OPPORTUNISTIC INFECTIONS AMONG HIV/AIDS PATIENTS SEEKING HEALTH CARE AT NATIONAL INSTITUTE FOR PHARMACEUTICAL RESEARCH AND DEVELOPMENT, ABUJA, NIGERIA.

*Ya’aba Y., †Izebe K.S., ‡Mohammed S.B., §Baba J. and ¶Usoroh M.

1Department of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development (NIPRD) Idu-Abuja, Nigeria.  
2Department of Microbiology, Ibrahim Badamasi Babangida University, Lapai, Niger state, Nigeria.

*Corresponding Author Email Address: yakayabig@yahoo.com Phone: +2349067002373  

ABSTRACT

Individuals with advanced HIV infection are prone to other infections called opportunistic infections (OIs). This study determines the prevalence of OIs among HIV infected patients. Five hundred and ninety-six (596) HIV positive Anti-Retroviral Therapy (ART) naive subjects referred to the Microbiology and Biotechnology Department, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria for diagnosis were recruited into the study. The socio-demographic and OIs isolated from patient’s data were collected and analysed by Statistical Product and Service Solution (SPSS) software (version 17.0, SPSS, Chicago, USA). ANOVA was used to compare the groups and (p ≤ 0.05) was set to be statistically significant. 203 (34.1%) patients presented with OIs. Isolated sputum bacterial pathogens included Mycobacterium tuberculosis (4.9%), Streptococcus pneumonia (7.4%), Staphylococcus aureus (10.0%), Pseudomonas aeruginosa (2.5%). Urinary tract infection pathogens are Escherichia coli (19.7%), Klebsiella aerogenes (9.9%), Proteus vulgaris (2.5%). Stool pathogens are: Salmonella typhi (8.9%), Shigella spp (7.9%). High vaginal swab (HVS) pathogens are Candida albicans (7.4%), Staphylococcus aureus (1.0%) and Streptococcus faecalis (1.5%). The presence of each pathogen in the various anatomical sites was statistically significant (p < 0.05). The study established OIs among HIV/AIDS patients and the need to have treatment options apart from the use of ART.

Keywords: Opportunistic infections, NIPRD, immunity, Prevalence, Anti-Retroviral Therapy, HIV/AIDS

INTRODUCTION

Individuals with advanced HIV infection are prone to opportunistic infections (OIs) and other malignancies due to the opportunity offered by depletions of the CD4 T cells count that cause continual weakening of the body immune system (Gangadhara and Ramesh, 2014). Skin challenges and upper respiratory tract infections may develop after an initial asymptomatic phase; and these patients begin to lose weight. Frequent feverish conditions, fungal or other repeated bacterial infections, and tuberculosis (TB) may surface. Although, reports from some studies showed that considerable co-morbidities associated with HIV/AIDS are candidiasis, proceeded opportunistic infections and pathologic organisms which cause gastrointestinal diseases leading to chronic diarrhea (Alemayehuel et al., 2017). Mild OIs and herpes zoster are most commonly found at the initial stages of the disease and some life threatening infections such as central nervous system (CNS), toxoplasmosis and cryptococcal meningitis appear at the advanced stage of the disease progression; and there is also possibility of recurrent of bacterial pneumonia and tuberculosis at the early as well as late WHO stages of the disease (Sharma et al., 2005).

There are over 20 specific OIs that are associated with HIV/AIDS infection (WHO, 2007); and patients are usually prone to condition called co-infections at the course of their diseases (Losina and Freedberg, 2011). HIV affiliated OIs are usually accompany with significant morbidity and mortality and almost none can be eliminated after some acute infections, thereby requiring life-long suppressive therapy. Prevention and treatment of such illness with the use of primary prophylaxis is therefore necessary (Gangadhara and Ramesh, 2014).

