PREVALENCE OF PULMONARY ASPERGILLOSIS AND DRUG SUSCEPTIBILITY TESTING AMONGST ADULT HIV PATIENTS AT KISUGU HEALTH CENTRE III, MAKINDYE DIVISION, IN UGANDA, A CROSSECTIONAL STUDY.

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Abstract

Background:

Pulmonary aspergillosis is one of the respiratory conditions that are missed to be diagnosed and neglected in Uganda and it has been reported to be common worldwide, especially among people living with HIV. With azole resistance common to aspergillus fumigatus becoming a global concern; a species that causes invasive disease, epidemiological data are necessary to guide clinical practice. This study determined the prevalence of pulmonary aspergillosis and drug susceptibility testing amongst adult HIV patients at Kisugu Health Centre III

Methods:

A cross-sectional study was carried out at Kisugu Health Centre III among 254 HIV clients. Early morning sputa were cultured on Sabroud Dextrose Agar at 30^0C for 7 days using a high-volume culture technique. Microscopy using lactophenol cotton blue stain was carried out to identify the species. Antifungal susceptibility testing was done using the agar-based disc diffusion method, and data were analyzed as proportion.

Results:

There were 79.5%(N=202) female participants. Participants' mean age was 34.20±10.27 (range: 18-67) years. The prevalence of pulmonary aspergillosis was 42.5% (N=108) with the commonest species being aspergillus niger (28%, N=71), fumigatus (9%, N=23), terreus(4.7%, N=12), co-species infection at 1.2% (N=3). 98.6% of aspergillus niger isolates were sensitive to voriconazole,87.5% of aspergillus fumigatus isolates were sensitive to voriconazole and 100% of the aspergillus terreus isolates were sensitive to voriconazole. Major resistance in this study was seen in aspergillus fumigatus with 12.5% resistance to itraconazole, resistance was also noticed amongst 8.3% of aspergillus terreus isolates on amphotericin B.

Conclusion:

The prevalence of Pulmonary aspergillosis was high, and different species were isolated. Moreover, a varied antifungal susceptibility pattern was established

Recommendation:

Routine testing for pulmonary aspergillosis should be included in HIV clinics.

Keywords: Pulmonary Aspergillosis, Drug Susceptibility testing, HIV, Uganda, Submitted: 2023-03-15 Accepted: 2023-03-20

1. INTRODUCTION:

Pulmonary aspergillosis (PA) is a collective term used to describe invasive and non–invasive forms of aspergillosis disease, it is an opportunistic mycosis amongst the immunocompromised. Common species that cause pulmonary aspergillosis are; *Aspergillus flavus, Aspergillus niger,* and *Aspergillus fumigatus.* Members of the genus Aspergillus produce spores that are easily inhaled and thrive in a variety of climates around the world. The fungus grows in the lungs. Moreover, it mainly affects the lungs and can decimate other organs during the progressive stages of the disease, it can be classified into; Chronic Pulmonary Aspergillosis (CPA), Covid -19 (Corona Virus Disease-2019) Associated pulmonary aspergillosis (CAPA); which is the latest definition of the pulmonary disease (Hoenigl et al., 2018) allergic bronchopulmonary aspergillosis (ABPA) which is closely associated with asthma patients(Singh et al., 2018), Invasive Pulmonary Aspergillosis (IPA); seen in tissue samples (histology), chronic necrotizing pulmonary aspergillosis (CNPA). For a clinician to sufficiently classify or type the form of pulmonary aspergillosis, X-ray and CT (Computed tomography) scans, clinical signs, and symptoms accompanied by in-depth laboratory investigations must be taken into consideration.

