ARC Journal of AIDS
Volume 2, Issue 1, 2017, PP 12-18
www.arcjournals.org

Assessment of Bacterial Profile in the Stool of HIV Positive Individuals Attending ART Clinic in a Tertiary Health Centre in Ekiti State, Nigeria

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Abstract: *In this study, 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. A total of 150 HIV patients were recruited for the study. Samples of their stools were collected for the investigation. Their stools were cultured on microbiological media and pure isolates were identified using standard microbiological techniques. HIV negative individuals were used as control. The results showed that the most frequently encountered bacteria in their stool are Pseudomonas aeruginosa, Morganella morganii, Aeromonas sp, Enterococcus sp. and Lactobacilli sp., All these bacterial spp however are absent from the stool of the control subjects. The isolated bacterial spp. was resistant to most of the conventional antibiotics tested. This study shows the importance of investigating associated bacterial pathogens in HIV patients before drug prescription to such patients in order to checkmate bacterial infections that may complicate the infection.*

Keywords: *HIV, bacterial isolates, HAART*

Abbreviations: *HAART: Highly Active Antiretroviral Therapy, HIV: Human immunodeficiency Virus, AIDS: Acquired Immunodeficiency Syndrome, EKSUTH: Ekiti State University Teaching Hospital*

1. INTRODUCTION

The Human Immunodeficiency Virus (HIV), a lentivirus and causal agent of Acquired Immunodeficiency Syndrome (AIDS), was first described in the early 1980s [1]. Based on their gene sequence homology, morphology and life cycle, Lentiviruses are capable of causing latent infection of cells in the long term, or cytopathic effects in the short term, slowly inducing progressive and fatal diseases [2]. There are two types of HIV; HIV-1 and HIV-2 (also Referred to as Lymphadenopathy-Associated Virus type 2), which produce similar clinical syndromes but differ in gene structure and antigenicity. HIV-1 is the most common cause of AIDS in the United States as well as in Brazil, while HIV-2 infection is predominantly found in West African countries [2].

Early HIV infection is the stage of undetected viral load or any clinical manifestation. It is the stage of rapid viral replication, intense immune response and immune destruction. It is also the stage where atypical micro biota has been observed in the gut of an infected individual [3].For example in their investigation they observed overrepresentation of pathogenic *Candida albicans* and *Pseudomonas aeruginosa* in early HIV patients.

Those species have been implicated in opportunistic infection observed in HIV infected individuals. In another study, species such as bifidobacteria and lactobacilli, with propensities to boost gut immune function by modulating the innate immune system in pathogenic infection, were observed to be depleted in the faecal microbiota of untreated HIV patients [4]

A shift in proportions of pathogenic and commensal microbes relative to viral load was also observed in another study carried out by Rossit et al, [5] involving early stage HIV patients, either treated or untreated. In this study imbalances in adherent gut bacterial communities leads to significant enrichment and depletion of the Enterobacteriaceae family and Bacteroides class, respectively, in untreated HIV positive subjects compared to treated subjects

which portend development of diarrhea. However ART treated HIV patients were observed to exhibited a bacteria composition similar to that of negative individuals.

Gut bacteria normally associated with HIV patients includes the following *E. coli*, *Salminella* sp, *Shigella* sp, *Campylobacter jejuni*. For example several strains of *E. coli* have been reported to be associated with persistent diarrhoea in HIV patient. Among the *Shigella* genus, *S. sonnei* is of great significance for instance *S. sonnei* was detected in 11% of diarrhoeic HIV-infected children from the Northwestern region of São Paulo State, Brazil [5]. Approximately the same percentage of *Shigella* sp. was reported for adult HIV seropositive diarrhoeic individuals from Southern India [6]. *Salmonella* sp. especially non-typhoidal typies considered another important pathogen for the HIV-infected group as these enterobacteria are 20 to 100 times more frequently isolated among HIV individuals compared to the immunocompetent population [7, 8]. Enteric salmonellosis was also more frequently seen in patients in the advanced stages of HIV infection and with impaired nutritional status [9].

2. MATERIALS AND METHODS

2.1. Collection of Sample

A total of 200 stool samples were used for this investigation. One hundred and fifty stool samples were collected from the sero-positive patients attending ART clinic at University Teaching Hospital Ado Ekiti, while fifty stool and blood samples were collected from uninfected individuals (control). The blood samples (4ml) from Suspected HIV negative individuals were collected through finger pricking with sterile lancet and used to screen them for their HIV status. The stool samples from both the HIV positive individuals and the control were subjected to both macrobiological and microbiological investigations. All samples were processed within 24 hours of collection.