Since the first case of HIV/AIDS the world is still facing continual threat with the dreaded virus. However, there has been concerted effort globally to thwart the threat (UNAIDS, 2016). This was achieved by paying more attention to the clinical management and care of the infected individuals using medications in order to prolong the life expectancy. The prevalence of HIV was estimated in Nigeria to be 1.8% in 1991 to 4.5% in 1996, 5.8% in 2001, 5.0% in 2003 and 4.4% in 2005. However, the national prevalence seemed to stabilize between 2005 and 2010 as shown by the reported prevalence 4.4% (2005), 4.6% (2006), and 4.1% (2010), which ranged from 1.0% in Kebbi State to 12.7% in Benue State (FMOH, 2011). Based on the overall national prevalence of 4.1% obtained in 2010, it is estimated that 3.1 million people in Nigeria are living with HIV/AIDS in 2010. Of these people, about 1.5 million require ARV drugs (FMOH, 2010). In 2014, the national prevalence was further reduced to 3.0%. Nevertheless, a prevalence of 3.0% HIV prevalence implied that over 3.4 million Nigerians are currently infected with the virus and about 2.5 million requiring ART (FMOH, 2015).

Opportunistic infections are so common in HIV/AIDS that they are continually with the definitive diseases in both paediatric and adult HIV/AIDS patients (UNAIDS, 2016). Therefore, most of the minor and major signs and symptoms of the WHO case definition of the disease shows the fundamental OIs (WHO, 2007). The Centre for Disease Control (CDC) case definition is dependent on the accuracy of methods used in the diagnosis of the OIs (UNAIDS,
They are found to occur in coexisting at uncommon sites in the body of the patient, and produce unusual tissue reactions in the body host, now with depleted body immune system (Gangadhara and Ramesh, 2014).

Considering the long term introduction and usage of Highly Active Anti-retroviral Therapy (HAART), there is significant reduction in OIs and AIDS related prognosis (Ayyagari et al., 1999; Sun et al., 2006; Habtamu et al., 2015). Although, the type of OIs which affects people living with HIV/AIDS varies from region to region (Sun et al., 2006). Hence, there are significant differences between OIs prevalent in Africa and those prevalent in United States of America and Europe (Mocroft et al., 2013). Of the all HIV/AIDS related infections or diseases, tuberculosis stands most common as seen across geographical boundaries and influencing about one third of the world’s people living with HIV/AIDS (PLWHA) (Sharma et al., 2005). This is may be due its worldwide distribution with endemicity in certain areas and cultures (Mocroft et al., 2013; Habtamu et al., 2015). Therefore, for the road map of HIV/AIDS related morbidity and mortality to be consolidated; their frequency, identification and distribution would play important task (WHO, 2007).

With the increase rate of reports on HIV/AIDS and OIs, there is the need for further study of other diseases affecting HIV/AIDS patients and thereby prevent the occurrence of OIs that can result in important gains in life expectancy and quality of life among this set of group. Thus, the aim of this work was to determine the prevalence of opportunistic infections associated with HIV/AIDS individuals in Abuja, FCT, Nigeria; so that policy makers, non-governmental organizations (NGOs) and international donors who have related setting can use the research findings for further planning and implementation of treatment options related to HIV/AIDS and other associated death.

MATERIALS AND METHODS

Study Area
This study was carried out in Microbiology and Biotechnology Department of National Institute for Pharmaceutical Research and Development (NIPRD) Idu – Abuja, FCT. The Federal Capital Territory (FCT) of Nigeria located on latitude 9°9’4”N and longitude 7°09’E. FCT is served by several health care centres, of which NIPRD facility were used for this study. This facility was chosen because of high frequency of attendance of clients for HIV voluntary counseling and testing (HVCT), rendering of secondary health services to the public and referral centre for HIV/AIDS treatments PEPFAR in August, 2006 for Abuja and its environs.

Study Design
This research work was a hospital based prospective study conducted from April, through July, 2015. The aim, objectives and benefits of the research work were explained in details to the participating patients consented to participate in the study. Five hundred and ninety-six (596) HIV positive anti-retroviral therapy (ART) naïve subjects referred to the Microbiology and Biotechnology Department, National Institute for Pharmaceutical Research and Development (NIPRD), Idu-Abuja, Nigeria for ART initiation. Parameters (CD4+ counts, haematological analysis and Viral Load estimations) were included in the study after they were given health talk on HIV/AIDS, OIs and consented to participate.

The recruitment was non-randomized and questionnaires were administered on those that consented to participate in the study. Data collected after informed consent obtained were strictly confidentially and other responses were kept during and after the study period. The patients were taken into the study when found eligible for ART as per the national guidelines on ART and fulfillment of the eligibility criteria for the study. The patients who were critically sick and wished not give their consent to be included were excluded from the study.

