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Profiling of potential pulmonary fungal pathogens and the prevalence of the association between pulmonary tuberculosis and potential fungal pathogens in presumptive tuberculosis patients referred to Saint Peter's Specialized Tuberculosis Referral Hospital, Addis Ababa, Ethiopia

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Abstract

Objective: The burden of pulmonary fungal infection is increasing and often misdiagnosed as pulmonary tuberculosis in developing countries where the prevalence of pulmonary tuberculosis is high. |Therefore, the purpose of this study is to determine the spectrum of potential pulmonary fungal pathogens and the prevalence of the association between pulmonary tuberculosis and potential fungal pathogens.

Materials and methods: A cross-sectional study was conducted between October 2018 and May 2019. Sputum was collected from 636 study participants. Part of the sputum was inoculated onto Brain Heart Infusion agar, and fungi were identified following standard microbiological procedures. The remaining part of the sample was used for the investigation of pulmonary tuberculosis.

Results: Among 636 sputum samples, 75.9% (483) and 25.6% (163) were positive for potential fungal pathogens and pulmonary tuberculosis, respectively. The prevalence of the association between pulmonary tuberculosis and potential fungal pathogens was 20.0%. Of fungal isolates, 81.4% were yeasts. The remaining 128 (18.6%) isolates were molds. The isolation rate of fungi was higher in males (51.6%) than in females (48.4%). There was no statistically significant association between the prevalence of potential pulmonary fungal pathogens and sex ($p = 0.239$). Patients in the age group of 35 to 44 and above were slightly more affected than younger age groups. The association between potential fungal pathogens and age was not statistically significant ($p = 0.50$).

Conclusion: High prevalence of potential pulmonary fungal pathogens and the association of tuberculosis and potential fungal pathogens recorded in this study will enforce health personnel to pay due attention to these conditions and arise the interest of researchers to conduct further work on the burden of the association between tuberculosis and potential fungal pathogens. Our study also revealed the need to employ conventional microbiology tests along with clinical and radiological evidence since clinical manifestations and radiological pictures of tuberculosis mimic that of pulmonary fungal infection.

Keywords

Fungal pathogens, pulmonary tuberculosis, respiratory diseases

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Introduction

Although the true burden of fungal respiratory tract infection is elusive, the frequency of the infection has been increasing in the last few decades.[1] Pulmonary tuberculosis (PTB), HIV/AIDS, chronic obstructive pulmonary disease, and extensive use of immunosuppressive drugs are incriminated for such an increase.[2] Globally, it is predicted that 1.2 million individuals live with chronic pulmonary aspergillosis (CPA) as a sequel to PTB, in which the prevalence of CPA is the highest in Africa, Western Pacific, and Southeast Asia.[3] The chronic nature of PTB along with prolonged chemotherapy with or without corticosteroids resulted in immune suppression in PTB patients eventually leading PTB patients susceptible to fungal infection.[4]

Pulmonary fungal infection is an infectious disease of the lungs that is caused by fungi. The infection develops after the colonization of the lungs by fungi or their spores through inhalation, or the reactivation of latent infection, or via hematogenous dissemination. Fungi or their spores are abundant and exist virtually everywhere in the human environment.[5,6] Given the ubiquitous nature of fungi and their spores, colonization or infection of the lungs is unavoidable, but mechanisms of differentiating fungal colonization from fungal infection are not well established, and hence the subject remains a serious challenge. In this article, isolation of fungi alone or in association with *Mycobacterium tuberculosis* from presumptive tuberculosis (TB) patients (i.e. patients presenting signs and symptoms of PTB and having radiological pictures characteristics of PTB) was considered as potential fungal infection. Many fungal species have been reported as etiological agents of lung infection. In most literature, species of *Aspergillus*,[7] *Candida*, *Cryptococcus*,[8] *Pneumocystis*,[9] and thermally dimorphic fungi[10] are the most significant. However, the epidemiology of fungi causing lung infection has been changing. Accordingly, many mycelial fungi, such as *Scedosporium* spp., *Fusarium* spp., *Penicillium* spp., dematiaceous filamentous fungi, zygomycetes, and yeasts other than *Candida albicans*, have emerged as etiological agents of respiratory disorders. While these fungi are rarely recovered in the respiratory tract of immune-competent individuals, they may disseminate to other systemic organs producing life-threatening invasive fungal diseases in individuals already experiencing serious illness.[11]

