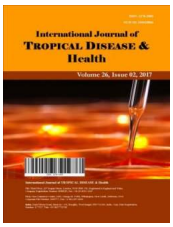

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Prevalence of Pulmonary Mycoses among HIV Infected Clients Attending Anti-Retroviral Therapy Clinic at Kisoro District Hospital, Western Uganda

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JM, BM, DAH, SB, CA and IMT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BM, CA and IMT managed the analyses of the study. Authors JM, DAH, SB and IMT managed the literature searches. Author IMT critically revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: For determining the prevalence of pulmonary mycosis among HIV infected clients attending anti-retroviral therapy clinic at Kisoro district Hospital in Western Uganda.
Study Design: This was a cross- sectional study.
Place and Duration of Study: This was conducted in the anti-retroviral therapy (ART) clinic at Kisoro District Hospital (KDH) during the months of March to July, 2016.
Methodology: We collected sputum samples from HIV sero positive clients that were enrolled in the ISS clinic. We did microscopic examination using Ziehl Neelsen (ZN) staining technique and sputum culture on sabroid dextrose agar (SDA), and identified to isolate fungal pathogens. Further, we obtained about 4.0 milliliters of ethylene-di-amine tetra acetic acid (EDTA) blood that was used to assay CD_4+ cell counts. Data was analyzed, and presented as proportions.

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Results: Of the 252 participants, 136 (53.97%) were females. The mean and median age was 38.94 (SD±12.64) and 38 years, respectively. The prevalence of pulmonary mycosis was 34/252 (13.49%; 95% CI=9.53-18.34), of which 12(35%; 95% CI=19.75-53.51) were molds and 22 (64.71%; 95% CI=46.49-80.25) were yeasts. The species isolated and their proportions were: *Candida albicans* (10/252, 3.97%), other *Candida* species (7/25, 2.78%), *Aspergillus* species (6/252, 2.38%), *Penicillium* species (5/252, 1.98%), *Cryptococcus species* (4/252, 1.59%), *Basidiomycetes* (1/252, 0.40%) and *Acremonium* species (1/252, 0.40%).
Conclusion: From this study, we report a high prevalence of pulmonary mycosis that is associated with deteriorating immunological state. In addition, it presents with signs and symptoms aping *Mycobacterium tuberculosis.*

Keywords: Opportunistic infections; pulmonary mycosis; HIV; Uganda.

ABBREVIATIONS

AIDS : Acquired Immune Deficiency Syndrome;
ART : Anti-Retroviral Therapy;
CD_4+ : Cluster of Differention-4cells;
HIV : Human Immune Deficiency Virus;
SDA : Sabouraud Dextrose Agar;
OIs : Opportunistic Infections;
WHO : World Health Organization.

LIST OF DEFINITIONS

Opportunistic fungal infections: Infections caused by fungal pathogens when the body defense mechanism has been weakened.
Pulmonary fungal infections: Infections of the lungs caused by fungal microorganisms

1. INTRODUCTION

The burden of human immune deficiency virus (HIV) infection in Uganda remains high [1]. This risks an estimated 60% of people living with HIV (PLWHIV) to acquire opportunistic infections (OIs) [2]. The OIs further weakens the immune system indicated by very low clusters-of differentiation-4 (CD_4+) cell counts, leading to acquired immune deficiency syndrome (AIDS) that may aggravate deaths [3,4]. Pulmonary mycosis is associated with 58% - 81% of morbidity and mortality among PLWHIV [5]. Infected individuals present with features similar to those of pulmonary tuberculosis (PTB), and this makes both diagnosis and management difficult [6]. Whereas prevention of pulmonary mycoses would offer a seemingly better approach among the at risk population, this is complicated by the virtue that fungi are ubiquitous, which propagates their rapid spread. Though it's commended to screen suspected cases of PTB for mycosis, Kisoro district Hospital (KDH) has no strategy for this. As such, there is missed diagnosis, delayed therapeutic intervention which augments poor outcome. Besides, cases which are treated with anti-TB therapy have a poor prognosis as these drugs have no effect on pulmonary fungal pathogens, also, such cases ought not to be initiated on anti-fungal empirically due to their hepatotoxicity [7]. To this, we sought to determine the prevalence of pulmonary mycosis, as a guide on infection burden and implication on diagnosis in a low resource set up.