2.2. Stool Sample Analysis

The collected stool samples were analysed both macroscopically (colour and texture of the stool e.g., whether watery, form or semi-form) and by culturing. Each stool sample was stirred together before a portion of each was transferred into a new sterile bottle containing peptone water and then homogenized. A loopful of each was then cultured on MacConkey agar except in the case of anaerobic culture where blood agar was used. Selenite F was then added to the remaining sample, incubated at 37^0C for 24 hours and then subculture on Deoxyckolate Citrate Agar (DCA). For anaerobic culturing Gas jar and pak was used and the samples were incubated for 24 hours at 37^oC.

2.3. Isolation of Bacteria

Both anaerobically and aerobically incubated plates were observed for visible colonies and then sub-cultured on fresh media by streaking method to obtain a pure isolates. The pure isolates were morphologically characterized and subculture into nutrient agar slant for identification and characterization [10].

2.4. Identification and Characterization of Isolates

The bacteria isolates were identified and characterized based on their colonial morphology, microscopic appearance, grams reactions and biochemical reactions, including carbohydrates utilization profile. The isolates were identified by comparing characteristics with those of known taxa as described by [10].

3. RESULTS

3.1. Distribution of HIV Infection among Patients Used for the Investigation Based on Gender Age

Table 1: *Distribution of HIV infection Among Patients used for the Investigation Based on Gender and Age*

Types of Bacterial Species Isolated from the Stool of HIV Pa

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)

Out of total number 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. Out of the females sampled, the infection was observed to be higher among the age group 36-45years while it was lowest among the age group 66-75 years. For the male counterpart, the infection was highest in the age group, 26-35 years while it was lowest in the age group <25 years (Table 1). In general, age group 26-35yerars had the highest incidence of HIV infection among the participants.

3.2. Types of Bacterial Species Isolated from the Stool of HIV Patient and the HIV Negative Individuals Investigated

The following bacterial species were isolated from the stool of the HIV patients used in this investigation. These are *Serratia marcescens, Escherichia coli, Salmonella typhi, Citrobacter freundii, Shigella* species, *Salmonella paratyphi* A, *Klebsiella pneumoniae, Enterobacter* species, *Proteus vulgaris, Yersinia enterocolitica, other Salmonella* sp., *Proteus mirabilis, Providencia s*pecies, *Pseudomonas aeruginosa, Morganella morganii, Aeromonas* species, *Staphylococcus aureus, Staphylococcus epidemidis, Streptococci* species, Lactobacillus sp and Enterococus sp. Almost the same types of bacterial species were also found in the stool of the control subjects except that *Pseudomonas aeruginosa, Morganella morganii, Aeromonas species, Lactobacillus sp* and *Enterococcus sp* were only found in the stool of the HIV patients but not in the stool of the control subjects while *Proteus vulgaris* was found only in the stool of the control subjects and not HIV patients. All the isolated bacteria had higher frequency of occurrence in HIV patients than the control subject except for *Proteus vulgaris* that was not found at all in their stool and other *Salmonellae* sp. that was found only 3(30%) of the HIV patients unlike 7(70%) occurrence found in the stool of HIV negative individual (Table 2).

Table 2: *Frequency of occurrence of the bacteria isolated from the stool of HIV patients and control individuals*

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

Percentages are represented in the parentheses

3.3. Frequency of Occurrence of Bacteria Isolated from Control, HIV Patients on HAART, HIV Patients not on HAART and the Control

The following bacterial isolates were common in the stool of both HIV patients on HAART, HIV patients who are on HAART and the control individuals investigated. These are *Escherichia coli, Salmonella typhi, Shigella* species, *Enterobacter* species, *Salmonellae* sp, *Providencia* species, *Coagulase positive Staphylococcus* and *streptococcus* sp. While *Serratia marcescens, Yersinia enterocolitica,*

Proteus mirabilis, coaguolase negative Staphylococcu, were only found in the stool of HIV patient on HAART and the control. Also *Pseudomonas aeruginosa, Morganella morganii* and *Lactobacilli* sp were only isolated in the stool of HIV patients who are on HAART while *Aeromonas* species was only found in HIV patients who are not on HAART. (Table3).