PTB is principally a disease of poverty, with 95% of cases and 98% of deaths occurring in developing countries.[12] Ethiopia stands 10th among the 30 high TB burden countries with an estimated incidence rate of 151/100,000.[13] The high rate of co-infection of pulmonary fungal infection with PTB further compounded the burden of PTB in these countries as the association of the two infections is responsible for a high rate of morbidity and mortality.[3] Therefore, proper diagnosis of fungal pathogen especially in PTB patients is critical.[14] To this end, the spectrum of pulmonary fungal infections and their association with PTB in Ethiopia is lacking.

Furthermore, similarities in clinical and radiological presentation of pulmonary fungal infection and PTB have made definite diagnosis between these two infections difficult. Persistent cough for more than 3 weeks is a common symptom of pulmonary disorders caused by *M. tuberculosis* and fungal pathogens. Lack of definite diagnosis between PTB and fungal lung infection may lead to empirical treatment in which fungal infections are treated with anti-TB chemotherapy with poor clinical outcomes, as anti-TB drugs do not affect fungal pathogens.[11] As in most developing countries, treatment of PTB patients in Ethiopia is empirical. Because of this, determining the spectrum of potential fungal pathogens from the sputum of patients presenting clinical and radiological characteristics similar to PTB and the magnitude of the association between TB and potential fungal pathogens is an active field of research. Therefore, the objectives of the present study were determining the distribution of potential fungal pathogens associated with the lower respiratory tract (lung) infection and the prevalence of the association between TB and potential fungal pathogens in presumptive PTB patients referred to the Saint Peter's Specialized Tuberculosis Referral Hospital.

Methods

Study area and design

The present study is a hospital-based, cross-sectional study conducted at the Saint Peter's Specialized Tuberculosis Referral Hospital located in Addis Ababa Administrative region, the capital city of Ethiopia between October 2018 and May 2019.

Population

Source of population. The source of population is those people seeking health service at Saint Peter's Specilized Tuberculosis Referral Hospital and those referred from other health institutes.

Study population. The population of this study is those patients who were assessed clinically and by radiological procedures by attending physicians in the hospital.

Inclusion and exclusion criteria. Patients with clinical manifestation of pulmonary infection (particularly of those with a persistent cough for more than 3 weeks and with radiological characteristics mimicking that of PTB) were included in the study. Healthy individuals with no clinical symptoms, no radiological picture mimicking of PTB, and those who were under antifungal treatment were excluded from the study.

Sample size determination. The minimum sample size of this study was determined based on a single population proportion formula, $n = Z^2P(1-P)/d^2n$, where n = sample size,

z=95% statistic for level of confidence (1.96), P=population proportion (42.3% or 0.423), and d=margin of error (degree of accuracy desired (d=0.05), using previous prevalence of 42.3%[15] pulmonary fungal infection). The sample size was estimated to be $(1.96)2 \times 0.423(1-0.423)/(0.05)2=375$. Therefore, by adding 10% contingency, the minimum number of study participants was 413. However, the sample size of study participants was increased to 636.

Sampling method. A convenient sampling method was utilized to achieve the estimated sample size. All PTB presumptive patients visit Department of Microbiology Laboratory of Saint Peter's Specialized Tuberculosis Referral Hospital within the specified time of the study.

Data collection procedure

Demographic data. The sociodemographic data—age, gender, and clinical symptoms—were obtained from a standard laboratory request form completed by physicians.

Data quality assurance quality control. The quality of sputum samples, GeneXpert kit and reagents, and mycological culture media were inspected before they are used. The sterility of culture media and the growth performance of each culture medium were evaluated as per standard procedures.

Statistical analysis. All data from the investigation were coded, double entered, and analyzed using SPSS version 20. Descriptive statistics and logistical regressions were used to estimate the crude ratio with a 95% confidence interval to the different variables. The p value <0.05 was considered significant.