2. MATERIALS AND METHODS

2.1 Study Site

The study was conducted in the anti-retroviral therapy (ART) clinic at Kisoro District Hospital (KDH) during the months of March to July, 2016. KDH is government hospital located in Kisoro District, Kisoro Town Council in Western Uganda along Kabale-Bunagana road. The clinic has 3,012 clients from within the district, neighboring districts, parts of Democratic Republic of Congo (DRC) and Rwanda. It attends to approximately 250 clients daily.

2.2 Sample Size Determination

We used Slovin's formula (1960), and from the hospital records it found that Kisoro Hospital Laboratory on an HIV clinic day receives over 15 patients from HIV clinic presenting with pulmonary symptoms for TB examination only (Kisoro Hospital Laboratory TB register, 2016). On substituting into the formular: $n=N/(1+Ne^2)$ where n stands for the number of samples to be used in the study; N being the population size; e as the margin of error estimated at 5%; then a sample size of 252 study participants was considered.

2.3 Sampling Technique

Convenient sampling technique was used where by all HIV positive patients who would come to the ART clinic with pulmonary symptoms and willingly gave an informed consent were sampled.

2.4 Selection Criteria

2.4.1 Inclusion criteria

All HIV sero-positive patients aged 18 years and above presenting with pulmonary symptoms and were able to give an informed consent.

2.4.2 Exclusion criteria

HIV sero-positive patients who were already on anti-fungal therapy after they had been diagnosed with pulmonary mycosis were excluded from the study.

2.5 Sample Collection

2.5.1 Sputum

Samples were obtained after one had inhaled deeply 3 times before coughing from their chest and carefully spat into the container and screwed tightly.

2.5.2 Blood

We collected about 4.0 mililitres (mL) of blood into a well labeled ethylene di-amine tetra acetic acid (EDTA) vacutainer. This was collected by applying a tourniquet to the upper arm, then disinfecting the site with a moist 70% alcohol swab. Venipuncture was then done to the prominent vein, with care to avoid hemolysis.

2.6 Laboratory Sample Analyses

Microscopic wet preparations using 10% Potassium Hydroxide (KOH) was done and examined. In addition, India ink preparation and Lacto phenol cotton blue (LPCB) for morphological features were done. These were stained using Gram staining technique for fungi shape and size of fungal arrangement. Culture was done on SDA with Chloramphenicol (CONDA pronadisa, Spain) for general fungal characteristics, and *Candida albicans* were identified by a positive Germ tube. Urease test was performed to identify other fungal isolates. CD_4+ cell counts were determined using a Becton, Dickson and company (BD) FACSCount (BD 2008, USA). The following approved standard operating procedures were used;

2.6.1 Identification of fungal pathogens

2.6.1.1 10% Potassium Hydroxide (KOH)

Using a clean glass slide, a drop of homogenized sputum sample was mixed with a drop of 10% KOH solution. The mixture was then incubated in a moist petri dish at room temperatures for 30 minutes. This was then microscopically examined for identification features.

2.6.1.2 India ink preparation

This was done by mixing a drop of sputum and a drop of India ink. The mixture was covered with a coverslip and microscopically examined using x10 and followed by x40 objectives looking for capsule of *Cryptococcus* species which appeared as round cells, against the dark back ground.

2.6.2 Culture on SDA with chloramphenicol

We dried SDA plates at 37℃ for 15 minutes. This was followed by streaking the sputum sample onto the surface of SDA plate containing chloramphenicol. We then incubated the plates at 37℃ for 2 weeks and were examined for growth after every two days.