Table 3: *Frequency of concurrency of bacteria isolated from HIV patients on HAART, not on HAART and the control.*

Bacterial species	HIV patients on HAART (%)	HIV patient not on HAART (%)	Control (%)	Total (%)
Serratia marcescens	8(72.7)	0(0.0)	3(27.3)	11
Escherichia coli	73(61.3)	20(16.8)	26(21.8)	119
Salmonella typhi	13(68.4)	2(10.5)	4(21.1)	19
Citrobacter freundii	17(60.7)	8(28.6)	3(10.7)	28
Shigella species	15(75.0)	2(10.0)	3(15.0)	20
Salmonella paratyphi A	3(75.0)	0(0.0)	1(25.0)	4
Klebsiella pneumonia	29(80.6)	1(2.8)	6(16.7)	36
Enterobacter specie	7(50.0)	1(7.1)	6(42.9)	14
Proteus vulgaris	0(0.0)	0(0.0)	1(100.0)	1
Yersinia enterocolitica	3(75.0)	0(0.0)	1(25.0)	4
Salmonellae sp	2(20.0)	1(10.0)	7(70.0)	10
Proteus mirabilis	12(66.7)	0(0.0)	5(27.8)	18
Providencia species	8(72.7)	1(9.1)	2(18.2)	11
Pseudomonas aeruginosa	8(100.0)	0(0.0)	0(0.0)	8
Morganella morganii	7(100.0)	0(0.0)	0(0.0)	7
Aeromonas species	0(0.0)	1(100.0)	0(0.0)	1
Coagulase positive Staphylococcus	3(50.0)	1(16.7)	2(33.3)	6
Coagulase negative Staphylococcus	1(50.0)	0(0.0)	1(50.0)	2
Streptococcus sp	4(50.0)	1(12.5)	3(37.5)	8
Lactobacilli sp	3(100.0)	0(0.0)	0(0.0)	3
Enterococi sp	3(60.0)	2(40.0)	0(0.0)	5
Total percentage	**219(65.4)**	**42(12.5)**	**74(22.1)**	**335**

4. DISCUSSION

In this study, the high prevalence of HIV infection among the female gender observed was in agreement with the report of Mabayoje and Akinleye [11] in the study they carried out among the people in 15 local government areas within Osun State in South West Nigeria. The high prevalence of HIV infection among this gender could be due to the fact that some women may be unaware of their male partner's HIV status and the gender violence against young females which is on the increase [12]. Also the risk of having HIV infection in female is higher than that of the male because of the anatomic structure of their sexual organ.

Age group 26-35years had highest prevalence of HIV infection (35.3%). This contradict 53% reported in a similar study conducted in West Virginia among age group 35-44years in 2013 Surveillance [13], though higher than 4.4% reported by NARHS Plus II, [14] among age group 35-39 years. This present result however show correlation with recent HIV Surveillance report by United States where prevalence was high among the age group 20-24 years followed by 25-29years [15]. The difference in the result may be due to different geographical location and the age when the individual started having sex. The high prevalence of HIV infection recoded among this age group in this study could be attributed to multiple sex partners and lack of the knowledge of the predisposing factors to this infection.

Escherichia coli (35.5%) accounted for the most frequently encountered bacterial sp. isolated from HIV patients and the HIV negative individuals (control) followed by *Klebsiella pneumoniae* (10.7%) agree with the report of Marbou, [16]; Fredrick *et al*. [17]. It also agree with the reported of Samie *et al.* [18] 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 (0.4%) in this study correlates the result of Hayath *et al.* [19] who recorded low number of *Aeromonas* among hospitalized HIV infected patients in southern India.

Moreover, the high occurrence of *Pseudomonas aeruginosa* 8(100%), *Shigella sp (85%), Klebsiella* species (83.3%), *Salmonella typhi (78.9%), E. coli (78.2%), Salmonella paratyphi A* (75%), *Serratia marcescens* (72.7%), *Staphy aureus (66.7%)* and *Enterobacter* Species (57.1%) among HIV positive patients on HAART in this study agrees with report of the work of Yu-Jie *et al.* [20] who reported that these bacteria were highly enriched in the mucosa of HIV patients, and they are known as opportunistic pathogens and sources of bacteremia in HIV-infected subjects. It also agrees with the report of Estes *et al.* [21] who observed that there was a persistence of an HIV-associated microbiota in some individuals on long-term ART. The presence of these bacteria among the group could indicate the inability of HAART to total by restore the gut microbiota though this work did not put in to consideration the duration of the antiretroviral therapy of the patients. This observation however is at variance with the report of Obi and Bessong, [22] who recorded a low percentage of these organisms. However, in 2007, Zajac *et al.* [23] reported that these bacteria shared more than 90% sequences of HIV-1, thus involved in the pathogenesis of HIV.