Laboratory investigation

Sputum collection. Patients were instructed to wash their mouth gently with tap water prepared for this purpose more than once and then purulent sputum was collected by sterile falcon tube by breathing deeply three times. Part of the early morning sputum was used for the detection of *M. tuberculosis* while about 0.5 m of the remaining sample was used for fungal culture. Sample collection was carried out under the supervision of a qualified medical laboratory technologist.

Fungal isolation and characterization. Unprocessed sputum was inoculated directly onto duplicate Brain Heart Infusion agar tubes supplemented with chloramphenicol (Oxoid, Basingstoke, UK) under safety cabinet level II at Saint Peter's Specialized Tuberculosis Referral Hospital. All inoculated tubes were then transported to the Department of Medical Laboratory Science, College of Health Science, Addis Ababa University. One of the tubes was incubated at 25°C while the other one was incubated at 37°C aerobically for up to 4 weeks. Culture plates were examined twice a week for any fungal growth.

Identification

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 Lactophenol Cotton Blue (LPCB) staining procedure. Briefly, a drop of LPCB stain was placed on a clean glass slide. A piece of fungal culture was placed on clean glass slides containing LPCB for the staining process. A stained preparation was then covered by a cover slide and examined for microscopic characteristics such as macro- and micro-conidia, chlamydospores, the morphology of reproductive structures, and the nature of hyphae by using 10× and 40× objectives of the microscope. Features seen in the stained slide were compared with established characteristics of fungal features using mycology atlases.[16,17]

Yeast identification. Yeasts were identified by employing an array of standard biochemical and assimilation test procedures,[18] germ tube production, and using CHROMagar Candida culture medium (Becton Dickinson, Paris, France) as per the instruction of the manufacturer.

Detection of M. tuberculosis. Detection of *M. tuberculosis* from sputum specimens was determined by using the GeneXpert Mycobacterium tuberculosis (MTB)/Resistance to refampicin (RIF) assay machine (Cepheid, Sunnyvale, CA, USA). Briefly, 4 mL of sputum was mixed with 8 mL of sample reagent, vortexed for 15 s, and allowed to stand for 10 min at room temperature. The preparation was vortexed again and allowed to stand for another 5 min. Then 2 mL of the processed sample was transferred into a multichambered plastic Xpert MTB/RIF cartridge using a Pasteur pipette provided with the kit. Then the cartridge with the specimen was loaded into the GeneXpert machine, and an automatic process completes the remaining assay steps. After 2 h, results were collected from the GeneXpert computer.

Results

Gender, age, and clinical symptoms of study participants

As shown in Table 1, the current study enrolled 636 study participants of which 327 (51.4%) were males and 309 (48.6%) were females. The ages of the study participants varied from 1 to 94 years with a mean age of 41 years. Table 1 also shows the percentage of patients with each symptom. The most common presenting symptom was cough (97.95%), followed by expectoration (78.45%) and weight loss (43.86%).

Distribution of fungi with gender and age

As shown in Table 2, out of 636 sputum samples collected and analyzed, fungal species were recovered from 483

Table 1. Demographic profile and clinical symptoms of study participants.

Variables	*n*	%
Gender		
Male	327	51.4
Female	309	48.6
Age groups in years		
1–14	31	4.87
15–24	85	13.36
25–34	125	19.65
35–44	133	20.91
45–54	82	12.89
55–64	93	14.62
>65	87	13.67
Clinical symptoms		
Cough	623	97.95
Expectoration	499	78.45
Weight loss	279	43.86
Fever	270	42.45
Shortness of breathing	237	37.26
Anorexia	221	34.74
Chest pain	178	27.98
Fatigue	100	15.72
Night sweats	94	14.77

samples. Among 483 culture-positive sputum samples, 51.6% (249/483) and 48.4% (234/483) were collected from male and female study participants, respectively. Consequently, the isolation rate of fungi was higher in male than in female study participants. However, there was no statistically significant association between the prevalence of pulmonary fungal isolation rate and the sex of patients ($p=0.239$). The distribution of fungal isolates varies by age. In general, the percentage positivity rate per age group depicted that patients in the age group of 35 to 44 and above were slightly more affected than younger age groups. The association of fungal isolation rate and age was not statistically significant ($p=0.50$)

