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# PREVALENCE AND PROFILE OF PULMONARY FUNGAL PATHOGENS AMONG HIV-INFECTED PATIENTS ATTENDING ALERT HOSPITAL, ADDIS ABABA ETHIOPIA

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## ABSTRACT

**Background:** Many fungi cause pulmonary disease in HIV-infected patients. Pulmonary infections due to mycosis remain a major cause of both morbidity and mortality among HIV patients. HIV infection reduces the number and functionality of CD4 helper lymphocytes that direct and coordinate acquired immunity against most pathogens. The decrease in CD4+ T lymphocyte count leads to various opportunistic infections (OI) in HIV infected persons. Identification of the fungal species is important, as it helps in the initiation of specific antifungal therapy which improves the outcome of treatment. Systemic fungal infections are more frequent and sever in patients with HIV infection. **Objective:** The aim of this study was to determine the prevalence of pulmonary fungal pathogens and their association with CD4+ lymphocyte count among HIV-infected patients. **Method**: A prospective cross sectional study was conduct at ALERT hospital from September, 2021 to April, 2022. Consented participants' information was collected using structured questionnaire. A total of 385 sputum and blood samples from HIV infected patients were collected aseptically by convenient sampling method and the sputum samples were inoculated into the sputum samples into SDA (Sabrouad's Dextrose Agar). Fungal culture identification was performed by studying the macroscopic and microscopic characteristics of their colonies on SDA medium. Also Germ tube production test, CHROM agar test, Indian ink test and Lacto phenol cotton blue stain were used for further identification of fungal isolates and CD4+ T lymphocyte count were determined using BD FACSPristo machine. The data were entered and analyzed using SPSS software version 20. Descriptive statistics and logistic regressions were used and P-value < 0.05 was considered as significant. **Result:** The prevalence of pulmonary fungal pathogens among HIV infected patient was 69.6% and nine different pulmonary fungal species were isolated from 268 cultures positive isolates. *Candida Albicans* and *Aspergillus niger* are among the predominant pulmonary fungal species and *Cryptococcus species* and *Mucor specie* showed the least. The highest pulmonary fungal isolates growth rate were observed from patients that have CD4+ T lymphocyte counts <200 cells/ml. **Conclusion**: The findings in this study reveal a high prevalence of pulmonary mycosis. It is higher among HIV infected patients with their immune status indicated by CD4+ cell counts <350 cell/ml. Pulmonary fungal infections in AIDS patients are a common and unrecognized problem. Early diagnosis is essential for effective management of the patients.

**KEYWORDS:** CD4 status, fungal etiologies, HIV infection.

## INTRODUCTION

Mycosis is a disease of humans and animals caused by fungus that invades the tissues, causing superficial, cutaneous, subcutaneous, and systemic disease.[1] Pulmonary mycosis is a systemic fungal infection that occurs when fungi cause diseases of the lungs through direct infection of the pulmonary tissues, by invading pulmonary airspaces or lung cavities, through their ability to trigger an immunological reaction when fungal material is inhaled.[2]

Opportunistic pulmonary mycoses comprise a large group of fungal diseases, the etiologic agents of which are usually potential pathogens in the immune-compromised patients like HIV infected patients.[3] In HIV infected patients, opportunistic and pathogenic fungi, such as *Candid*a species, *Aspergillus* species, *Penicillium marneffei*, *Cryptococcus neoformans*, *Penicillium marneffei*, *Pseudosporium apiospermum*, *Histoplasma capsulatum*, and *Coccidioides immitis*, 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.[4]

Diagnosis of pulmonary fungal infection can be difficult because the signs and symptoms of disease can be nonspecific. Pulmonary fungal infected individuals present with Persistent cough for more than three weeks is similar with a common symptom of pulmonary disorders caused by a wide range of pathogens, including Fungi, Bacteria, and even viruses. Although active pulmonary mycoses may denote advanced immune suppression from HIV infected patients.[5]

Acquired immunodeficiency syndrome (AIDS) is the late stage of *human immunodeficiency virus* (HIV) infection that occurs when the body's immune system is badly damaged because of the virus. HIV infection reduces the number and functionality of CD4 helper lymphocytes that direct and coordinate acquired immunity against most pathogens. Individuals with AIDS are vulnerable to opportunistic infections and common infections. Pulmonary Fungal infections are common causes of mortality and morbidity in patients diagnosed with HIV infection with account for up to 70% of illness in AIDS cases. The range of illness varies from asymptomatic mucosal candidiasis to overwhelming disseminated infections.[6]

The Objective of the present report is to indicate the Prevalence of pulmonary fungal pathogens and their association with CD4+ T Lymphocyte Count among HIV-infected patients attending ALERT Hospital, Addis Ababa, Ethiopia.