Demographic Information
The information collected for the purpose of this study from the patients includes age, sex, occupation, marital status, educational background, phone number and residential address through the administration of questionnaires.

Ethical Consideration
Ethical approval for the study was sought and obtained from Health Research and Ethics Committees (HREC) of Federal Capital Territory Abuja and was endorsed by the authority of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu-Abuja, Nigeria were the prospective study was developed.

Sample Collection
The urine, stool and sputum samples were collected in sterile universal bottles and a sterile swab stick was used for the collection of high virginal swab (HVS). The samples were microbiological analyzed for the presences of microorganisms in the laboratory using standard laboratory procedures.

Examination of stool samples containers for pathogenic protozoan infections.
Collected stool samples containers were firstly examined macroscopically for odor, colour, consistency, (formed unformed, watery or mucoid) and for presence of worms, and were also then look into microscopically for possible presence of any form or type of protozoa, fungi or bacteria. The cysts, larva and eggs of parasites were probed by examination of stool samples in eosin and iodine preparations and by modified Ziehl Neelsen staining for oval Cryptosporidium oocysts (Cheesbrough, 1991).

Bacterial Cultivation and Identification
The stool samples were sub-cultured in Salmonella Shigella agar and incubated at 37°C for 24 hours. The colonies with whitish appearance were suggestive of presence of pathogens. The further identification involved use of biochemical tests (Cheesbrough, 1991). Drug susceptibility testing was done on all the pathogens isolated.

Urine Microscopy and Culture
Mid-stream urine in sterile containers was obtained from all the patients that participated in the study. Each urine sample was inoculated onto sterile blood agar and McConkey agar plates and incubated at 37°C for 24 hours. The isolated pathogens of medical significant were identified and characterized according to the methods of Cruickshank, (1980); Cheesbrough, (1991). For all the identified pathogens, drug susceptibility testing was also done using disc diffusion technique.
Screening of Sputum Samples
Sputum samples collected were checked into for appearance: purulent, muco-purulent, cheesy, mucoid or muco-salivary, white coloured, yellow or bloody or bloodstained and then Giemsa stained for sporozoites, trophozoites or cysts of pneumocystis carinii as described by Cheesbrough (1991). Purulent, mucopurulent or cheesy specimens were initially digested with Potassium hydroxide solution and incubated for 1 hour to liquefy as described by Cheesbrough, (2000) for identification of mycelia of fungi. Acid Fast Bacilli (AFB) were then screened for using the Ziehl Neelson Staining technique as described by Cheesbrough, (1991).

Screening for bacterial and fungal pathogens of the respiratory tract
Digested sputa and nasopharyngeal secretions were cultivated on MacConkey, Blood, and Chocolate agar to which optochin discs (ethylhydrocuprein hydrochloride) were incorporated, and incubated in a carbon dioxide enriched atmosphere (10% CO2) at 37°C, for 48hr, and look into for growth after overnight incubation (Cheesbrough, 1991). The isolates were identified by biochemical tests as described by Cheesbrough, (1991).

Fungal pathogens of the respiratory tract were screened following digestion of sputa, naso-pharyngeal secretions and high vaginal swabs (HVS) in 5ml of 10% potassium hydroxide solution and subsequent examination on physiological saline and Lacto-phenol cotton blue for fungal budding cells, hyphae and pseudohyphae. The samples were further Giemsa stained and looked into for the presence of intracellular yeasts of Histoplasma capsulatum as described by Cheesbrough (1991).

Fungal Cultivation and Identification
The samples contained in sputa, nasopharyngeal secretions and HVS were firstly inoculated onto Sabouraud Dextrose Agar (SDA) incorporated with chloramphenicol and incubated at both ambient temperature (25-30°C), and at 37°C for 2-7 days. The rapidly growing colonies on SDA were looked into with a hand lens and further on drops of Lactophenol cotton blue for fungal budding cells, hyphae and pseudohyphae. The samples were further Giemsa stained and looked into for the presence of intracellular yeasts of Histoplasma capsulatum as described by Cheesbrough (1991).

RESULTS

Socio-demography characteristics data of participants
A total of five hundred and ninety-six (596) HIV/AIDS patients over the age ranges of 18 years presented at ART clinic to be initiated on ART at NIPRD Idu-Abuja participated in the study, of which 242 (40.6%) were males and the rest 354 (59.4%) were females. The ratio of male to female was 1: 1.32 and mean age of both sexes 38.0 years.

