Prevalence and profile of pulmonary fungal pathogens among HIV-infected patients attending University of Maiduguri Teaching Hospital, Nigeria

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Background
Fungal infections in lungs are being diagnosed with increasing frequency. This is related to the increased immunocompromised status and hygienic factors among patients. In view of this, the present study sought to isolate, identify, and determine the prevalence of pathogenic fungi in HIV-infected patients attending the University of Maiduguri Teaching Hospital, Nigeria.

Materials and methods
Between 5 April 2016 and 27 August 2016, three consecutive sputum samples in the early morning were collected from individual participants. The samples were inoculated onto Petri dishes containing sabouraud dextrose agar in triplicates for isolation. The fungal isolates were identified using standard mycological stains and reagents. Self-administered questionnaires and participants' hospital cards were used to assess demographic and clinical variables, respectively.

Results
The prevalence of pulmonary fungal infection among the patients was at 68%. Of the infected patients, male patients accounted for 59.8%, whereas female patients accounted for 40.1%. However, there was no significant association between the prevalence of pulmonary fungal infection and sex of subjects (P=0.630). *Candida albicans* [24 (23.5%)] accounted for the highest proportion of the fungal isolates, followed by *Aspergillus* spp. [19 (18.6%)], with the least being *Torulopsis dattila* [one (0.9%)], *Torulopsis glabrata*, [one (0.9%)], and *Microsporum canis* [one (0.9%)]. Subjects with ages between 31 and 35 years that the highest prevalence of pulmonary fungal infections, 28 (27.5%), whereas those between 56 and 60 years, had the least prevalence, 1 (20.0%). Among the 102 participants coinfected with HIV/tuberculosis (TB), 92 (90.2%) had fungal coinfections, representing a relatively higher prevalence compared with those without TB, 10 (20.8%). There was statistical association between the prevalence of pulmonary fungal infections and TB status and age of patients ($P<0.05$).

Conclusion
Findings from this study revealed a high prevalence of pulmonary fungi in HIV/TB coinfected patients, with *C. albicans* being the leading causal fungi responsible for symptoms of pulmonary diseases.

Keywords:
coinfections, immunosuppression, mycosis, opportunistic infections

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Introduction

Fungal respiratory infections are important causes of mortality and morbidity among HIV-positive individuals. They account for up to 70% of illness in AIDS cases [1]. The range of illness varies from asymptomatic mucosal candidiasis to overwhelming disseminated infections. In these patients, dissemination of fungus leads to very serious outcomes, hence it is important to have the knowledge of prevailing profile of fungi that cause infections, so that they can be treated at the onset [1]. Fungal infections in the lungs are being diagnosed with increasing frequency. This is related to the increased immunocompromised and other susceptible patient groups. Heightened awareness of fungal lung infection and appropriate use of the available diagnostic modalities permit appropriate treatment of these important clinical infections in immunocompromised individuals [2]. Very few of these fungi are capable of causing infection in a normal host. Vast progress has been achieved in the understanding of fungal pathogenicity, including the mechanism of adherence to the host tissue, penetration of the tissue, multiplication within the host,

and the interaction of fungal cells with host effector cells [3].

In addition to the increase in the infection rates in immuocompromised patients by opportunistic and pathogenic fungi, such as *Candida* spp., *Aspergillus* spp., *Cryptococcus neoformans*, *Histoplasma capsulation*, and *Coccidioides immitis*, many fungi that occur as saprophytes in the environment and which had previously been considered to be nonpathogenic are now being encountered as a causal agent in human infections [3]. The advent of these unusual infections has led to reappraisal of the diagnostic test involved in the investigation of fungal infections and has had important implications for the choice of treatment. Many of these fungi have a similar tissue appearance, and the organism must be isolated and identified to ensure that the most appropriate treatment is given [3].