## MATERIAL AND METHODS

### Study Population

During the period of September, 2021 to April, 2022, a total of 385 HIV infected Patients attending ART clinic follow up were selected. Study participants were informed about the study and the objective of the study were thoroughly explained to them and were asked for their volunteer participation. Then, written informed consent was obtained from volunteer study participants. Questionnaire was also filled by experienced laboratory technologists to collect socio-demographic and other relevant information.

### Sample Size Calculation

The sample size for this study was determined using a single population proportion formula. In Ethiopia there are no published studies on prevalence of pulmonary mycosis among HIV patients. Assuming prevalence of pulmonary mycosis among HIV patients is 50%, 95% CI and 5% margin of error. Therefore the sample size was given as follows:

$$n=\frac{Z^2\times P(1-P)}{d^2}=\frac{1.96^2 \times 0.5(1-0.5)}{0.05^2}=384.16\approx 385$$

Where: n = the sample size, (Zα/2).2 = at 95% confidence interval, Z value (α = 0.05). = 1.96
P = the proportion of occurrence of pulmonary mycosis 50% (0.5), and d = margin of error at 5% (0.05).

### Sputum collection

Patients were instructed to wash their mouth gently with tap water prepared for this purpose more than once and then collect purulent sputum by sterile falcon tube by breathing deeply three times. Sample collection was carried out under the supervision of a qualified medical laboratory technologist. Sputum samples were collected for mycological investigation with the assistance of experienced medical laboratory scientists and venous blood were also collect for CD4 Lymphocyte T cell count estimation

### Sample transportation

Sputum sample for mycological investigation was transported to mycology laboratory of ALERT(All African Leprosy, TB Rehabilitation and Training Center) Hospital within 2 hours of collection by following the appropriate sputum sample transportation method**.** The blood samples for CD4 Lymphocyte T cell count were transported to ALERT hospital laboratory within 1 hour.

### Fungal Isolation and characterization

Unprocessed sputum was inoculated directly onto Saborad Dextrose Agar tubes supplemented with Chloramphenicol or Gentamiycine under safety cabinet level II at ALERT Hospital laboratory. The tube inoculated at $37^0$C aerobically for up to four weeks. Culture plates were examined every two days for any fungal growth.

### Mold Identification

Mycelia fungi were identified by studying their microscopic and macroscopic characteristics. Pigmentation of the front and the reverse side, texture, topography, and rate of growth of each culture were

considered for macroscopic identification. Diagnostic microscopic features of mycelial fungi were determined by using a lacto phenol cotton blue staining procedure. Briefly, a drop of Lactophenol cotton blue (LPCB) stain was placed on a clean glass slide. A piece of fungal culture growth was placed on clean glass slides containing LPCB for the staining process. A stained preparation were then be covered by a cover slide and examined for microscopic characteristics such as macro and micro-conidia, chlamydo spores, the morphology of reproductive structures, and the nature of hyphae by using 10X and 40X objectives of the microscope. Features seen in the stained slide were compared with established characteristics of fungal features using mycology atlases.[7,8]

**Yeasts Identification**

Yeasts were identified by employing germ tube production test and using CHROM agar Candida culture medium (Becton Dickinson) as per the instruction of the manufacturer.

**CD4 count determination using FACS Pristo**

CD4 measurement is performed using photomicroscopy and multicolor fluorescence. Two LEDs are used as the excitation sources, one green and one orange. Whole blood stained with CD3 APC, CD4 PE-Cy™5, CD14 PE, and CD45RA APC. CD45 is used to identify the total population of all lymphocytes; while CD3 is used to identify the total population of T lymphocytes. Monocytes may also express significant amounts of the CD4 antigen. They are isolated via a CD14 antibody conjugate. This enables a count of all CD4 positive cells and then subtracts the monocytes to yield only CD4 positive T lymphocytes.[9]

**RESULTS**

A prospective cross sectional study was performed on Prevalence of pulmonary fungal pathogens among HIV-infected patients attending ALERT Hospital, Addis Ababa Ethiopia from September, 2021 to April, 2022.