However, there was limitation to this present study as it was greatly difficult to get equal number of HIV positive individuals who are on HAART and those not on HAART for proper comparison.

5. Conclusion

In this study it was observed that HIV infection was significantly higher among the females than males and among traders than other occupations investigated. It was also observed to more prevalent in the age group 26-35 years.

The types of bacteria isolated from the stool of |HIV positive individuals on HAART were almost similar to those found in HIV negative individuals. However there was a higher percentage of most of the pathogenic bacteria such as *E. coli, Shigella* species, *Salmonella typhi, Klebsiella pneumoniae, Salmonella paratyphi* A, *Yersinia enterocolitica, Proteus mirabilis, Enterococcus* spp, *Streptococcus* sp, and *Providencia* species among HIV patient. In addition to the ones that were found only in HIV patient screened which are *Pseudomonas aeruginosa, Morganella morganii, Aeromonas* species and *Enterococcus* spp

Recommendation

1. Awareness of HIV infection in relation to causative agent, mode of transmission and the risk factor is highly imperative in the community sampled in order to reduce the incidence rate of the infection
2. Combination of chemotherapy and nutrition is advocated to manage HIV infection.
3. Antibiotic should be prescribed only after pathological investigation of types of pathogenic microorganisms being harboured by HIV positive individuals to prevent indiscriminate use of antibiotic.
4. Early administration of HAART to HIV positive patients is highly recommended. This will help preventing the rapid replication of the virus.
5. A research into probiotic therapy is recommended to that can help reconstitute the shift in the gut microbiota

6. Contribution to Knowledge

This study has shown that although HAART is very important, it is not enough for the management of HIV infection, because the administration of HAART did not have significant effect on bacterial population in the stool of HIV positive individuals as compared to the bacterial population in the stool of control individuals with lower population, which is a reflection of the immune status of these seropostive individuals.

This research work however has shown that the bacteria isolated from HIV seropositive individuals are more sensitive to antibiotics than bacteria isolated from the HIV negative individuals.

Moreover, the investigation showed higher prevalence of the infection in the female gender than the males

It also showed that the highest prevalence of the infection was found in age group 26-35 years and it is also prevalent among traders.

REFERENCES

[1] Sandler N.G., and Douek D.C. (2012) "Microbial translocation in HIV infection: causes, consequences and treatment opportunities," Nature Reviews Microbiology, 10, (9) 655-66.

[2] Abbas, A..K.., Lichtman, A.H., and Pober, J.S. (1994). Congenital and acquired Immunodeficiency. In: Cellular and Molecular Immunology. 2 ed. Philadelphia: W.B. Saunders, 418-30.

[3] Gori, A, Tincati, C, and Rizzardini G. (2008) "Early impairment of gut function and gut flora supporting a role for alteration of gastrointestinal mucosa in human immunodeficiency virus pathogenesis," Journal of Clinical Microbiology, 46; 2: 757–758, 2008

[4] Pérez-Santiago, J, Gianella, S, and Massanella, M. (2013). "Gut Lactobacillales are associated with higher CD4 and less microbial translocation during HIV infection," AIDS. 27; 12: 1921–1931, 2013.

[5] Rossit, A. R,, Almeida, D. E. and Nogueira, M. T. (2007). Bacterial, yeast, parasitic, and viral enteropathogens in HIV-infected children from São Paulo State, Southeastern Brazil. Diagnostic Microbiological infectious Disease, 57: 59-66.

[6] Kownhar, H., Shankar, E. M., Rajan, R., Vengatesan,, A. and Rao, U. A. (2007). Prevalence of Campylobacter jejuni and enteric bacterial pathogens among hospitalized HIV infected versus non-HIV infected patients with diarrhoea in Southern India. Scand. Journal of Infectious Disease, 39: 862-866.\

[7] Lourenço, M. C., Dos Reis, E. F., Valls, R., Asensi, M. D, and Hofer, E. (2004).Salmonella enterica subsp houtenae serogroup O:16 in a HIV patient: case report. Reviewer Institute of Medical Tropical, 46: 169-170.