Globally, there are 1.2 billion fungal infections which have resulted in an estimated 1.5 -2 million deaths. Further, deaths related to chronic pulmonary aspergillosis globally are about 450,000 (Ibe, 2022). There have been several documented cases of all forms of pulmonary aspergillosis across Asia most especially in China and India. A study in Northern India amongst HIV (Human Immunodeficiency virus) patients showed that the prevalence rate was 13.3 % and common among women (Kaur et al., 2017). However, the study used conventional culture which according to present-day literature has a low sensitivity and specificity (Denning, 2021). A recent study showed that in 14 African countries, there were about 1,247 cases of CPA (Olum et al., 2021)

According to recent findings, 87% of medical centers in Eastern and South Eastern Europe have access to aspergillus species diagnostics, including mold-active antifungals. It is estimated that there are about *240,000* active cases of CPA across Europe according to (Denning et al., 2016)

In Uganda there have been several studies about PA and other forms of aspergillosis, there is no nationwide prevalence rate for PA since the Ministry of Health has not adopted any guidelines for the diagnosis and patient management (Kwizera et al., 2021). A recent study amongst active Tuberculosis patients showed a prevalence rate of 31% for Aspergillus Specific Immunoglobulin-G (IgG) tests, for sputum culture re; positivity rate was at 23%, 29.6 % of the study participants were HIV positive (Namusobya, Bongomin, Mukisa, Olwit, Batte, Mukashyaka, Mande, Kwizera, David W Denning, *et al.*, 2022). There have also been case studies of CPA in the Gulu district that identified patients on TB treatment but tested positive for Aspergillus Ig G (Kwizera et al., 2021). The rise in the prevalence of pulmonary aspergillosis in the immunocompromised has resulted in a global healthcare problem that, if not diagnosed and treated, has the potential to spread and lead to a devastating invasive disease condition. There are numerous symptoms associated with pulmonary aspergillosis. Cough, chest pain, arthralgia, fever, difficulty breathing, and headache are examples of symptoms. The non-specific symptoms of this disease make diagnosis difficult for clinicians. Beyond this limited data, there is a need for more epidemiological data to guide policy regarding Aspergillosis infections among individuals with the symptoms of tuberculosis and their drug susceptibility pattern. The objective of our study was to determine the prevalence of pulmonary aspergillosis, speciate at the phenotyphic level and establish the antifungal susceptibility patterns of the known aspergillus species amongst HIV adults.

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2. METHODS:

2.1. ***Study design***

The study was a descriptive hospital-based cross-sectional study design that employed the use of quantitative data collection methods, this was suitable for the study given that it was more of an epidemiological study. Sputa were collected from only HIV patients that matched our inclusion criteria. The study was carried out from October to December 2022.

2.2. ***Study area***

The study was carried out at Kisugu Health Centre III, Makindye division, Kampala district, the health center III is surrounded by slums such; as Kasanvu, Kanyogoga, Kisugu, and Namwungo.

2.3. ***Study population***

Patients that visited Kisugu Health Centre III, HIV clinic, or TB clinic from within Kampala.

2.4. ***Selection criteria***

2.4.1. ***Inclusion criteria:***

Patients that consented to participate in the given study. Patients that had HIV, a history of pulmonary TB, or living with TB. HIV patients between the ages of 18-80 years. HIV Patients exhibited the following signs, symptoms, and conditions: cough (with blood), chest pain, breathlessness, TB drug resistance, diabetes, long term smokers. HIV patients that had lung conditions (e.g., Asthma, Chronic Obstructive Pulmonary Disease (COPD), cystic fibrosis) and were on corticosteroids. HIV patients that had undergone solid organ transplants or stem transplants. HIV patients that had a COVID-19 history. HIV patients that had been on antifungal treatment and also those that are undergoing antifungal treatments.

2.4.2. ***Exclusion criteria:***

- Patients that were HIV positive and were in a critical state or need emergency.
- Patients that were HIV positive but are not mentally sound.

2.5. ***Sampling.***

- Using clinical findings, a random probability sampling method was used. HIV patients that matched our inclusion criteria were identified and their consent was sought, once obtained their sputa samples were retrieved from storage, and various tests were carried out.

2.6. ***Bias***

Different research assistants participated in recruiting the study participants, this was to avoid individual bias.

Patient data were double-checked with clinical records to ensure the validity of the data.