A total of 385 study participants were included in this study. Socio-demographic data is given on Table1.The study participants were in the age range of 12-84 years. Relatively the majority of participants 112 (29.1%) were in the age group 41 to 50 years, followed by 31 to 240 years 100(26.0%) and age greater than 60 years accounted the least 27(7.0%). Of the participants, male were 175(45.5%), whereas female participants were accounted for 210(54.5%). The marital status among the study participants were married 219(56.9%), followed by single 87(22.6%), widow 67(17.4) and separated 12(3.1%). Among 385 participants were their educational status 136(35.3) were high school, 120(31.2) were elementary, 68 (17.7) were illiterate and 61 (15.8) were above college.

Out of 385 participants in the study 268(69.6%) showed fungal growth while the remaining 117(30.4%) samples yielded no growth after four weeks of incubation. Of the 69.6% pulmonary fungal isolates; 127/268 (47.4%) isolated from males and 141/268 (52.6%) from females. Table 2 shows frequency of single pulmonary fungi isolate from study participants. 225/268(83.9%) yielded single fungal isolates which had a growth of only one fungal species. Table 3 shows the various fungal isolates, among them 144 (53.7%) were yeast, 100 (37.3%) were mold (mycelia) and the other 24(9.0%) were mixed (which contain growth of both mold and yeast) fungal isolates.

Nine different pulmonary fungal species were isolated from 268 culture positive isolates, within individual study participant sputum sample, while 43(16.1%) identified with mixed isolates, in this case which had growth of two pulmonary fungal species found within individual study participant sputum sample.

The dominant pulmonary fungal isolates that observed from single fungal isolate were *Candida albicans* 84 (37.3%), followed by *Aspergillus niger* 51 (22.7%), and the least were *Cryptococcus species 6(2.7%)* and *Mucor species* 5 (2.2%).

**Table 1: Prevalence by Socio demographic and Clinical variable of study participant and their associated with growth of fungal culture isolates using Multivariate analysis, at ALERT Hospital, Addis Ababa, Ethiopia, 2022 (n=385)**

| Characteristics | | No of participant | No of fungal isolates, *n* (%) | AOR(95% CI) | p-value |
|---|---|---|---|---|---|
| Sex | Male | 176 | 127(72.6%) | 0.87(0.53, 1.44) | 0.591 |
| | Female | 209 | 141(67.5%) | Reference | |
| | Total | 385 | 268(**69.6%**) | | |
| Age | 10-20 | 28 | 19(67.8%) | 0.77(0.19, 3.18) | 0.72 |
| | 20-30 | 40 | 32(80.0%) | 0.53(0.14, 2.01) | 0.35 |
| | 31-40 | 100 | 73(73.0%) | 0.69(0.25, 1.88) | 0.47 |
| | 41-50 | 112 | 80(71.4%) | 0.71(0.28, 1.84) | 0.48 |
| | 51-60 | 78 | 47(60.2%) | 1.29(0.49, 3.39) | 0.61 |
| | >60 | 27 | 17(63%) | Reference | |
| | Total | 385 | 268(**69.6**%) | | |
| Marital status | married | 219 | 162(74.0%) | 0.29(0.08, 0.99) | 0.048 |
| | single | 87 | 61(70.1%) | 0.46(0.11, 1.89) | 0.282 |

| | | | | | |
|---|---|---|---|---|---|
| | widow | 67 | 40(59.7%) | 0.53(0.14, 1.98) | 0.346 |
| | separated | 12 | 5(41.7%) | Reference | |
| | Total | 385 | 268(**69.6**%) | | |
| Level of education | Illiterate | 68 | 51(75%) | 0.93(0.68, 1.89) | 0.583 |
| | Elementary | 120 | 80(66.7%) | 0.74(0.44, 1.50) | 0.298 |
| | High school | 136 | 93(68.4%) | 0.45(0.17, 1.02) | 0.337 |
| | College and above | 61 | 43(70.5%) | Reference | |
| | Total | 385 | 268(**69.6**%) | | |
| CD4 count level | 0-50 | 8 | 8(100.0%) | 0.99(0.61, 2.10) | 0.000 |
| | 51-100 | 19 | 17(89.4%) | 0.26(0.07, 0.94) | 0.040 |
| | 101-200 | 37 | 34(91.9%) | 0.05(0.01, 0.35) | 0.003 |
| | 201-350 | 72 | 59(81.9%) | 0.33(0.17, 0.66) | 0.002 |
| | 351-500 | 60 | 36(60.0%) | 0.98(0.53, 1.80) | 0.944 |
| | >500 | 189 | 114(60.3%) | Reference | |
| | Total | 385 | **268(69.6%)** | | |