The spectrum of fungal isolates

In the present study, of a total of 636 sputum cultures, 483 were positive for potential fungal pathogens out of which 180 samples yielded more than one fungal species. A total of 690 fungal isolates were recovered. Among the isolates, 562 (81.4%) were yeasts comprising of *C. albicans* (260; 46.3%), non albican candida (*NAC*) species (296; 52.7%), and *Cryptococcus neoformans* (6; 1.0%). At the species level, however, *C. albicans* was the most prevalent species accounting for 46.2% of yeast isolates. The remaining 128 (18.6%) isolates were mycelial fungi, where *Aspergillus* spp. (79; 61.7%), *Penicillium* spp. (16; 12.5%), *Scedosporium apiospermum* (13; 10.2%), *Fusarium* spp. (10;7.8%), and *Mucor* spp. (5; 3.9%) being the major isolates with regard to mold isolates (Table 3).

TB and fungal co-infection

Among 636 study participants, 75.9% (483/636) were positive for potential fungal pathogens, and 25.6% (163/636) were positive for PTB. The association between PTB and potential fungal pathogens among the study participants (636) was 20% (127/636). With regard to PTB patients, the association between PTB and potential fungal pathogens was seen in 77.9% (127/163) of study participants (Table 4). The association between PTB and potential fungal pathogens was not statistically significant.

As shown in Table 5, about 183 potential fungal pathogens were recovered from PTB and potential fungal pathogens associated study participants of which 148 (80.9%) were yeasts while 35 (19.1%) were mycelial fungi. *C. albicans* and *Aspergillus* spp. were more prevalent among yeasts and molds, respectively.

Discussion

The global burden of pulmonary fungal infections caused by opportunistic fungal pathogens is increasing.[19] Sustaining of patients by drugs, chemicals, and mechanical processes that compromise physical barriers to infection, suppress immune mechanisms, or upset the balance of normal flora are responsible for rendering hosts more susceptible not only to pathogenic fungi but also to all fungi with which they come in contact. The increased age of the world population that resulted in more chronic diseases with their debilitating effects is also another attributing factor for an increase in fungal lung infection by an opportunistic fungal pathogen.[20] The impact of these factors may explain for the high prevalence of the association between potential fungal pathogens and TB reported in the present study.

Our result regarding the prevalence of PTB and potential pulmonary fungal pathogens was consistent with the findings of Sani et al.,[21] but PTB potential fungal pathogen association in our study was threefold (20.0% against 6%) from that of their report. On the contrary, PTB potential fungal pathogen association in the range of 18% to 40% was reported by other similar studies.[22,23] The prevalence of PTB potential fungal pathogen association in this study was also high. This may support that preexisting or residual cavity produced following TB infection are frequent places of fungal colonization, and the chromic nature of PTB along with prolonged chemotherapy makes PTB patients more susceptible to fungal infection.[4] The high prevalence of PTB and potential fungal pathogen association exhibited in this study may worsen the existing burden of PTB and, hence, due attention should be given.

Lower respiratory tract fungal infection (pulmonary fungal infection) such as aspergillosis frequently occurs in middle-aged to an elderly individual and is more commonly reported in male patients.[24,25] Our finding was in line with the findings of Kosmidis and Denning[24] and Kohno et al.[25] as the isolation rate of potential fungal pathogens was higher in

Table 2. Distribution of fungal isolates against gender and age.

Sex	Number tested	Negative	Positive	% Positivity per age group	*p*[a]
Male	327	78	249	51.6	0.239
Female	309	75	234	48.4	
Age	Positive	Negative	Positive	% Positivity per age group	*p*[a]
1–14	31	8	23	74.20	0.50
15–24	85	21	64	75.30	
25–34	125	46	79	63.20	
35–44	133	24	109	81.95	
45–54	82	17	65	79.30	
55–64	93	24	69	74.00	
>65	87	13	74	85.00	

[a]Statistical association as determined by χ^2 test.