2.7. ***Sample size estimation***

The sample size was estimated using the kish and Leslie formula

$$= \left(Z_{(1-\propto)}\right)^2 \left(\frac{P\,(1-P)}{D^2}\right)$$

Where Z was = 1.96 (at 95% confidence interval)

P = prevalence from a previous study which 19.8% (obtained from a previous study carried out in kampala) (Namusobya et al., 2022)

D = percentage points of 5% (0.05)

Minimum sample size of 244 participants but 254 were recruited

2.8. ***Data collection and research tools.***

- Consent forms were provided to those willing to participate in the study.
- Aspergillus detection and speciation (Using High Volume Culture)
- Early morning sputa were collected, and the color, and thickness of the sputa were noted down.
- No slide was made to view fungi before culture using potassium hydroxide solution.
- The sputum (about 1 ml) was inoculated on Sabroud dextrose agar (SDA) with 0.5% Chromophenical to ensure only the growth of fungi, the sputa were cultured for 7 days at 300C but observed at 24-hour intervals for growth.

- Once sufficient growth was observed, pure colonies were sub-cultured for proper identification, the colonies were later observed under microscopy with lactophenol cotton blue stain, under x10 and x40 objective lenses. The arrangement of hyphae, conidiophores, and conidia was noted down as well as the color of colonies on the SDA.

- The color of colonies, arrangement of hyphae, conidiophores, and conidia was used to speciate Aspergillus.

2.9. ***Antifungal susceptibility:***

- Once speciation had been successfully done, anti-fungal susceptibility was carried out.

- The conidia were mixed with 2- 4 drops of 0.9% normal saline, and the optical density was adjusted to 0.09 – 0.011 using a spectrophotometer at a wavelength of 530 nm. (as per CLSI standards)

- The mixture was poured on non-supplemented Mueller Hinton Agar (MHA) and later allowed to dry

- The following antifungal discs were inoculated on the MHA plates; amphotericin B, voriconazole, and itraconazole and cultured at 300C for 48 hours. Resistance was determined/interpreted by the CLSI minimum Inhibitory Values (MICs).

- The antifungal discs were prepared in-house from antifungal tablets and vials

2.10. ***Ethical approval:***

Ethical approval was obtained from the CIU (Clarke International University) Ethical Review Committee before proceeding with the study under the reference number CLARKE-2022-466. Permission from the administration of Kisugu Health Centre III was obtained and participants that gave consent were only accepted to take part in the study and were also allowed to withdraw from the study.

2.11. ***Quality controls.***

Sterility and viability tests were carried out on all culture media used.

2.12. ***Data analysis and management.***

The data was collected in registers and later on, entered into a Microsoft excel spreadsheet

The collected data was analyzed using SPSS 25 software. The data about aspergillus culture positivity rate, age, sex, and antifungal susceptibility patterns were analyzed.

3. RESULTS

3.1. ***Socio-demographic characteristics***

A total of 254 adult HIV seropositive patients were considered for this study. This study was carried out among patients from within Kampala visiting Kisugu Health Centre III HIV clinic, and the TB clinic. More, fifty-two (20.5%) of the respondents were male, while 79.5% (202) were female. The mean age of the respondents was 34.20±10.27 (range: 18 to 67) years.

3.2. Prevalence of Pulmonary aspergillosis.

The prevalence of Pulmonary aspergillosis amongst the study population was 42.52%(N=108). (95% confidence interval: 36.4 – 48.9).

The commonest cause was Aspergillus Niger (28%; N= 71. There were 3 cases of co-infection with Aspergillus Niger and fumigatus (1.2%; N=3) , aspergillus fumigatus (9.1%; N=23) , aspergillus terreus (4.7; N=12). figure 3 summaries the distribution of isolates.

3.3. Antifungal susceptibility testing of aspergillus species.

257 isolates were obtained from the study and the following drugs were used amphotericin B , voriconazole and itraconazole , 98.6% of aspergillus niger isolates were sensitive to voricon-azole ,87.5% of aspergillus fumigatus isolates were sensitive to voriconazole and 100% of the aspergillus terreus isolates were sensitive to voriconazole . Major resistance in this study

