Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Profiling of Pulmonary Fungal Pathogens and the Prevalence of Pulmonary Tuberculosis Fungal Co-Infection in Presumptive Tuberculosis Patients Referred to Saint Peter's Tuberculosis Referral Hospital, Addis Ababa, Ethiopia

Adane Bitew (Adane.bitew@aau.edu.et)

Department of Medical Laboratory Science, College Health Sciences, Addis Ababa University, Ethiopia Box 1176 Addis Ababa University, Addis Ababa Ethiopia

Solomon Bati

Saint Peter's Specialize Tuberculosis Referral Hospital, Addis Ababa Administrative region, Addis Ababa, Ethiopia, Box 21494/30178

Research Article

Keywords: Pulmonary mycosis, pulmonary tuberculosis, fungal pathogens, co-infection

Posted Date: January 4th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-135396/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Abstract

Background: The burden of pulmonary mycosis is increasing and often misdiagnosed as pulmonary tuberculosis in developing countries where the prevalence of pulmonary tuberculosis is high.

Objective: To determine the prevalence of pulmonary mycosis and pulmonary tuberculosis fungal co-infections.

Methods: A hospital-based, cross-sectional study was conducted between October 2019 and May 2019. Sputum was collected from 636 study subjects. 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 by GeneXpert.

Results: Among 636 sputum samples, 75.9% (483), 25.6% (163), and 20% (127) were positive for fungal pathogens, pulmonary tuberculosis, and pulmonary tuberculosis fungal co-infection, respectively. Of 690 fungal isolates, 81.4% were yeasts compromising of 46.3% C*andida albicans* 52.7% Non-a*lbicans Candida* species, and 1.0% *Cryptococcus neoformans.* The remaining 128 (18.6%) isolates were molds, where *Aspergillus* species (79; 61.7%), *Penicillium* species (16; 12.5%), *Scedosporium apiospermum* (13; 10.2%), and *Fusarium* species (10;7.8%) being the major isolates. 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 pulmonary fungal pathogens and sex (P=0.239). Patients in the age group of 35-44 and above were slightly more affected than younger age groups. The association of fungal pathogens and age was not statistically significant (P=0.50).

Conclusions: Our study revealed a high prevalence of pulmonary fungal pathogens (75.4%) and pulmonary fungal co-infection (20%). The isolation rate of Non-*albicans Candida* species out-numbered that of *C. albicans*. High prevalence of pulmonary fungal pathogens in our study, especially in cases where sputum for pulmonary tuberculosis was negative alerts, the need to employ conventional microbiology tests along with clinical and radiological evidence.

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 South-East Asia [3]. The chromic 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 (pulmonary mycosis) 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 paper, isolation of fungi alone or in association with *M. tuberculosis* from presumptive tuberculosis patients (i.e., patients presenting signs and symptoms of pulmonary tuberculosis and having radiological pictures characteristics of PTB) was considered as fungal infection or pulmonary mycosis.

Many fungal species have been reported as etiological agents of fungal 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.*, P*enicillium SPP.*, dematiaceous filamentous fungi, zygomycetes, and yeasts other than *C. albicans* have emerged as etiological agents of respiratory fungal 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].

Pulmonary tuberculosis 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/ 100000 [13]. The high rate of co-infection of pulmonary mycosis 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 differential diagnosis between these two infections difficult. Persistent cough for more than three weeks is a common symptom of pulmonary disorders caused by a wide range of pathogens, including fungi and other bacteria. Lack of differential diagnosis may lead to empirical treatment in which fungal infections are treated with anti-tuberculosis chemotherapy with poor clinical outcomes, as anti-tuberculosis drugs do not affect fungal pathogens [11]. As in most developing countries treatment of PTB patients in Ethiopia is empirical. In view of this, determining the spectrum of fungal pathogens from the sputum of patients presenting clinical and radiological characteristics similar to PTB and the magnitude of fungal and PTB co-infection is an active field of research. Therefore, the objectives of the present study are determining the distribution of fungal pathogens associated with the lower respiratory tract (lung) infection and the prevalence of fungal pathogens and PTB co-infections in presumptive PTB patients referred to the Saint Peter's Specialized Tuberculosis Referral Hospital.

High prevalence of pulmonary fungal pathogens in our study, especially in cases where sputum for *M. tuberculosis* was negative and study subjects with PTB 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 pulmonary fungal infection and its association with Pulmonary tuberculosis which is a neglected disease in most developing countries.