2.6.3 Gram staining technique

With a sterile wire loop, we picked a pure single colony, then spread it evenly over an area of 2x1 cm on a clean slide and left it to air dry. This was heat fixed by slowly passing the slide smear uppermost, three times through a flame and allowed to cool. We then flooded the slide with Gentian violet stain for 60 seconds, washed the stain with clean water, and tipped off excess water. We added Gram's iodine for 60 seconds, washed the stain with clean water and tipped off excess water. We decolorized using 50% Acetone Alcohol for 5 seconds and washed off immediately with water. Flooded the smear with dilute carbol fuschin (1 in 20) stain for 3 minutes then washed off the stain with clean water and wiped the back of the slide clean and air dried. We examined the smear microscopically under the X100 objective for fungi shape and size of fungal arrangement.

2.6.4 Urease test on yeasts

We dried urease agar in the hot air oven at 37℃ for 30 minutes and then streaked the colonies

from the growth on SDA media. We incubated at 37℃ with the lid of the universal bottle loosely tightened for 24 hours and examined for color change to pink for *Cryptococcus* species and no color change for *Candida* species. This was done to differentiate between the *Cryptococcus* species from *Candida* species among yeast growth on SDA media.

2.6.5 Germ tube technique

To 1 ml of human serum in a labeled small tube, we emulsified yeast colonies and the mixture was incubated at 37℃ for one hour then transferred a drop of serum onto the slide for microscopic examination under X40 objective for the presence of Germ tube. This was used to differentiate *Candida albicans* from other *Candida* species whereby Candida albicans produced a short hyphal extension that arose laterally from a yeast cell and were reported as Germ tube positive and other non-*Candida albicans* were negative.

2.6.6 Determination of CD_4+ counts

We labeled each reagent tube according to the patients' identification number and vortexed upside down and upwards for 6 seconds each. We opened the reagent with the coring station by sliding the reagent tubes upright into the coring station. We then transferred the tubes from the coring station to the workstation, keeping the tubes upright. We then added 50 µl of the patient's blood to 50 µl of a fixative solution into the tube, then vortexed upright for 6 seconds. We then switched the on the BD FACSCount machine then entered the patient identification number and ran samples.

2.7 Quality Control

Sample analysis was done in strict adherence to SOP. In addition, standard strains of *Candida albicans* (ATCC 60193) as a positive control, *Staphylococcus aureus* (ATCC-25923) and *Escherichia coli* (ATCC-25922) were included as a negative control for performance of culture media. We tested for sterility of the culture media, and daily controls for the BD FACS Count machine were done before running patient samples.

2.8 Data Management and Analysis

Data was entered in Microsoft Excel, and imported to STATA 11.0 software for analysis. Data was presented using frequencies and percentages, the differences in proportions were assessed using the Chi-square test at 95% CI.

3. RESULTS

We enrolled 252 adult HIV sero-positive participants. Of these, 136 (53.97%) were females. Their mean and median age were 38.94 (SD±12.64) and 38 years, respectively. Out of the 252 sputum samples cultured, 34/252 (13.49%; 95% Confidence Interval=9.53-18.34) had growth that tested positive for fungal infections. Twelve (35%; 95% CI=19.75-53.51) were molds and 22 (64.71%; 95% CI=46.49-80.25) were yeasts. The species isolated were *Candida albicans* (10/252, 3.97%), other *Candida* species (7/25, 2.78%), *Aspergillus* species (6/252, 2.38%), *Penicillium* species (5/252, 1.98%), *Cryptococcus species* (4/252, 1.59%), *Basidiomycetes* (1/252, 0.40%) and *Acremonium* species (1/252, 0.40%) as shown in Fig. 1.

When a comparison with CD_4+ cell count was made, majority (33/34, 97.1%) had CD_4+ less than 350 cells/µl (X^2 = 80.37, *p-value*=<0.001), as indicated in Table 1.

4. DISCUSSION

The prevalence of pulmonary mycosis was 13.49%. This is lower than 42.96% that was earlier reported in India [8], 68.5% in Nigeria [9], 48% in Cameroon [10] and 44.18% in Kenya [11].The lower prevalence in this study is attributed to two factors: Firstly, our participants had CD_4+ cell count above 350 [5]. Secondly, unlike the previous studies, the 'test and treat' HIV care approach in our set up implies that our participants were enrolled on ART, which lessens the risk of OIs [9].

