacter freundii</i>	AUG,AMP	1
15	7	<i>Citrobacter freundii</i>	CAZ,OFLO,AUG,NIT,AMP	4
16	29	<i>Providencia species</i>	AUG,NIT,AMP	2
17	68	<i>Providencia species</i>	OFL,AUG,AMP	2
18	76	<i>Pseudomonas aeruginosa</i>	ALL	4
19	88	<i>Pseudomonas aeruginosa</i>	CAZ,CRX,GEN,OFL,AUG,NIT,AMP	4
20	86	<i>Serratia marcescens</i>	CAZ,CRX,GEN,OFL,AUG,AMP	4
21	15	<i>Salmonella typhi</i>	CAZ,CRX,GEN,AUG,NIT,AMP	4

Table 7: Pre-cured antibiogram profile of selected multiple resistant isolates from HIV positive individuals. **Note:** Gentamicin (GEN), Ofloxacin (OFL), augmentin (AUG), Nitrofurantoin (NIT), Ceftanidime (CAZ), Cefuroxime (CRX), Cefprofuxacin (CPR), and Ampicillin (AMP) **Cephalosporins-** (CRX 2nd, CAZ 3rd), **Aminoglycosides-** (GEN), **Fluoroquinolones-** (OFL, CPR, NIT), **Penicillins-** (AMP, AUG).

Serial Numbers	Codes	Organism	Antibiotics	Groups
1.	8A	<i>Escherichia coli</i>	CAZ,CRX,CRP,AUG,AMP	3
2	9B	<i>Escherichia coli</i>	CAZ,OFLAUG,AMP	3
3	17C	<i>Escherichia coli</i>	ALL	4
4.	26B	<i>Escherichia coli</i>	CAZ,OFLAUG,AMP	3
5.	31A	<i>Escherichia coli</i>	CRX,AUG	2
6.	3B	<i>Proteus mirabilis</i>	ALL	4
7.	29B	<i>Providencia species</i>	CAZ,CRX,AUG,AMP	2
8.	37A	<i>Salmonella typhi</i>	CAZ,CRX,AMP	2

Table 8: Pre-cured antibiogram profile of selected multiple resistant isolates (Control). **Note:** Gentamicin (GEN), Ofloxacin (OFL), augmentin (AUG), Nitrofurantoin (NIT), Ceftanidime (CAZ), Cefuroxime (CRX), Cefprofuxacin (CPR), and Ampicillin (AMP) **Cephalosporins-** (CRX 2nd, CAZ 3rd), **Aminoglycosides-** (GEN), **Fluoroquinolones-** (OFL, CPR, NIT), **Penicillins-** (AMP, AUG).

Serial Numbers	Codes	Isolates	Antibiotics Resistant to:
1.	8A	<i>Escherichia coli</i>	AUG,AMP
2	9B	<i>Escherichia coli</i>	AUG,AMP
3	17C	<i>Escherichia coli</i>	CRX,OFL,AUG,AMP
4.	26B	<i>Escherichia coli</i>	CAZ,CRX,AUG,AMP
5.	31A	<i>Escherichia coli</i>	AUG,AMP
6.	3B	<i>Proteus mirabilis</i>	GEN,AUG,NIT,AMP
7.	29B	<i>Providencia sp</i>	CAZ,CRX,AUG,AMO
8.	37A	<i>Salmonella typhi</i>	CAZ,AUG,AMP

Table 9: Post cured antibiogram profile of selected multiple resistant isolates (seropositive). **Note:** Gentamicin (GEN), Ofloxacin (OFL), augmentin (AUG), Nitrofurantoin (NIT), Ceftanidime (CAZ), Cefuroxime (CRX), Cefprofuxacin (CPR), and Ampicillin (AMP). **Cephalosporins-** (CRX 2nd, CAZ 3rd), **Aminoglycosides-** (GEN), **Fluoroquinolones-** (OFL, CPR, NIT), **Penicillins-** (AMP, AUG).

Discussion

The high prevalence of HIV infection among the female gender observed was in agreement with the report of [16] and the observation

that the highest prevalence (35.3%) is found in the age group 26-35 years contradict the result from West Virginia where age group 35-44 years (53%) were reported to have the highest prevalence of the infection in 2013 Surveillance [16,17]. The high prevalence of HIV infection among this gender and age group could be due to the fact that some men and women may be unaware of their partner's HIV status and the gender violence against young females which is on the increase [18].