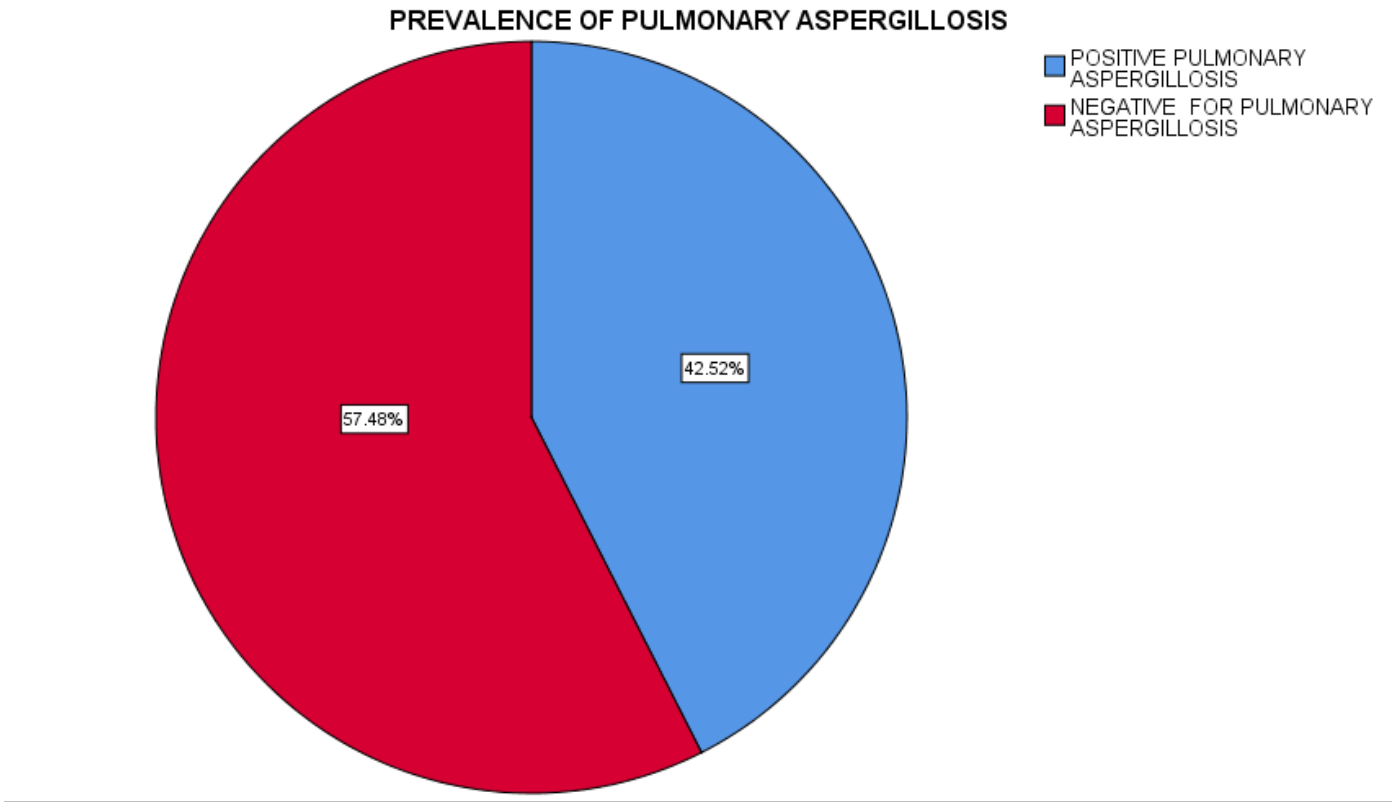

Figure 1: (Source: Primary data, 2022) Figure 1: Pie chart showing the prevalence of Pulmonary aspergillosis