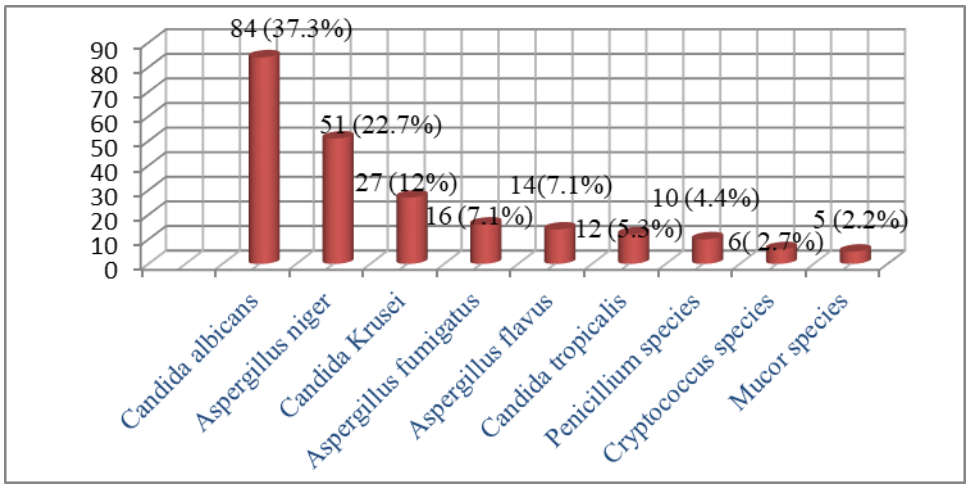


**Figure 2: Frequency of single pulmonary fungi isolate from study participants, at ALERT Hospital, Addis Ababa, Ethiopia, 2022.**

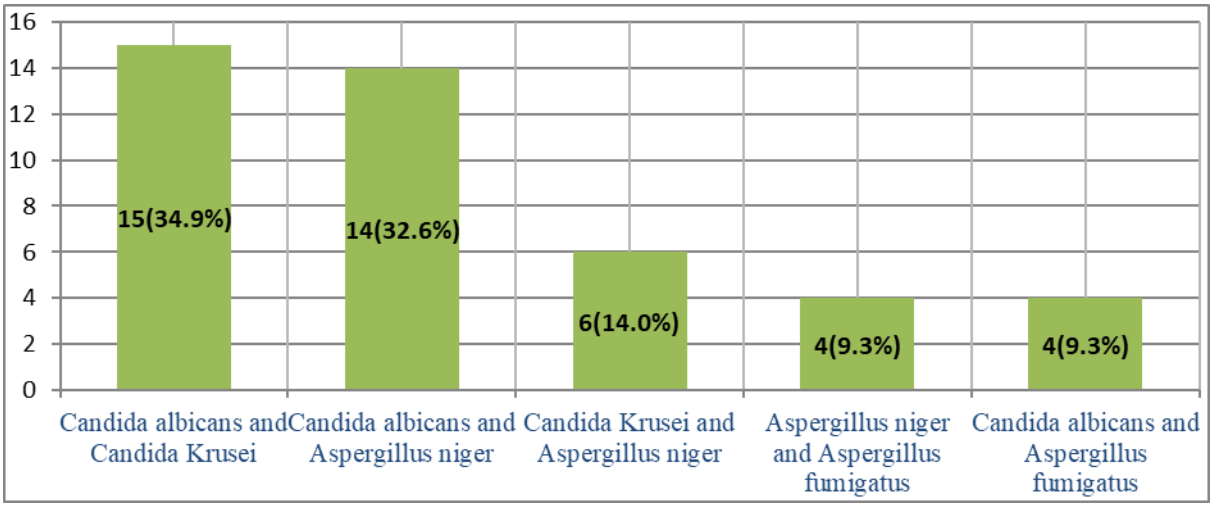


**Figure 3: frequency of mixed pulmonary pathogenic fungi isolate from study participants, at ALERT Hospital, Addis Ababa, Ethiopia, 2022.**