Escherichia coli (35.5%) accounted for the most frequently encountered bacterial sp. isolated from HIV patients and the control individuals followed by *Klebsiella pneumoniae* (10.7%) this agree with the report of Marbou [19]; Fredrick et al. [20]. It also agree with the reported of Samie et al. [21] who worked on the diarrhoeagenic bacterial pathogens in HIV-positive patients in Rural Communities of Limpopo Province, South Africa. The low occurrence of *Aeromonas* species 1 (0.4%) in this study correlates the result of Hayath et al. [22] who recorded low number of *Aeromonas* among hospitalized HIV infected patients in southern India.

In relation to CD4 counts which measure the degree of immunosuppression in HIV positive patients about 40% of HIV patients recruited for this study were observed to have CD4 count between 200-350 cells/mm³. This did not agree with the report of Akinsegun et al. [23], who observed 67.4% individuals with CD4 count greater than 350 cells/mm³. The reason for this difference may be due to the fact that majority of the HIV infection cases in this present study was only detected when there was appearance of symptoms as a result of opportunistic infections.

The relationship between CD4 count and types of bacteria present show that *Enterobacter* species (50%) and *Escherichia coli* (44.1%) were found in double fold among HIV patients with CD4 count level of ≥ 350 cells/mm³ as compared with patients with CD4 count level 200-350 cells/mm³ and less 200 cells/mm³. *Morganella morganii* (71.4%), *Enterococcus* sp (50%) and coagulase positive and negative *Staphylococcus* were also observed to be high among patients with CD4 count 500 cells/mm³. This agrees with the report of Estes et al.

[6]. Also *Aeromonas* species (100%), *Pseudomonas aeruginosa* (50%) and *Serratia marcescens* (50%) were observed to be high among HIV patients with CD4 count level 200 cells/mm³, the occurrence of these pathogens among this group is an indication of weak immunosystem. *Lactobacilli* sp (66.7%), *Salmonella paratyphi* A (66.7%), *Klebsiella pneumoniae* (63.3%), *Citrobacter freundii* (48%), *Shigella* species (41.2%) and *Salmonella typhi* (40%) were found to be in double fold among patients with CD4 count level 200-350 cells/mm³.

In general it was observed that patients with CD4 count 200-350 cells/mm³ had higher bacterial isolates (37.9%) followed by patients with CD4 count \geq 350 cells/mm³ (35.2%). This contradicts the high percentage of bacterial isolates recorded by Arun et al. [24] among patients with CD4 count below 200 cells/mm³ in North India.

The bacteria isolates in this study demonstrated a varying pattern of antibiotic susceptibility though statistically there was no significant difference in their sensitivities to the antibiotics used ($p > 0.05$). Maximum resistance was observed by both isolates from HIV positive and HIV negative individuals in this study against Ampicillin 9 (69.2%) and augmentin 7 (53.8%). All the *Escherichia coli* isolates from the HIV positives participant had low resistance to all the antibiotics used than those isolated from the control sample. This agree with a previous work on antibiotic resistance profiles and relatedness of enteric bacterial pathogens isolated from HIV/AIDS patients with and without diarrhoea in rural communities in Limpopo Province South Africa by Obi et al. [25]. Also *Proteus mirabilis* isolated from HIV positive was found to have lower resistance to ceftanidime (15.4%), cefuroxime, gentamicin, ciprofloxacin, ofloxacin and nitrofurantoin (0.0% each) except against Ampicillin 9 (69.2%) and augmentin 7 (53.8%). The *Proteus mirabilis* from HIV negative participants had 1(20.0%) resistance to all the antibiotic used except against ampicillin where 3(60.0%) resistance was observed.

The observation that *S. marcescens* isolates from HIV positive participant had higher resistance against ceftanidime 4 (50.0%), gentamicin, ciprofloxacin and Ofloxacin 2 (25.0%) however there was no difference against augmentin, and ampicillin (100.0%) when compared with *S. marcescens* from HIV negative participants. This agree with Hayath et al. [22].