The incidence of invasive fungal infections has increased considerably in recent years because of the increase in population having HIV infection [4,5]. Survival of patient from such life-threatening infections depends on early diagnosis, but clinical manifestations of invasive fungal infections are nonspecific and laboratory methods are often unable to diagnose the infections in its early stages [6]. In view of these, the present study sought to isolate, identify, and determine the prevalence rate of pathogenic fungi in HIV-infected patients attending the University of Maiduguri Teaching Hospital, Nigeria.

Patients and methods

Study area

The prospective study was conducted at the HIV/AIDS special clinic of the University of Maiduguri Teaching Hospital, Borno State, Nigeria. Maiduguri, the capital city of Borno State, is situated at the North-East of Nigeria sharing borders with neighboring countries such as Niger Republic, Chad, and Cameroon. It also shares borders with neighboring states such as Adamawa, Yobe, and Gombe states. This city is in the Sahel savannah with high temperature of over 39°C for almost 7 months of the year and very little rainfall.

Procedure for fungal identification

Potassium hydroxide (KOH) wet mount: a clean grease-free glass slide was taken, and a large drop of KOH solution was placed on the slide with a Pasteur pipette. Small quantity of the specimen was transferred with a loop into the KOH drop. A clean coverslip was then placed over the preparation gently to avoid air bubble. The slide was kept in a moist chamber at room temperature for 15 min and then observed under ×10 and ×40 objectives [8].

Lactophenol cotton blue staining (needle mounts preparation): a drop of lactophenol cotton blue stain was placed on a clean grease-free glass slide. A small fragment of cottony, woolly, or powdery colony was picked from the midpoint of the culture using a sterile straight wire and placed on clean glass slides for the staining process. A clean coverslip was applied avoiding air bubbles. Excess stain was removed with blotting paper and the preparation was examined using ×10 and ×40 objectives of the microscope. Microconidia, macroconidia, chlamydospores, and hyphae that appeared spiral, pertinate, and antler-like structures were investigated. Features seen in stained slide were compared with established characteristic fungal features using mycology atlases [7,8].

Indian ink preparation: this stain was used to identify *C. neoformans*. The preparation was made in the center of a clean grease-free glass slide. A drop of the ink was placed on the slide, and a loopful of the specimen was placed close to the drop and mixed well. A coverslip was held vertically such that one edge just touches the fluid on the slide. Keeping that edge in contact with the fluid surface, the coverslip was gently dropped on the fluid so that air bubble was not trapped. Thereafter, immediate microscopic examination followed [7,8].

Germ tube test: this test was used for the identification of *Candida albicans* from other species. Approximately 0.5 ml of sterile human serum was dispensed into a test tube using a sterile wire loop; the serum was lightly inoculated with the test organism. The test tube was then incubated at 37°C for 2–3 h, after which a drop of the serum yeast culture was transferred to a clean grease-free glass slide and covered with a coverslip. It was then examined microscopically using ×10 and ×40 objectives with the diaphragm closed sufficiently to give a good contrast. Sprouting or tube-like outgrowth from the cells indicates that the organism is *C. albicans* [7,8].

Statistical analysis

The results from self-administered questionnaire and mycology results were analyzed using Statistical Package for Social Sciences (SPSS Version 21, IBM California Inc., USA) for χ^2-test. P values of less than 0.05 were considered statistical significant for comparison between demographic variables and prevalence of fungi from HIV-infected patients.