Of the two hundred and three (203) (34.1%) HIV/AIDS seropositive patients that were found infected with one or more of the OIs, in relation to age brackets, it was observed that at age brackets of (28 – 37) years had the highest rate of 81 (39.9%) of isolated OIs patients, followed by (38 – 47) years with 57 (28.1%), (18 – 27) years with 39 (19.2%), (48 – 57) years with 23 (11.3%) and least observed at above 58 years with 3 (1.5%). More than half 149 (73.4%) are from urban settlement while 93 (45.8%) were living in a marital association. About 144 (70.9%) of study patients that OIs were isolated educational level was above secondary school whereas 71 (35.0%), 32 (15.7%), 57(28.1%) and 43(21.2%) of them were occupationally business, civil servant, house wife and others (daily laborer, farmers), respectively shown in Table 1.

<table>
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<tr>
<th>Variable</th>
<th>Number (n=203)</th>
<th>Percentage (%)</th>
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<td><strong>Sex</strong></td>
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<tr>
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<td>&gt;58</td>
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<td>1.5</td>
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<td>57</td>
<td>28.1</td>
</tr>
<tr>
<td>Others*</td>
<td>43</td>
<td>21.2</td>
</tr>
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</table>

* Daily laborer, Farming

From this study, it was observed that out of the two hundred and three (203) (34.1%) HIV seropositive patients that were found infected with one or more of the OIs using urine, stool, sputum and high vaginal swab specimens; the females 123 (50.6 %) had highest prevalence of OIs cases while male had 80 (33.0%). The mostly frequently found OIs in both gender was Escherichia coli (19.7%) which were followed closely by Staphylococcus aureus (10.0%) and Klebsilla aerogene (9.9%) shown in figure 1.

**Figure 1:** Gender wise distribution of OIs among HIV/AIDS ART naïve patient’s in NIPRD, Abuja

The prevalence of related pathogens isolated from HIV/AIDS patient’s specimens.

**Urine specimens:**
Some frequently occurring bacterial pathogens that were isolated from urinary tract specimen included Escherichia coli (19.7%), Klebsilla aerogene (9.9%) and Proteus vulguria (2.5%). All infections were concomitant with HIV infection.

**Stool specimens:**
The most important pathogen encountered from the stool specimen were Salmonella typhi (8.9%) and Shigella Spp (7.9%).

**Sputum specimens:**
Some important clinical micro-organisms: Mycobacterium tuberculosis (4.9%), Streptococcus pneumonia (7.4%), Staphylococcus aureus (10.0%) and Pseudomonas aeruginosa (2.5%) were isolated from sputum specimen.

**High Vaginal Swab (HVS) specimens:**
The most frequently isolated fungal pathogen in this study was Candida albicans (7.4%); and bacterial included Staphylococcus aureus (1.0%) and Streptococcus faecalis (1.5%). The prevalence of these pathogens isolated from the specimen used in the study are shown in figure 2.

**Figure 2:** The prevalence of related OIs among HIV/AIDS ART Patient’s in NIPRD, Abuja.

**DISCUSSION**
The prevalence of opportunistic infections is very important in infection prevention and control like HIV/AIDS. These may due to the facts that the infections occurred possibly as a result of depletions and concession in the body immunity of the infected individual.

It was observed in this study that gender distribution of male and females were infected with various OIs. There was higher proportion of females, 242(40.6%), as compared to males, 203(34.1%) as compared to males, with the female to male ratio of 1.32 to 1.0. A total of two hundred and three (34.1%) of 596 HIV/AIDS ART naïve infected patients studied presented with variety of OIs.