In the present study, the most dominant isolates were *Candida* species which was in line with other findings reported in India [6], Cameroon [10] and Nigeria [5]. Among the *Candida* species, *Candida albicans* was the most isolated which is agrees with findings from other studies in India [6], Kenya [11], and Nigeria [5,9]. Candida is a normal flora that becomes opportunistic following low immunity [12,13], and this could be the basis as to why it was the most common isolate. *Aspergillus* species were the second most prevalent with a prevalence of 2.38% which was a similar observation in Kenya [11] and India [6]. This could have been due to presence of spores

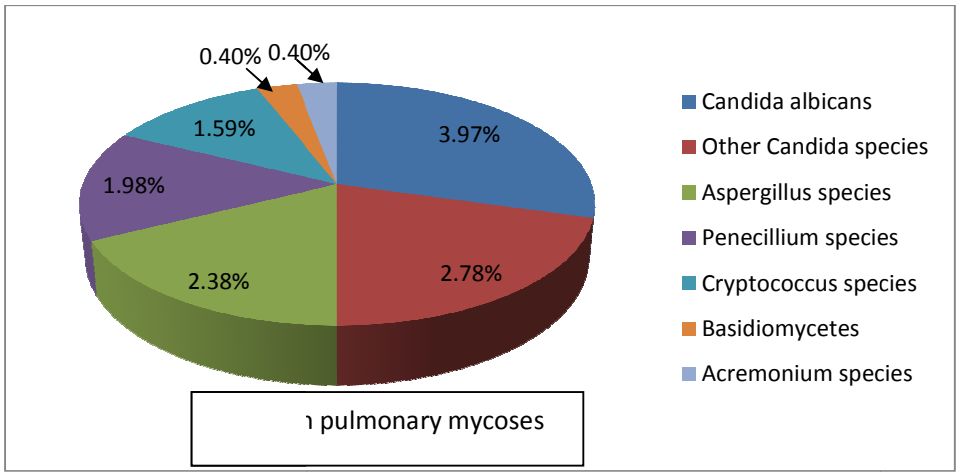

Fig. 1. A pie chart illustrating common pulmonary mycoses

Table 1. Frequency of the relationship between CD4 counts and pulmonary mycoses

Variables	Culture results		Total	X^2	P. *Value*
	Growth (%)	No growth (%)			
CD4 counts					
<350	33 (97.06)	45 (20.64)	78 (30.95)	80.365	<0.001
≥350 cells/µl	1 (2.94)	173 (79.36)	174 (69.05)		
Total	34 (100.00)	218 (100.00)	252 (100.00)		

CD4: Cluster of differentiation-4 marker; X^2: Chi-square test

of *Aspergillus* species in the atmosphere, their ability to grow in abundance everywhere, production of small conidia that easily penetrate deep in the alveoli region and grows at 37℃ [14], also the possibility of ingesting contaminated grains. The isolates of *Penicillium* species were in agreement with a study in Cameroon [10] but slightly lower compared to 4.1% in Nigeria [5] and 6.25% in India [15]. Cryptococcus species was among the less common isolates in our study with a low rate, similar to studies in Cameroon [10] and India [5], but highly differing from other studies in India [8] and in Nigeria [9]. Soils and bird droppings predispose immune-suppressed patients to Cryptococcosis, and this could be the basis for the observed high prevalence [12]. Whereas a study in India isolated *Histoplasma capsulatum* and *Pneumocystis jirovecii* [6], we did not isolate them in this study. This is probably due to their isolation requirements (like scrapings from foci of infection, bronco-alveolar lavage and pulmonary biopsies) which we never performed. Further, their identification techniques like, complement fixation test, Wright's stain, Giemsa stain, direct immunofluorescence were not done; Albeit we cannot rule out their occurrence in our set up.