Methods

Study Area and design

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

Study Population

All PTB suspected patients seeking health services at Saint Peter's hospital and those referred from other health institutes.

Inclusion and exclusion Criteria

Patients with clinical manifestation of pulmonary tract infection, particularly of those with a persistent cough for more than three weeks and consented to participate in the study were included in the study. Apparently, healthy individuals with no clinical symptoms of pulmonary infection, 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, $n = Z^2 P (1- P)/ d^2 n$, 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 study participants were 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 visiting Saint Peter's Specialized Hospital Department of Microbiology Laboratory within the specified time of the study.

Data Collection Procedure

Demographic data

The socio-demographic data age, gender, history of previous pulmonary fungal infection, and treatment of each study participant were obtained from a standard laboratory request form completed by physicians. Assent and consent forms are filled during sample collection.

Informed consent, and ethical consideration

The following activities were performed before sample collection. The aim of the study and its benefits were explained to the study participants. Study participants who did not consent to participate in the study are not obliged to participate in the study and can withdraw from the study at any time of the course of the study. Then written informed consent for the study subject with an age of 19 years or above was completed by the study participant while written informed consent for the study subject with an age of 18 years or below was completed by parents/guardians. The study was started after obtaining an ethical clearance (Protocol number: DRER/393/19/MLS/2019) from the Internal Review Board of the Department of Medical Laboratory Science College of Health Science Addis Ababa University and permission from research directorate of the hospital.

Data Quality Assurance Quality Control

The quality of sputum samples, Xpert kit and reagents, and mycological culture media were inspected before they are used. The sterility of culture media and 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 crude ratio with a 95% confidence interval to the different variables. 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 collect purulent sputum by sterile falcon tube by breathing deeply three times. Part of early morning collected sputum was used for the detection of *M. tuberculosis* while the remaining sample 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 Hospital. All inoculated tubes were then transported to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University. One of the tubes was incubated at 25^0 C while the

other one was incubated at 37^0 C aerobically for up to four 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 staining procedure. Briefly, a drop of lactophenol cotton blue (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 10X and 40X 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) as per the instruction of the manufacturer.

Detection of M. tuberculosis

Detection of *M. tuberculosis* from sputum specimens was determined by using the GeneXpert MTB/RIF assay machine (Cepheid, Sunnyvale, CA, USA). Briefly, 4 ml of sputum was mixed with 8 ml of sample reagent, vortexed for 15 seconds, and allowed to stand for 10 minutes at room temperature. The preparation was vortexed again and allowed to stand for another 5 minutes. 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 two hours, results were collected from the GeneXpert computer.

Results

Distribution of fungi in relation to gender and age

The current study enrolled six hundred thirty- six (636) study participants of which 327 (51.4%) were males and 309 (48.6%) were females (Table 1). The ages of the study subjects varied from 1 to 94 years with a mean age of 41 years. As shown in Table 1, out of 636 sputum samples collected and analyzed, fungal species were recovered from 483 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 subjects. 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 with respect to age. In general, the percentage positivity rate per age group depicted that patients in the age group of 35–44 and above were slightly more affected than younger age groups (Table 1). The association of fungal isolation rate and age was not statistically significant (P = 0.50)

Table 1
Distribution of fungal isolates against gender and age

Sex	Number tested	Negative	Positive	% positivity per age group	P-value*
Male	327	78	249		0.239
Female	309	75	234		
Age	Positive	Negative	Positive		
‹ 15	31	8	23	74.20	
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	0.50
*Statistical association as determined by X^2 test					

Distribution of fungal isolates

In the present study, of a total of 636 sputum cultures, 483 were positive for fungal pathogen 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 compromising of C. *albicans* (260; 46.3%), 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 regards to mold isolates (Table 2).