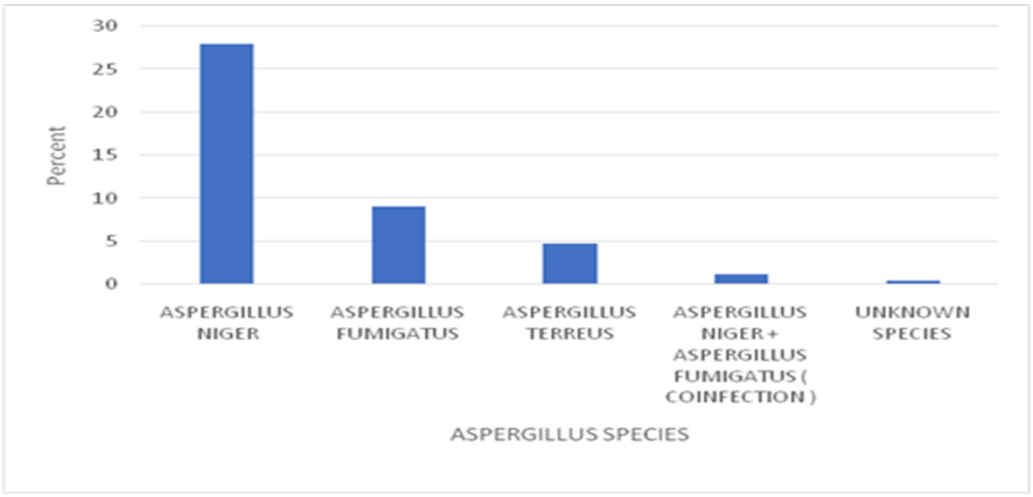

Figure 2: Showing the distribution of aspergillus isolates

Table 1: Aspergillus species and antifungal susceptibility patterns

drug/aspergillus species		A. NIGER		A.FUMIGATUS		A. TERREUS	
		Count	Frequency	Count	Frequency	Count	Frequency
ITRACONZOLE (10micrograms)	INTERMEDATE	2	2.8%	6	25.1%	1	8.3%
	RESISTANT	1	1.4%	3	12.5%	0	0.0%
	SUSCEPTIBLE	67	94.4%	14	58.3%	11	91.7%
AMPHOTERICIN B (10micrograms)	INTERMEDIATE	3	4.2%	4	16.6%	5	41.7%
	RESISTANT	0	0.0%	0	0.0%	1	8.3%
	SUSCEPTIBLE	69	94.6%	19	79.2%	6	50.0%
VORICONAZOLE (10 micrograms)	INTERMEDIATE	1	1.3%	0	0.0%	0	0.0%
	RESISTANT	0	0.0%	2	8.3%	0	0.0%
	SUSCEPTIBLE	71	98.6%	21	87.5%	12	100.0%

(source: Primary data 2022)

was seen in aspergillus fumigatus with 12.5% resistance to itraconazole, resistance was also noticed amongst 8.3% of aspergillus terreus isolates on amphotericin B. Table 3 summarizes the anti-fungal activity of the aspergillus species

4. Discussion:

The findings show that the prevalence of pulmonary aspergillosis amongst HIV patients was 42.5%, this is higher compared to the reported values in another study in Uganda that had a prevalence rate of 0.3 % amongst HIV patients (Page et al., 2019) however the methods of detection differed with our study, the latter study used aspergillus antibody assay to detect pulmonary aspergillosis which is according to Namusobya et al., 2022 have low sensitivity due to species and genetic variations. Findings from a study in southwestern Uganda to assess the burden of pulmonary fungal infections found that 17.2% of infections were attributed to aspergillus species this was lower compared to our prevalence value. This difference could be also attributed to the fungal culture method (Njovu et al., 2021). Another study form Nigeria showed that 18.6 % of pulmonary infections were due to aspergillus species (Talle et al., 2017). Aspergillus pulmonary infection in a study in Ethiopia was at 61%, higher compared to our findings (Bitew and Bati, 2021). The prevalence in our study is almost similar to the prevalence of aspergillus antibodies in Taiwan at 43.2% (Lee et al., 2020) and higher than findings from India with a prevalence of 13.3% (Kaur et al., 2017.), though the study used conventional culture techniques which according to Denning, 2021have a low diagnostic yield. The variation in the findings is attributed to the sample size, study setting, and laboratory methods.

The most common species isolated in our study was *Aspergillus Niger* at 28 % and 9 % *Aspergillus fumigatus.* Studies conducted by Namusobya et al., 2022 on CPA in Uganda also agree with our findings, 23.4 % of aspergillus species isolated were *Aspergillus Niger* and 8 % were Aspergillus *fumigatus*, this study also employed the use of high-volume culture methods, this strongly suggests that high volume culture in the diagnosis of fungal infections is very beneficial. Our speciation results differed from those from a study in Nigeria where A*spergillus fumigatus* was the most prevalent (43.4%) followed by *niger* at 16.4 % (Sani et al., 2020). , Another study carried out by Mbarara University teach-