[8] Zilberman, S. G., Niv, Z., Shlomik, I., Stavros, B., Hila, E. and Eran, E. (2016). The gut microbiome in human immunodeficiency virus infection. Biomedical Medicine. (2016) 14:83 1-11

[9] Tacconelli, E., Tumbarello, M, and Ventura, G. (1998). Risk factors, nutritional status, and quality of life in HIV-infected patients with enteric salmonellosis. Italian Journal of Gastroenteritis Hepatology, 30: 167-172.

[10] Cheesbrough, M. (2006).District Laboratory Practice in Tropical Countries. Cambridge University Press, p. 434.

[11] Mabayoje, V. O. and Akinleye, C. A. (2016). Incidence of HIV infection in 15 localgovernment areas within Osun State in South West Nigeria. HIV and AIDS Review, 15(1): 33-35\

[12] Abdulazeez, A., Alo E. and Naphthali, R.(2008).Concurrent Infection of HIV-1 and HIV-2 Serotypes in Adamawa State Nigeria. World Journal of Medical Sciences, 3(1): 15-18

[13] Bureau for Public Health. (BPH). (2014). West Virginia HIV/AIDS Surveillance Report www.hiv.wv.gov retrieved on 15/6/2015.

[14] Federal Ministry of Health [Nigeria] (2013). National HIV & AIDS and Reproductive Health Survey, 2012 (NARHS Plus). Federal Ministry of Health Abuja, Nigeria. www.naca.gov.ng>narhs-plus-2012-final

[15] WHO. (2000). Second Generation Surveillance for HIV/AIDS' source from: http://www. avert.org/worldwide-hiv-aids-statistics.htm# sthash.Xos6Aeyd.dpuf. Retrieved 31/5/2015.

[16] Marbou, W. J. (2016). textBacterial resistance and immunological profiles in HIV-infected and non-infected patients at Mbouda AD LUCEM Hospitalin Cameroon. Journal of Infection and Public Health, page: http://www.ncbi. nlm.nih.gov/m /pubmed /27133911/#fft. Retrieved on 24/3/2016.

[17] Fredrick, O., Tura G., Arvelo, W., Onesmus, M. M., Evalyne, W. K., Juliana C. T., Samwel, A. and Waqo, B. (2014). Antimicrobial resistance: capacity and practices among clinical laboratories in Kenya, 2013. The Pan African Medical Journal, 19: 332.

[18] Samie, A., Bessong P. O., Obi, C. L., Dillingham, R. and Guerrant, R. L. (2011). Bacterial and Parasitic Agents of Infectious Diarrhoea in the Era of HIV and AIDS - The Case of a Semi Rural Community in South Africa, Microbes, Viruses and Parasites in AIDS Process, VladimÃr Zajac (Ed.). Www.intechopen.com. Retreived on 23/6/2015.

[19] Hayath, k., Esaki M. S., Ramachandran R., Appasamy, V. and Usha, A. R. (2007). Prevalence of Campylobacter jejuni and enteric bacterial pathogens among hospitalized HIV infected versus non-HIV infected patients with diarrhoea in southern India. Scandinavian Journal of Infectious Diseases, 39: 862-866.

[20] Yu-Jie, Z., Sha L., Ren-You G., Tong Z., Dong-Ping, X., and Hua-Bin, L. (2015). Impacts of Gut Bacteria on Human Health and Diseases. International Journal of Molecular Science, 16(4): 7493–7519.

[21] Estes, Li. Q., Duan, J. D., Jessurun, L., Pambuccian, J., Forster, S., Wietgrefe, C., Zupancic, S., Schacker, M., Reilly, T., Carlis, C. and Haase, J. V. (2008). Simian immunodeficiency virus-induced intestinal cell apoptosis is the underlying mechanism of the regenerative enteropathy of early infection. Journal Infectious Disease, 197: 420–429.

[22] Obi, C. L. and Bessong, P. O. (2002). Diarrhoeagenic bacteria pathogens in HIV-positive patients with diarrhoea in rural communities of Limpopo province South Africa.Journal of Health and Population.Nutrition, 20(3): 230- 234.

[23] Zajac, V., Stevurkova, V., Matelova, L. and Ujhazy, E. (2007). Detection of HIV-1 sequences in intestinal bacteria of HIV/AIDS patients. Neuroendocrinology Letter. 28: 591–595

Citation: *Ojo, B. A, Adebolu T. T., Okebugwu, Q. C. Assessment of Bacterial Profile in the Stool of HIV Positive Individuals Attending ART Clinic in a Tertiary Health Centre in Ekiti State, Nigeria. ARC Journal of AIDS. 2017; 2(1):12–18.*

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