Table 2
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 marnefei	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 Subtotal	**227**	**335**	**562**
A.niger + Aspergillus terreus	-	1	1
A. niger + Fusarium SPP.	-	1	1
A. niger + P. marneffei	-	1	1

Fungal species	Single (pure) isolates	Mixed with other fungi	Total isolates
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
C. albicans + A. fumigatus	-	1	1
C. albicans + A. niger	-	4	4
C. albicans + Aspergillus SPP. + Fusarium SPP.	-	1	1
C. albicans + C. krusie	-	3	3
C. albicans + C. krusei + A. fumigatus	-	1	1
C. albicans + C. Krusei + other NAC SPP.	-	2	2

Table 2
spectrum of fungal isolates in presumptive pulmonary tuberculosis patients referred to pulmonary tuberculosis (N + 636) cont'd

Fungal species	Single (pure) isolates	Mixed with other fungi	Total isolates
Molds	-		
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. krusie + A. fumigatus	-	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. krusi + 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

Fungal species	Single (pure) isolates	Mixed with other fungi	Total isolates
C. tropicalis + other NAC SPP.	-	3	3
Total no. samples with mixed culture	-	180	180

Tuberculosis and fungal co-infection

Among 636 study participants75.9% (483/635) were positive for fungal pathogen, 25.6% (163/636) were positive for PTB. Pulmonary tuberculosis fungal co-infection among the study subjects (636) was 20% (127/636). With regards to PTB patients the prevalence of PTB fungal co-infection was seen in 77.9% (127/163) study subjects (Table 3). The association between fungi and PTB patents co-infection was statistically significant (Table 3).

Table 3
Prevalence of tuberculosis, fungal pathogens, and co-infections among study subjects (n = 636)

	Prevalence	P-value*
Culture positive	483(75.9)	
Tuberculosis infection	163(25.6%)	
Fungal-tuberculosis co-infection	127(20%)	0.147
Only tuberculosis infection	36 (5.7%)	
Only fungal infection	447 (70.3%	
Culture negative samples	153 (24.1%)	
*Statistical association as determined by X^2 test		

As shown in Table 4, about 183 fungal pathogens were recovered from PTB fungal co-infected study subjects 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.

Table 4
Spectrum of fungi in fungus-tuberculosis co-infected patients (n = 127)

Fungal species	Number	%
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**
Grand total	**183**	**100**

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 opportunistic fungal pathogen [20]. The impact of these factors may explain for the higher prevalence of fungal pulmonary infection reported in the present study.

The present study revealed that the prevalence of PTB, pulmonary fungal pathogens and PTB-fungal pathogen co-infection were found out to be 25.6%, 75.9%, and 20.0%, respectively. Our result regarding the prevalence of PTB and pulmonary fungal pathogens was consistent with the findings of Sani et al

[21] but PTB- fungal pathogen co-infection in our study was three-fold (20.0% against 6%) from that of their report. On the other hand, PTB fungal coinfection in the range of 18–40% was reported by other similar studies [22, 23]. Among 163 study subjects that were positive for *M. tuberculosis*, 123 (77.9%) were co-infected with fungal pathogens. The high prevalence of PTB fungal pathogen co-infection in study subjects with tuberculosis in our study may support that pre-existing or residual cavity produced following tuberculosis 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 fungal co-infection exhibited in this study may worsen the existing burden of PTB and, hence, due attention should be given.

Lower respiratory tract fungal infection (pulmonary mycosis) such as aspergillosis frequently occurs in middle-aged to an elderly individual, and are 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 fungal pathogens was higher in 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 20–34 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 pulmonary fungal infection with age and sex in our study was not statistically significant with respective *P*- values of 0.239 and 0.50.

In the present study, of a total of 636 sputum cultures, 483 were positive for fungal pathogen and a total of 690 fungal isolates were recovered. Furthermore, out of 483 positive culture, 43 samples yielded more than one fungal species. Among the isolates, 562 were yeasts where NAC species comprised the most predominant yeast isolates (52.7%). 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 pulmonary tuberculosis [24, 25, 28, 29].

Even though *Candida* species were noted as the most frequent fungal species recovered from the sputum of patients with tuberculosis 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]. Correspondingly, our study depicted that *C. albicans* was the most prevalent yeast in PTB yeast co-infected patients accounting for 64.6% of yeast isolates. Similar other several studies [31–33] demonstrated a high prevalence isolation rate of *C. albicans* ranging from 45–92% 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 2nd and the 3rd most prevalent yeasts in PTB-yeast co-infected 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 chronic-pulmonary aspergillosis [21, 36–38], although its incidence appears to be decreasing in recent years with an increase in cases by other no-*fumigatus* 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 co-infected accounting for 14.96% of mold isolates.