Table 3. Spectrum of fungal isolates in presumptive pulmonary tuberculosis patients ($N = 636$).

Fungal species	Single (pure) isolates	Mixed with other fungi	Total isolates
Molds			
Aspergillus niger	24	17	41
Aspergillus fumigatus	22	4	26
Aspergillus flavus	1	1	2
Aspergillus glacus	1	—	1
Aspergillus spp.	3	5	8
Aspergillus terreus		1	1
Penicillium marneffei	3	2	5
Penicillium spp.	5	6	11
Scedosporium apiospermum	9	4	13
Mucor spp.	2	3	5
Fusarium spp.	3	7	10
Acremonium spp.	1	1	2
Alternaria spp.	1	1	2
Bipolaris spp.	1	—	1
Mold sub-total	76	52	128
Yeasts			
Candida albicans	164	96	260
Candida krusei	43	78	121
Candida tropicalis	10	103	113
Other *NAC* spp.	9	53	62
Cryptococcus neoformans	1	5	6
Yeast sub-total	227	335	562
Mixed cultures			
A. niger + *Aspergillus terreus*	—	1	1
A. niger + *Fusarium* spp.	—	1	1
A. niger + *P. marneffei*	—	1	1
A. niger + *Penicillium* spp.	—	3	3
A. niger + *Mucor* spp.		1	1
A. niger + *C. neoformans*	—	1	1
A. niger + *Acremonium* spp.	—	1	1
A. fumigatus + *Fusarium* spp.	—	1	1
Aspergillus spp. + *Fusarium* spp.	—	1	1
Aspergillus spp. + *Paecilomyces* spp.	—	1	1
Aspergillus spp. + *S. apiospermum*	—	1	1
C. albicans + *A. flavus*	—	1	1

(Continued)

Table 3. (Continued)

Fungal species	Single (pure) isolates	Mixed with other fungi	Total isolates
C. albicans + *A. fumigatus*	—	1	1
C. albicans + *A. niger*	—	4	4
C. albicans + *Aspergillus* spp. + *Fusarium* spp.	—	1	1
C. albicans + *C. krusei*	—	3	3
C. albicans + *C. krusei* + *A. fumigates*	—	1	1
C. albicans + *C. krusei* + other *NAC* spp.	—	2	2
C. albicans + *C. krusei* + *C. tropicalis*	—	5	5
C. albicans + *C. tropicalis*	—	60	60
C. albicans + *C. tropicalis* + other *NAC* spp.	—	1	1
C. albicans + *C. tropicalis* + other *NAC*	—	6	6
C. albicans + *C. tropicalis* + *Penicillium* spp.	—	1	1
C. albicans + *Cryptococcus neoformans*	—	1	1
C. albicans + *Fusarium* spp.	—	1	1
C. albicans + other *NAC*	—	5	5
C. albicans + *Penicillium* spp.	—	1	1
C. albicans + *Mucor* spp.	—	2	2
C. krusei + *A. fumigates*	—	1	1
C. krusei + *C. tropicalis*	—	22	22
C. krusei + *A. niger* + *Penicillium* spp.	—	1	1
C. krusei + *C. neoformans* + *A. niger*	—	1	1
C. krusei + *C. tropicalis* + *A. niger*	—	1	1
C. krusei + *C. tropicalis* + other *NAC* spp.	—	3	3
C. krusei + *C. tropicalis* + other *NAC* spp.	—	3	3
C. krusei + *S. apiospermum*	—	2	2
C. krusei + *C. neoformans* + *Aspergillus* spp.	—	1	1
C. krusei + other *NAC* spp.	—	30	30
C. krusei + other *NAC* + *Alternaria* spp.	—	1	1
C. krusei + other *NAC* + *P. marneffei*	—	1	1
C. krusei + *Fusarium* spp.	—	1	1
C. tropicalis + other *NAC* spp.	—	3	3
Total number of samples with a mixed culture	—	180	180

NAC: non albican candida.