In this study, it was observed that majority of the patients with isolations of OIs were in the age group of 28-37 years and all these patients are found below the age of 60 years. This is in agreement with the findings of Garcia et al (1998), Who observed in their studies that the frequency of OIs are usually higher in the sexually active age group of the society. This show that a trend of young and productive generation being affected; a reflection of the serious challenges that India will face as the younger generation work force is affected. The findings from this study showed that HIV/AIDS patients in Abuja, FCT have a variety of OIs, which included the isolated Mycobacterium tuberculosis, Streptococcus pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa, Eschericha coli, Klebsilla aerogenes, Proteus vulgaris, Salmonella typhi, Shigella spp, Candida albicans, Staphylococcus aureus and Streptococcus faecalis. Although, it was found that the mostly frequently OIs in both gender was Escherichia coli (19.7%) which were closely followed by Staphylococcus aureus (10.0%) and Klebsilla aerogene (9.9%). The pattern of these OIs in a particular geographical area helps the attending physicians to be on the search for them and take prompt treatments options. Concurrently, specific health education of people living with HIV/AIDS regarding early detection of OIs and importance of antimicrobial prophylaxis to reduce the morbidity and mortality can be undertaken (Gangadhara and
The prevalence of coliform bacilli: E. coli (19.7%), Proteus species (2.5%), Klebsiella aerogenes (9.9%) observed in this study from the urinary tract, may be extremely associated with the malfunction of the urinary tract in the HIV/AIDS infected persons examined. This observation is in agreement with the findings of Kathleen and Wizburg (1996), on the traditional bacterial infections associated with compromised immune system. These infections were apparent to be associated with general unhealthy sanitary habits, sexual contact and due to infection from feces on contact with urethral opening.

The frequency of HIV/AIDS related OIs in this study was even higher (83.6%). This finding was in agreement with that of Seyler and co-researchers (Seyler et al., 2007). Although, there was no information about the burden of OIs during pre-HAART and HAART initiation period, the high rate of 47.6% reported by Sun et al., (2006) in Taiwan may be attributable to the high OI prevalence of 77.7% in their patients before HAART initiations.

The presences of Staphylococcus aureus (10.0%), Streptococcus pneumonia (7.4%), and Pseudomonas aeruginosa (2.5%) in this study on sputum, may be seen as OIs in Aids-Defining Conditions (ADC); with is in conformity with the report of Duguid et al., (1978) and Jacobson et al., (1998), in a case study of HIV/AIDS infected persons in eastern Nigeria. Although, Staphylococcus aureus was identified by Duguid et al., (1978), as a normal flora of the skin, urethra and vagina of about 10-30% healthy individuals. The Pseudomonas aeruginosa opportunistic abilities was seen by its prevalence rate of 2.5%. The organism has ability to cause skin infections at burn sites, ulcers, and wounds as its secondary invader. It also has high resistance ability to a wide range of disinfectants, and antibiotics, and the ease of infectivity was therefore established.

The individual frequency of OIs in this study was low. Although, Mycobacterium tuberculosis (TB) was one of the most frequent OIs seen in our patients studied; and are not in conformity with those reported in patients taking HAART in other low-income settings (Sun et al., 2006; Seyler et al., 2007; Mzileni et al., 2007; Manosuthi et al., 2007). The prevalence of OIs in this study was not similar to what was reported by (Salami et al., 2006; Daniyam et al., 2011), in HAART-naive patients in Nigeria. Although, lack of change in the frequency of OIs post-HAART compared to the pre-HAART era has been reported by some authors (Forrest et al., 1995; Ledergerber et al., 1999; Sun et al., 2006; Manosuthi et al., 2007). It is of important to mention here that it is unusual of a lot of these studies to show that TB was not the commonest OI among our patients, despite the well-known high burden of TB in Nigeria. Globally, TB diagnosis continuous to remains a serious challenge in people living with HIV/AIDS because of the absence of classical features, high rate of smear negative disease, and relatively high burden of extra-pulmonary disease.

Conclusion

From the findings, this study has showed the involvement of some OIs as aetiological agents in the prognosis to AIDS in individuals living with HIV/AIDS in Abuja, FCT, Nigeria. Therefore, it is recommending that early detection of these infections, clinical outcome, diagnosis, as well as knowledge on the epidemiology and proper treatment options would be useful in the proper management in Nigeria.

Acknowledgement

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Conflict of interest

The authors declared no conflict of interest.

List of abbreviations

HIV, Human Immunodeficiency Virus
HAART, Highly Active Anti-retroviral Therapy
PEPFAR, President’s Emergency Plan for AIDS Relief
ART, Antiretroviral Therapy
NIPRD, National Institute for Pharmaceutical Research and Development
HREC, Health Research and Ethics Committee
FCT, Federal Capital Territory
ARVs, Antiretroviral Drugs
HVS, High Vaginal Swab
OIs, Opportunistic Infections
PLWHA, People Living With HIV/AIDS.

REFERENCES