ing hospital on pulmonary infection reported several co-species infections though the most common co-aspergilli infection was aspergillus niger and flavus which our study didn't observe, however, *aspergillus Niger* was the common species isolated which agrees with our study (Njovu et al., 2021). This alone suggests that the chances of co-species infection are likely to be high due to the fact these species can co-exist in the same niche. Bitew & Bati, 2021 have similar findings with *A. Niger* being common isolates, the study was carried out in Ethiopia. This difference could be due to geographical locations. The results in the study also agree with a case finding in Japan whose culture results yielded *Aspergillus Niger*. Our study found that one sample could not be speciated but according to microscopic features it belonged to the genus *aspergillus* because hyphae were arranged at acute angles and were septate, this shows that species that cause pulmonary aspergillosis are diverse within the aspergillus genus hence the use of PCR techniques that can recognize diverse genes hence accuracy. The observed variations in species isolated are due to differences in climatic patterns and geographical locations.

In our study, voriconazole was the drug that most of the isolates (95.3%) were sensitive to followed by itraconazole at 81.5 % and amphotericin B at 74.6%, with 87.5 % *of Aspergillus fumigatus isolates* being sensitive to voriconazole. *Aspergillus fumigatus* cross-resistance rate for itraconazole and voriconazole was found to be at 10.4 % in our study, this is lower compared to the 46.2% rate from a study conducted in Japan (Takeda et al., 2021)this could be attributed to the difference in genes amongst the isolates, the methods used to detect resistance in the latter study were molecular methods. Resistance of *Aspergillus terreus* in our study was minimal (8.3 %), this disagrees with the finding of, Lass-Flörl, 2018 that showed that it was intrinsically (by gene mutations) resistant to amphotericin B, her findings also pointed out 80-90% treatment failures with amphotericin B. This kind of difference in *Aspergillus terreus* and amphotericin B could be attributed to genetic variation due to different geographical locations. Voriconazole also exhibited high antifungal activity in a study in Nigeria(Sani et al., 2020.), which is similar to our findings, agar-based disc diffusion method was also applied in their study. Our results show that azoles should be recommended for the treatment of pulmonary aspergillosis instead of Amphotericin B due to its side effects.

5. Conclusion

Our findings show a high prevalence of pulmonary aspergillosis (42.5%) amongst HIV patients, Aspergillus niger was the most common species isolated (28 %) followed by fumigatus at 9% and terreus at 4.7% with 3 cases of Niger and fumigatus coinfection. Voriconazole was identified with the highest antifungal activity. The prevalence obtained from our study gives a strong signal that the disease could be looked at amongst HIV patients, this coincides with Denning, 2021 findings; that this disease is under-reported globally. This calls for attention from all HIV experts to re-evaluate the burden of this disease using more sensitive and accurate techniques.

6. Recommendations.

Regular culture and sensitivity testing should be done on sputa samples of HIV patients that exhibit respiratory problems. there is a need to train laboratory personnel on the identification of aspergillus pathogenic fungi. A bigger multicentric study is desirable involving more sensitive analytical techniques to establish the nationwide burden of pulmonary aspergillosis. Also, health policymakers ought to include pulmonary aspergillosis as one of the silent killers amongst immunocompromised persons to reduce mortality.

7. Limitations.

Our study did not speciate one mold; we suggest the inclusion of molecular techniques in the detection of species. The international fungal bodies and associations strongly suggest the use of micro broth dilution techniques to assess for

antifungal activity which is costly and labor intensive but more accurate which our study didn't apply. The study could not assess genes responsible for resistance in some species. This study did not establish the associated factors to pulmonary aspergillosis, this ought to be explored.

8. Acknowledgement.

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9. List of abbreviations.

AHD Advanced HIV Disease
HIV Human Immunodeficiency Virus
CIU Clarke International University
CPA Chronic Pulmonary Aspergillosis
GAFFI Global Action Fund against Fungal Infections
Ig G Immunoglobulin Gamma
IPA Invasive Pulmonary Aspergillosis
MICs Minimum Inhibitory Concentration
MoH Ministry of Health Uganda
PA Pulmonary Aspergillosis
REC Research and Ethics Committee
WHO World Health Organization

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