The resistance observed in *Pseudomonas aeruginosa* isolates in this study against augmentin 8(100.0%), ceftanidime 6 (75.0%), nitrofurantoin and ampicillin 5 (62.5%) agrees with Ehiaghe et al. [26] who recorded similar sensitivity among clinical *Pseudomonas aeruginosa* in Benin City, Nigeria. The resistance observed may be due to the reduction of Extended-Spectrum Beta-Lactamases (ESBLs), the enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams [27]. Ceftazidime is a third generation Cephalosporins that work by inhibitors of cell wall synthesis and which is active against isolates of *Enterobacteriaceae* even *Pseudomonas aeruginosa* [27]. In general the low resistance observed among the isolates from HIV positive individuals as compared to isolates from HIV negative individuals could be as a result of strict adherence to only drug prescribed by their physicians.

The resistance observed among *E.coli* may be due to the mutations in the genes encoding ribosomal P1 protein and this has been reportedly associated with decreased permeability of the cell envelop in enteric bacteria by antibiotics, including plasmid-mediated mechanisms. Cross-resistance due to decrease permeability or other factors have been noted among antibiotics [25].

The plasmids from isolates show similar molecular size (23130 bp). This agrees with report of Obi et al. [25] and Shahriar et al. [28], who observed that majority of their bacterial isolates were having the same base pair. The heavy plasmid size observed in this investigation was greater than those recorded in a previous work by Umofia [29], who observed plasmids with base pair range between 3162 and 3981bp among bacterial isolated from HIV positive people.

Seven 7 (87.5%) of the control isolates sample possess plasmid out of eight that were selected while 20 (95.2%) clinical isolates displayed the presence of resistance plasmid. This is higher than 13.0% detected among the clinical isolates recorded by Ehiaghe et al. [26].

The variation in resistance pattern of some of these isolates to selected antibiotics may have other factors associated with their resistance in addition to presence of plasmid. This was confirmed in this study with the result of the post cured antibiogram, which shows that resistance plasmid is not the only factor responsible for the antibiotic resistance in this study. This equally agrees with the work of Carattoli [30]; Yah et al. [7] and Umofia [29]. The test shows that the resistance of *Escherichia coli* to ceftanidime, cefuroxime, ofloxacin, augmentin, ampicillin, ciprofloxacin and gentamicin were not generally plasmid based [31].

Also it was observed that some of the isolates developed post-cure antibiotic resistance to the same drug to which they were sensitive at pre-cure antibiotic test. This agrees with the work of Shahriar et al. [28] who recorded resistance to *Klebsiella* sp (512 Kleb (6 s)) to drug previously sensitive to, but contradicts Ehiaghe et al. [26], who reported decrease in antibiotic resistance from post cure antibiogram test. augmentin 9 (n = 21) has the highest occurrence of this new resistance development among *Escherichia coli* 6 (n = 21), followed by nitrofurantoin, 2 (n = 21), ceftanidime 3 (n = 21) and cefuroxime 2 (n = 21), while gentamicin 1 (n = 21) and ofloxacin 1 (n = 21) occur in *Pseudomonas aeruginosa* and *Escherichia coli* respectively. Though, the reason for this development could not be ascertained in this study, but it could be the effect of chemical used during curing.

Conclusion and Recommendation

HIV infection is prevalent among female and age group 26-35 years, thus there is need for more awareness about the causative agent, risk factors and mode of transmission. Most pathogenic bacteria were found among HIV patients with CD4 count level 200-350 cell/mm³ and 200 cell/mm³ which is an indication of weak immunosystem. Proper monitoring of their ART treatment and administration of antibiotic drug is recommended. The low resistance to antibiotic drug among bacterial isolates from HIV positive participant could be as a result of adherence to any drug prescribed by their physicians. To prevent indiscriminate use of antibiotics Government should make a police that will prevent peoples' access to antibiotic drugs without physician's prescription.

Limitation

The level of CD4 count was not determined for the HIV negative participants, which made it difficult to draw strong inference about the diversity of bacterial isolates found in the HIV positive as compared with HIV negative patients.

Acknowledgement

I will like acknowledge Mr. O. E. Olabiyi the chief medical laboratory scientist of the department of microbiology, Ekiti State University Teaching Hospital Ado Ekiti and other staffs of the department for all the assistance rendered to make the research work a success.

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