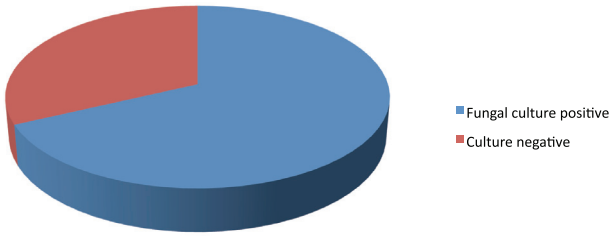
Results

Of the 150 sputum samples analyzed, 102 (68%) were positive for fungal infection (Fig. 1). Of the infected patients, males accounted for 61 (59.8%), whereas females accounted for 41 (40.1%). Consequently, the prevalence of pathogenic fungi was higher in male than in female patients (Table 1). However, there was no significant association between the prevalence of pulmonary fungal infection and sex of patients (P=0.630). The distribution of the pathogenic fungi in the infected population reveals that *C. albicans* [24 (23.5%)] accounted for the highest proportion of the isolates, followed by *Aspergillus* spp. [19 (18.6%)], and the least were *Torulopsis dattila* [one (0.9%)], *Torulopsis glabrata* [one (0.9%)], and *Microsporum canis* [one (0.9%)] (Table 2). The study revealed that in the age group between 31 and 35 years, 28 (27.5%) had pulmonary fungal infection, representing the highest prevalence, whereas in those between 56 and 60 years, one (20.0%) had pulmonary fungal infection, representing the least prevalence (Table 3). There was statistical association between the prevalence of pulmonary fungal infections and age of patients (P=0.033). Overall, 92 (90.2%) persons infected with HIV along with tuberculosis (TB) had pulmonary fungal infection, which represents a relatively higher prevalence compared with those without TB, 10 (20.8%) (P=0.00) (Tables 4 and 5).

Discussion

HIV-infected persons often have weak immune function, hence opportunistic pulmonary fungal infections are expected. This present study reported a higher prevalence of pulmonary fungi at 68% in HIV-infected persons attending UMTH. This is higher to the prevalence reported in Kano and Calabar by Taura *et al.* [9] and Ogbaa *et al.* [10], respectively, and also higher than the prevalence report by Shailaja *et al.* [11], Diaz-Fuentes *et al.* [12] reported a prevalence of 41% in HIV-positive patients on autopsy in New York. The high prevalence rate recorded in the present study could reflect the fact that three consecutive spontaneous sputum samples in the early morning were collected from each patient and processed immediately after collection, so that pathogens were not lost in the process of preservation. It could also be associated with the inadequate medical care

Figure 1



Sputum culture result among the studied population

Table 1 Prevalence of pathogenic fungi by sex of HIV-infected patients

Sex	Number of patients	Patients with fungal infections [*n* (%)]	*P* value
Male	88	61 (69.3)	
Female	62	41 (66.1)	
Total	150	102 (68.0)	0.680

Statistical association as determined by χ^2-test.

Table 2 Frequency of occurrence of pathological fungi among the positive culture samples

Pathogen	*n*=102 [*n* (%)]
Trichosporun cutaneum	3 (2.9)
Candida parapsilosis	6 (5.9)
Candida albicans	24 (23.5)
Cryptococcus neoformans	8 (7.8)
Mucor spp.	10 (9.8)
Rhodotorula rubra	7 (6.9)
Microsporum audouinii	4 (3.9)
Aspergillus spp.	19 (18.6)
Trichophyton verrucosum	4 (3.9)
Penicillium spp.	7 (6.9)
Candida tropicalis	5 (4.9)
Torulopsis candida	2 (1.9)
Torulopsis dattila	1 (0.9)
Torulopsis glabrata	1 (0.9)
Microsporum canis	1 (0.9)
Total	102 (100)

Table 3 Prevalence of pathogenic fungi by age of HIV-infected patients

Age (years)	Infected patients [*n* (%)]	Studied patients (*N*)	*P* value
15–20	9 (60.0)	15	
21–25	16 (72.2)	22	
26–30	28 (80.0)	35	
31–35	15 (83.3)	18	
36–40	20 (66.7)	30	
41–45	10 (66.7)	15	
46–50	2 (28.6)	7	
51–55	1 (33.3)	3	
56–60	1 (20.0)	5	
Total	102 (68)	150	0.026

Statistical association as determined by χ^2-test.

Table 4 Prevalence of pulmonary fungi and tuberculosis coinfection among HIV-infected patients

Clinical presentations	Patients with fungal infection [*n* (%)]	Number of patients	*P* value
Tuberculosis	92 (90.2)	102	
No tuberculosis	10 (20.8)	48	
Total	102 (68)	150	0.000

Statistical association as determined by χ^2-test.