Pulmonary fungal infections have long been recognized as a significant complication and 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 supports 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 subjects infected with human immunodeficiency virus [41] while *Penicillium SPP.* other than *Penicillium marneffei* have 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, it 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 [Chowdhary [6, 45, 46]. Isolation of *Alternaria* and *Bipolaris* species in our study supports the findings of Chowdhary et al [6], Bush and Prochnau [45], and Chowdhary et al [46].

Conclusions

Our study revealed a high prevalence of pulmonary fungal pathogens (75.4%) and PTB fungal co-infection (20%). Although *C. albicans* has long been reported the dominant yeast associated with PTB, our study, however, demonstrated that the isolation rate of NAC out-numbered that of *C. albicans*. High prevalence of pulmonary fungal pathogens in our study, especially in cases where sputum for *M. tuberculosis* was negative and study subjects with PTB alerts, 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.

Declarations

Ethics approval and consent to participate

All methods were carried out in accordance with the declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects WMA General Assembly, Seoul, Korea, October 2008. Briefly the aim of the study and its benefits were explained to the study participants. Study participants who did not consent to participate in the study are not obliged to participate in the study and can withdraw from the study at any time of the course of the study. Then written informed consent for the study subject with an age of 19 years or above was completed by the study participant while written informed consent for the study subject with an age of 18 years or below was completed by parents/guardians. The study was started after obtaining an ethical clearance (Protocol number: DRER/393/19/MLS/2019) from the Internal Review Board of the Department of Medical Laboratory Science College of Health Science Addis Ababa University and permission from research directorate of the hospital.

Consent for publication

The study contains no individual person’s data in any form

Availability data and materials

All the data and materials included in the manuscript

Competing interest

The authors declare that no financial and non-financial competing interest for the manuscript.

Funding

No fund was obtained for the work

Author Contributions:

AB: Conceived and designed the work, analysis and interpretation of data, drafting of the article and revising it critically; Solomon SB: Acquisition of data, analysis and interpretation of data, drafting of the article, data collection, part of laboratory work. Both authors commented on the initial manuscript and approved the final manuscript.

Acknowledgments:

The authors would like to thank the Department of Medical Laboratory Science, College Health Sciences, Addis Ababa University, Ethiopia, and Saint Peter's Specialize Tuberculosis Referral Hospital for allowing the authors to use laboratory facilities for free. The authors also would like to acknowledge study subjects for their participation in the study. We are also very grateful to the medical Laboratory technologists for their help during sample collection.

References

1. Fauci AS, Morens DM. The perpetual challenge of infectious diseases. N Engl J Med. 2012; 366: 454–461. doi: 10.1056/NEJMra1108296 PMID: 22296079.
2. Chakrabarti A. Microbiology of systemic fungal infections. J. Postgrad. Med. 2005; 51: 16–20.
3. Denning DW, Pleuvry A, Cole, DC. Global burden of chronic pulmonary aspergillosis as a sequel to pulmonary tuberculosis. Bull. World Health Organ.2011; 89: 864–872.
4. Ellner J. “Immunological aspect of Mycobacterial infection”. J. Infect. Dis. 1982; 11: 450–455.
5. Woolnough K, Fairs A, Pashley CH, Wardlaw AJ. Allergic fungal airway disease: pathophysiologic and diagnostic considerations. Curr. Opin. Pulm. Med. 2015; 21: 39–47. doi: 10.1097/MCP.0000000000000129
6. Chowdhary A, Agarwal K, Meis JF. Filamentous fungi in respiratory infections. What lies beyond aspergillosis and mucormycosis? PLoS Pathog. 2016;12: e 1005491. doi: 10.1371/journal.ppat.1005491.
7. Yao Z, Liao W. Fungal respiratory disease. Curr Opin Pulm Med. 2006; 12:222–227. doi: 10.1097/01.mcp.0000219272.57933.01
8. Kronstad JW, Attarian R, Cadieux B, Choi J, D’Souza CA, Griffiths EJ, et al. Expanding fungal pathogenesis: Cryptococcus breaks out of the opportunistic box. Nat Rev Microbiol. 2011; 9:193–203. doi: 10.1038/nrmicro2522
9. Udwadia ZF, Doshi AV, Bhaduri AS. Pneumocystis pneumonia. N Engl J Med. 2004; 351:1262 3. doi: 10.1056/NEJM200409163511222
10. Lortholary O, Denning DW, Dupont B. Endemic mycoses: a treatment update. J Antimicrob Chemother.1999; 43:321–31. doi: 10.1093/jac/43.3.321.
11. Nucci M. Marr K.A. Emerging fungal diseases. Clin. Infect. Dis. 2005; 41: 521–526.
12. Sharma SK, Mohan A. Multi-drug resistant tuberculosis. Indian J. Med. Res. 2004;120: 354–376.
13. WHO. Global tuberculosis report 2019