**Table 2: Distribution of pulmonary fungal isolate in relation with CD4+ T lymphocyte count from study participants, at ALERT Hospital, Addis Ababa, Ethiopia, 2022 (n=268)**

| Fungal isolates | N | CD4+ T lymphocyte count levels | | | | | |
|---|---|---|---|---|---|---|---|
| | | <50 | 51-100 | 101-200 | 201-350 | 351-500 | >500 |
| | | 30±14.5 | 82.7±13.5 | 158.1±28.5 | 269.9±43.4 | 434±45.6 | 771.4±229.5 |
| *Candida albicans* | 84 | 4(4.8%) | 4(4.8%) | 13(15.5%) | 17(20.2%) | 9(10.7%) | 37(44.0%) |
| *Candida Krusei* | 27 | 0(0.0%) | 1(3.7%) | 4(14.8%) | 3(11.1%) | 6(22.2%) | 13(48.1%) |
| *Candida tropicalis* | 12 | 0(0.0%) | 0(0.0%) | 1(8.3%) | 3(25.0%) | 0(0.0%) | 8(66.7%) |
| *Cryptococcus species* | 6 | 1(16.7%) | 1(16.7%) | 3(50.0%) | 1(16.7%) | 0(0.0%) | 0(0.0%) |
| *Aspergillus niger* | 51 | 1(2.0%) | 2(3.9%) | 1(2.0%) | 15(29.4%) | 8(15.7%) | 24(47.1%) |
| *Aspergillus flavus* | 14 | 0(0.0%) | 0(0.0%) | 1(7.1%) | 3(21.4%) | 4(28.6%) | 6(42.9%) |
| *Aspergillus fumigatus* | 16 | 0(0.0%) | 1(6.2%) | 2(12.5%) | 4(25.0%) | 2(12.5%) | 7(43.8%) |
| *Penicillium species* | 10 | 0(0.0%) | 1(10.0%) | 0(0.0%) | 5(50.0%) | 2(20.0%) | 2(20.0%) |
| *Mucor species* | 5 | 0(0.0%) | 0(0.0%) | 1(20.0%) | 0(0.0%) | 1(20.0%) | 3(60.0%) |
| *Aspergillus niger and Aspergillus fumigatus* | 4 | 1(25.0%) | 1(25.0%) | 0(0.0%) | 0(0.0%) | 0(0.0%) | 2(50.0%) |
| *Candida albicans and Aspergillus fumigatus* | 4 | 1(25.0%) | 1(25.0%) | 1(25.0%) | 0(0.0%) | 0(0.0%) | 1(25.0%) |
| *Candida Krusei and Aspergillus niger* | 6 | 0(0.0%) | 1(16.7%) | 2(33.3%) | 0(0.0%) | 1(16.7%) | 2(33.3%) |
| *Candida albicans and Aspergillus niger* | 14 | 0(0.0%) | 4(28.6%) | 3(21.4%) | 5(35.7%) | 1(7.1%) | 1(7.1%) |
| *Candida albicans and Candida Krusei* | 15 | 0(0.0%) | 0(0.0%) | 3(20.0%) | 3(20.0%) | 2(13.3%) | 7(46.7% |

## DISCUSSION

The result of this study showed that the prevalence of pulmonary fungal infection was (69.6%). This indicated that HIV infected patients were vulnerable to pulmonary fungal infection. The current study finding was similar to the prevalence reported from Nigeria (68%) by Talle M, et al. (2) and from Uganda (71.3%) by Njovu IK et al.[10] In Contrary, to the present finding lower prevalence (41%) was observed from HIV-positive patients on autopsy in New York by Diaz-Fuentes et al.[11] The high prevalence rate recorded in the present study could be associated with the inadequate medical care of HIV-infected patients along with nutritional and hygienic factors. On the other hand, highest prevalence (86.1%) rate was reported from India by Chandwani et al.[6] The reason could be due to difference in health status of the study participants.

In our study the prevalence of pulmonary fungal infection by sex group of HIV-infected patients were 67.5% for female and 72.6% for males. Other study has been reported similar finding in Nigeria by Talle M, et al.[12] 66.1% for female and 69.3% for males which indicate that males are more prone to pulmonary pathogenic fungi infection than females. This also agrees with another finding reported from Nigeria by Sani FM et al[13] where (75.4%) of male study patients and (42%) females study participants were infected with pulmonary fungi infection.[13] This might be attributed to more exposure of males to external environment than females.