Table 5 Distribution of pathogenic fungi among positive patients with and without tuberculosis

Fungi	Patients with tuberculosis	Patients without tuberculosis
Trichosporon cutaneum	3	1
Candida parapsilosis	5	1
Candida albicans	24	0
Cryptococcus neoformans	7	1
Mucor spp.	10	0
Rhodotorula rubra	6	1
Microsporum audouinii	2	2
Aspergillus spp.	19	0
Trichophyton verrucosum	3	1
Penicillium spp.	6	1
Candida tropicalis	3	2
Torulopsis candida	2	0
Torulopsis dattila	1	0
Torulopsis glabrata	1	0
Microsporum canis	1	0
Total [*n* (%)]	92 (90.2)	10 (9.8)

of HIV-infected persons in Maiduguri owing to sociopolitical unrest the region experiences along with nutritional and hygienic factors.

Candida spp. globally remain the most frequently implicated opportunistic fungal pathogens in HIV/AIDS infection [13], probably because they are endogenous opportunists, and they were also the most prevalent fungal agent in our study, occurring in 16.0% of HIV-positive patients. These results are in agreement with the 11.8% reported by a study in Calabar [10], 19.0% by Aluyi *et al.*[14], and the 12.1% prevalence reported in India [13] but considerably lower than that reported in New York by Diaz-Fuentes *et al*. [12], which is 48%. However, in the New York study, *Candida* spp. was isolated from lung tissue at autopsy; this is a definitive diagnostic technique for pulmonary candidiasis, and it is likely that none of their isolates were lost.

In the present study, no autopsy was carried out if a participant died, so we were not able to confirm what infection was present. *C. albicans* was the species most commonly identified in our study, followed in descending order of prevalence by *Aspergillus* spp., *Mucor* spp., and *C. neoformans*. The variation in the aforementioned finding perhaps reflects variation in the geographical distribution of different *Candida* spp.

Regarding sex of the patients, male (69.3%) patients were found to have relatively higher prevalence of pulmonary fungi than the female (66.1%). When analyzed statistically, the association was found to be nonsignificant, which means that fungal infection does not correspond to sex. This correlates with previous finding [9,15] which reported that sex did not show any independent risk for developing pulmonary biopsies regarding fungal infection.

Pulmonary mycoses occur in all age groups as shown in the study; however, age group 31–35 years was found to have the highest prevalence (83.1%), whereas age group 56–60 years had the lowest (20.0%). When analyzed statistically, age distribution was found to be significant ($P<0.05$), which indicate that it is age related. This agrees with the findings of Aluyi *et al*. [14] in the analysis of pulmonary mycoses in patients with AIDS according to age groups in which they found out that nine species of fungi were isolated within ages 21–30 and eight organisms from 40–50 age groups and three organisms isolated from below 20 and above 50 age brackets (21–45 years). Possible reason for high prevalence could be that young adults mostly engage in outdoor activities where they could have been exposed to the airborne form of these fungi. The lower prevalence among ages between 51 and 60 years could be because very few individuals participated in this study.

Findings from this study revealed that patients with HIV/TB coinfections had relatively higher and significant prevalence of pulmonary fungal infections. In the present study, we reported a 90.2% prevalence of opportunistic fungal infections in HIV/TB coinfected patients. This value is higher than those reported by Taura *et al.* [9] and Yadu *et al.* [16]. Apart from the ideal opportunistic relationship between fungus and TB infections of the lungs, HIV infection also provides ideal immunosuppression that allows these fungi to thrive favorably [16].

Conclusion: Findings from this study revealed a high prevalence of pulmonary fungi HIV/TB coinfection, with *C. albicans* being the leading causal fungi responsible for symptoms of pulmonary diseases, followed by saprophytic and airborne fungi. A more detailed longitudinal (cohort) study may help formalize the use of $CD4^+$ T cell count as an indicator of HIV/AIDS with opportunistic mycoses and form an appropriate point for prophylactic and therapeutic measures in a resource-limited setting.

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Conflicts of interest

There are no conflicts of interest.

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