Table 4. Prevalence of tuberculosis and the association between tuberculosis and potential fungal pathogens among study participants ($N = 636$).

	Prevalence	p^a
Culture positive	483 (75.9%)	0.147
Tuberculosis infection	163 (25.6%)	
Fungal–tuberculosis association	127 (20%)	
Only tuberculosis infection	36 (5.7%)	
Only fungal infection	447 (70.3%)	
Culture negative samples	153 (24.1%)	

[a]Statistical association as determined by χ^2 test.

patients above 35 years than younger age groups, and male than female study participants. Other studies,[26,27] however, demonstrated that the age group of 20 to 34 years is most affected by fungal pathogens. Certainly, old age is a known risk factor for pulmonary fungal infection probably due to diminishing immune function as one gets aged.[20] The association of potential pulmonary fungal infection with age and

Table 5. Spectrum of fungi in fungal–tuberculosis associated patients ($n = 127$).

Fungal species	*n*	%
Aspergillus spp.	19	14.96
Penicillium spp.	4	3.1
Mucor spp.	2	1.6
Scedosporium spp.	5	3.9
Fusarium spp.	2	1.6
Acremonium spp.	1	0.79
Alternaria spp.	1	0.79
Bipolaris spp.	1	0.79
Total mycelial fungi	35	19.9
Candida albicans	82	64.6
C. krusei	25	19.6
C. tropicalis	29	22.8
Other *NAC* spp.	10	7.9
Cryptococcus neoformans	2	1.6
Total yeast species	148	80.9
Total	183	100

NAC: non albican candida.

sex in our study was not statistically significant with respective *p* values of 0.239 and 0.50.

In the present study, many species of mycelia fungi and yeasts were recovered from sputum. Among fungal isolates, *NAC* species were the dominant group. At the species level, however, *C. albicans* was the most prevalent species accounting for 46.2% yeast isolates. This was followed by *C. tropicalis* and *C. krusei*. Numerous studies have reported that *Candida* species are the most frequent fungal species recovered from the sputum of patients with PTB.[24,25,28,29] Even though *Candida* species were noted as the most frequent fungal species recovered from the sputum of patients with TB, its significance has always been a matter of debate because up to 32.5% of healthy individuals harbor *Candida* in their throat that can contaminate the sputum during sample collection.[30] To this effect, in our work, standard aseptic procedures were followed to reduce contamination of sputum from external sources starting from sample collection up to sample inoculation. Correspondingly, our study depicted that *C. albicans* was the most prevalent yeast in PTB-yeast associated patients accounting for 64.6% of yeast isolates. Similar to other several studies, Latha et al., Jain et al., and Baradkar et al.[31–33] demonstrated a high prevalence isolation rate of *C. albicans* ranging from 45% to 92% in PTB patients co-infected with yeasts. The existence of candidiasis concurrently with PTB patients is of paramount interest in the treatment of patients as *C. albicans* is supposed to enhance the virulence of PTB.[22] Although *C. albicans* continues to be the most predominant species in pulmonary candidiasis,[31–33] several *NAC* species are also reported in increasing frequency. *C. tropicalis* and *C. krusei* were isolated as the second and the third most prevalent yeasts in PTB-yeast co-associated patients with respective frequencies of 22.6% and 19.9%. Our result is in line with Latha et al.,[31] Jain et al.,[32] and Baradkar et al.[33] *C. tropicalis* is an emerging pathogen with higher rates of severe disease and deep tissue invasion than *C. albicans* in immune-debilitated individuals, and *C. krusei* is noted as intrinsically resistant emerging yeast pathogen to azole antifungal drugs particularly to that of fluconazole.[34,35]