This study also showed that pulmonary fungal infection on the basis of marital status; married participants had higher prevalence of fungal infection 162(74.0%) and the separated being the lowest 5(41.7%). This finding was aligned with study conducted in kano by Taura D.W et al married (50.45%), and separated were (4.5%).[2] The relationship for married group were found to be statistically significant ($p<0.05$). Those study participants who had a married were 29% more likely to be infected with pulmonary fungal infection compared to those separated marital status (AOR (95% CL) 0.29(0.08, 0.99).

In the present study, many species of yeasts and mycelia fungi were recovered from sputum of HIV patients as single and mixed isolates. At the species level *Candida albicans* were the most prevalent pulmonary pathogenic fungi species (n=84) identified from single fungal isolates, and also *Candida albicans* mixed with *Candida Krusei (*n=15), *Candida albicans* mixed with *Aspergillus niger (*n=14) and *Candida albicans* mixed with *Aspergillus fumigates (*n=4) identified from mixed isolates. Hence the overall frequency of *Candida albicans* identified from HIV infected patient were 117/268(43.6%) and from *Candida* species 117/177(66.6%). This finding was in agreement with the findings of Njunda AL et al in Cameron.[14] However, this prevalence was lower than a study conducted in Northwest Ethiopia by Mulu etal (81%)[15] and study conducted in central Ethiopia by Bitew A etal (80%).[16] This could be due to difference in the study subjects and anatomical Sample collection site. Many studies have also reported that *Candida* species are the most frequent fungal species recovered from the sputum of HIV infected patients that could be causing severe disease.[17,18]

In our study the second predominant pulmonary pathogenic fungi isolates were *Aspergillus niger* 69/268(25.7%) that identified from single isolates (n=51) and from mixed fungal isolates; *Aspergillus niger* mixed with *Candida albicans (*n=14) and *Aspergillus niger* mixed with *Aspergillus fumigates* (n=4). This indicates that *Aspergillus niger* were the most common species isolated among mold fungi. The study finding was consistent with similar studies conducted in India by Chandwani etal (27.7%)[6] and another study by Kaur etal.[19] In contrary, another study in Nigeria by Nasir etal reported that among *aspergilus species; Aspergillus fumigates* accounted for highest isolates, followed by *Aspergillus niger..*,[2] These variations could be due to variations in geographical locations and ecological niches. *Aspergillus* species are ubiquitous molds found in organic matter.[20] The fungal spores are transmitted to the human via inhalation and primarily affect the lungs. In lung Alveolar macrophages are the first line of defiance against inhaled *Aspergillus* conidia and commonly found in immune compromised cases like in HIV infection.[20] Since the findings of this study correlate significantly with many other report stated, *Aspergillus* species could be said to be the second most prevalent species in HIV patients. This could due to presence of spores of *Aspergillus* species in the environment, their ability to grow in abundance everywhere, production of small conidia that easily penetrate deep in the alveoli region and its ability grow at 37$^{o}$C and the possibility of ingesting contaminated grains.[21]

*Cryptococcus species (2.2%), Penicillium species (3.7%) and Mucor species (1.9%)* were among the less common isolates in this study. The finding for *Cryptococcus species (2.2%)* and *Mucor species (1.9%)* agreed with study conducted by Chandwani etal in India of *(3.2%) and (1.6%)* respectively.[6] However, slightly lower compared with *Penicillium species* (7.7%) by Chandwani etal[6] and *(9.7*% %) for Cryptococcus *species by* Aluyi etal in Nigeria.[22] Soils and bird droppings predispose immune-suppressed patients to Cryptococcosis, and this could be the basis for the observed prevalence.

## CONCLUSION AND RECOMMENDATION

We reported 69.6% prevalence of pulmonary fungal pathogens and nine different pulmonary fungal species were isolated from 268 cultures positive isolates. *Candida Albicans* and Aspergillus niger are among the predominant pulmonary fungal species and the least were *Cryptococcus species* and *Mucor specie.* The findings in this study reveal a high prevalence of pulmonary mycosis. It is more among those with deteriorating immune status indicated by CD4+ cell counts below350 cell/ml. Pulmonary fungal infections in AIDS patients are a common and unrecognized problem. Early diagnosis and producing updated information on local fungal pathogens has necessary for treatment and effective management of the patients.

The current study has indicated the prevalence of fungal infection in HIV infected patients, so we recommend fungal screening in order to get better treatment outcome in HIV infected patients. Furthermore, as our study provides only baseline information in terms of indicating the great possibility of fungal infection among HIV infected patients, we recommend further study to be done.

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