Pulmonary mycosis were seen more among patients with CD_4+ counts less than 350 cells/µl which was in agreement with a study in Nigeria [9]. Furthermore *Cryptococcus* species were isolated among patients whose CD_4+ counts were below 100 cell/ µl, and *Aspergillus* species at CD_4+ less than 350 cells/µl and this was similar to findings in Nigeria [3,9]. Our results fell short of the following: 1) the prevalence of pulmonary mycoses reported is for the HIV population irrespective of ART status. Thus we do not know the cofounding effecting of ART naïve as they are more prawn to opportunistic infections. 2) Although our participants had immunosuppression due to HIV, we did not investigate for other conditions such as cancer which could aggravate their status.

5. CONCLUSION

The findings in this study reveal a high prevalence of pulmonary mycosis. It is more among those with deteriorating immune status indicated by CD_4+ cell counts below 350 cell/µl. As this infection presents with signs and symptoms aping *Mycobacterium tuberculosis*, apt laboratory diagnosis is key to reduce its mismanagement, and its effects.

CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from research and ethics committee of International Health Science University. We ensured strict confidentiality and high ethical standards. We obtained written informed consent from each participant. Results were only availed to the attending doctor for proper management. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. UNAIDS. Access to antiretroviral therapy in Africa: Status report on progress towards 2015 targets. Geneva; 2014.
2. Usman AD, Uba A. A survey of bacterial and fungal opportunistic infections among HIV clients in Kano metropolis Bayero. Journal of Pure and Applied Sciences. 2011;4(1):148-152.
3. Ogba OM, Abia-Bassey LN, Epoke J. The relationship between opportunistic pulmonary fungal infections and CD4 count levels among HIV sero-positive patients in Calabar, Nigeria. Journal of Fungal Infections. 2013;1.
4. Parkes-Ratanshi RA, Kambugu A, Meya D, Denning D. Estimated burden of fungal disease in Uganda. Abstract 3635. Kampala, Uganda; 2015.
5. Aluyi FO, Iweriebor O. Incidence of pulmonary mycoses in patients with acquired immuno-deficiency diseases. Nigerian Journal of Clinical Practice. 2010;13(1):78-83.
6. Tshering Ongmu, Bhutia LA. Pulmonary mycoses among the clinically suspected cases of pulmonary tuberculosis. International Journal of Research in Medical Sciences. 2015;3(1):260.
7. World Health Organization. Report on pulmonary mycoses and their management. Geneva; 2014.
8. Sunita J, Bansod MR. Emerging of mycotic infection in patients infected with *Mycobacterium tuberculosis*. World Journal of Medical Sciences. 2008;3:74-80.
9. Nwako OF, Ofondu EU, Nwako AB, Dozie INS, Nwako CA. Mycological studies on the sputa of HIV positive clients attending HIV clinic in Federal Medical Centre Owerri, Nigeria. AIDS & Clinical Research. 2014;5:3.
10. Njunda AL, Nsagha DS, Assob JCN, Kamga HL, Teyim P. *In vitro* antifungal susceptibility patterns of *Candida albicans* from HIV and AIDS patients attending the Nylon Health District Hospital in Douala, Cameroon. Journal of Public Health in Africa. 2012;3:e2.
11. Mwaura NE, Elizabeth VM, Christine B. Mycological findings of sputum samples from pulmonary tuberculosis patients attending TB clinic in Nairobi, Kenya. Virol Mycol. 2013;2:119.
12. Kayser HF, Bienz KA. Medical microbiology. Germany: Georg Thieme Verlag Stuttgart. 2005;10.
13. Damtie D, Yismaw G, Woldeyohannes D, Anagaw B. Common opportunistic infections and their CD4 cell correlates among HIV-infected patients attending at antiretroviral therapy clinic of Gondar University Hospital, Northwest Ethiopia. BMC Research Notes. 2013;6:534.
14. BodoWanke M, MarcioNucci L. Fungal infections in the immuno compromised host. MemInst Oswaldo Cruz, Rio de Janeiro. 2000;95(1):153-158.
15. Bharathi B, Siva Sankar S, Swamidoss D. Incidence of bacterial and fungal coinfections in some HIV infected Indian population. Indian J. Biotechnol. 2010;3(2): 199.

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