Fungal infections of the respiratory tract by large are considered to be identical with invasive pulmonary infections caused by *Aspergillus* spp.[6] Our finding was consistent with this report because out of 128 mycelial fungi recovered in the present study, 61.7% (79) of the isolates were *Aspergillus* species. Among a hundred species of *Aspergillus*, *A. fumigatus*, *A. flavus*, *A. niger*, and *A. terreus* are pathogenic species to man. Most previous studies reported that *A. fumigatus* is the most common cause of CPA,[21,36–38] although its incidence appears to be decreasing in recent years with an increase in cases by other non-*fumigatus Aspergillus* species, especially *A. flavus*, *A. niger*, and *A. terreus*.[39] In the present study, *A. niger* was the most frequently isolated *Aspergillus* species followed by *A. fumigatus* and *A. flavus*. Our finding was in line with the findings of Park et al.[40] According to Park et al.,[40] non-*fumigatus Aspergillus* species are known to cause all forms of aspergillosis. Correspondingly, our study depicted that *Aspergillus* species were the most prevalent mycelial fungi in PTB mold associated patients accounting for 14.96% of mold isolates.

Pulmonary fungal infections have long been recognized as a significant complication and are mainly caused by *C. albicans* and *Aspergillus* spp. Within the past few decades, however, infections due to infrequently encountered fungi (e.g. *Penicillium* spp., *Scedosporium* spp., dematiaceous filamentous fungi, and zygomycetes) have become increasingly common in immunocompromised hosts.[6] Our finding supported the report of Chowdhary et al.[6] in that the isolation rate of mycelial fungi other than *Aspergillus* spp. was considerable. Among 128 mycelial fungi isolated, 49 (38.3%) were mycelial fungi other than *Aspergillus* spp. Among non-*Aspergillus* isolates, 16 (12.5%) were represented by *Penicillium* spp. of which 5 (31.25%) of the isolates were *P. marneffei* and the remaining 11 (68.75%) were other *Penicillium* spp. *P. marneffei* is an emerging dimorphic fungal agent that can cause a deadly systemic mycosis in participants infected with HIV[41] while *Penicillium* spp. other than *Penicillium marneffei* has been recovered most frequently in the clinical laboratory as culture contaminants. They have, however, been emerged as opportunistic pathogens in an immunocompromised individual and consequently, they should not be regarded as a contaminant without a thorough investigation.[42]

In our study, *S. apiospermum* and *Fusarium* spp. accounted for 10.2% (13) and 7.8% (10) of the isolates of mycelial fungi, respectively. *S. apiospermum* is among the most common filamentous fungi colonizing the lungs of cystic fibrosis patients with a frequency of 9%.[43] *Fusarium* species once considered to be important plant pathogens are known to cause a broad spectrum of infections, including mycotic keratitis and onychomycosis. Lung involvement is common in invasive fusariosis occurring among immunocompromised patients with disseminated infection.[44] Species of *Alternaria*, *Bipolaris*, *Curvularia*, and *Exserohilum* have been reported to cause different types of human respiratory tract infections, including invasive lung disease (isolation of *Alternaria* and *Bipolaris* species in our study supported the findings of Chowdhary et al.,[6] Bush and Prochnau,[45] and Chowdhary et al.[46]).

Limitations of the study

The increased prevalence of fungal lung infections now than in the past is largely related to increased numbers of immune-compromised human hosts. Lack of information about the immune status of study participants was the major limitation of the study as most of human fungal pathogens are opportunistic. Due to lack of facilities and resources, unable to determine the antifungal susceptibility profile of

the fungal isolates in the present study was another limitation of our study.

Conclusion

High prevalence of potential pulmonary fungal pathogens in our study, especially in cases where sputum for *M. tuberculosis* was negative, and study participants with PTB will enforce health personnel to pay due attention to these conditions and arise the interest of researchers to conduct further work on the burden of pulmonary fungal infection and its association with PTB which is a neglected disease in most developing countries. Our study also revealed the need to employ conventional microbiology tests along with clinical and radiological evidence since clinical manifestations and radiological pictures of PTB mimic that of pulmonary fungal infection.

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Ethical approval

Ethical approval of this study was obtained from the Internal Review Board (IRB) of the Department of Medical Laboratory Science, College of Health Sciences, Addis Ababa University (Protocol Number: DRER/393/19/MLS/2019).

Informed consent

Written informed consent from all participants or their legally authorized representatives was obtained prior to study initiation.

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