14. Kaur R, Dhakad MS, Goyal R, Bhalla P, Dewan R. Spectrum of opportunistic fungal infections in HIV/AIDS patients in tertiary care hospital in India. Can J Infect Dis Med Microbiol 2016; 2016:2373424.
15. Muluneh T. Spectrum of fungal pathogens in sputum samples from pulmonary tuberculosis suspected patients at Kotebe primary hospital Addis Ababa Ethiopia Master thesis. Available at: http://www.etd.aau.edu.et/
16. Ellis D, Davis S, Alexiou H, Handke R, Bartley R. Description of medical fungi. North Adelaide, USA: Mycology Unit, Women's and Children's Hospital; 2007.
17. Ochie J, Kolhatkhar A. Laboratory techniques in mycology examination of sputum. Medical Laboratory Science, Theory and Practice. New Delhi, India: Tata McGraw Hill Publishing Co. Ltd; 2005. pp. 105–3
18. David E, Stephen D, Helen A, Rosemary H, Robyn B. Descriptions of medical fungi. 2nd. edi. Mycology unit Women□s and children□s hospital School of molecular & Biomedical science University of Adelaide Australia; 2007:8-188
19. Enoch DA, Ludlam HA, Brown NM. Invasive fungal infections: a review of epidemiology and management options, J. Med. Microbiol. 2006; 55: 809–818.
20. Evans EGV and Gentles JC. Essentials of Medical Mycology. Churchill, Livingston, Edinburgh: 1985.pp 1-196.
21. Sani FM, Uba A, Tahir F, Abdullahi IN, Adekola HA, Mustapha J, et al. Spectrum of Pulmonary Fungal Pathogens, Associated Risk Factors, and Anti–fungal Susceptibility Pattern among Persons with Presumptive Tuberculosis at Gombe, Nigeria. Int J of Mycobacteriol.2020; 9: 144–149.
22. Shome SK, Upreti HB, Singh MM, Pamra SP. Mycosis associated with Pulmonary Tuberculosis. Ind J Tub. 1976; 23:64–68
23. Bansods, Rai M. Emerging of mycotic infection in patients infected with mycobacterium tuberculosis world. Journal of Med. Sciences. 2008; 3:74–80.
24. Kosmidis C, Denning DW. The clinical spectrum of pulmonary aspergillosis. Thorax 2015; 70:270–277.
25. Kohno S, Izumikawa K, Ogawa K, Kurashima A, Okimoto N, Amitani R, et al. Intravenous mica- fungin versus voriconazole for chronic pulmonary aspergillosis: a multicenter trial in Japan. J Infect 2010; 61:410–418.
26. Ogba OM, Abia–Bassey LN, Epoke J. The relationship between opportunistic pulmonary fungal infections and CD4 count levels among HIV–seropositive patients in Calabar, Nigeria. Trans R Soc Trop Med Hyg. 2013; 107:170–175.
27. Ofonime MO, Lydia NA, James E, Baki IM, Godwin DI. Characterization of *Candida* species Isolated from cases of lower respiratory tract infection among HIV/AIDS Patients in Calabar, Nigeria. World J AIDS 2013; 3:201–206.
28. Roohani AH, Fatima N, Shameem M, Khan HM, Khan PA, Akhtar A. Comparing the profile of respiratory fungal pathogens amongst immunocompetent and immunocompromised hosts, their

susceptibility pattern and correlation of various opportunistic respiratory fungal infections and their progression in relation to the CD4 + T–cell counts. Indian J Med Microbiol 2018; 36:408–15.

29. Kali A, Charles MP, Noyal MJ, Sivaraman U, Kumar S, Easow JM. Prevalence of Candida co–infection in patients with pulmonary tuberculosis. Australas Med J 2013;6:387–91.
30. Santiwongkarn P, Kachonboon S, Thanyasrisung P, Matangkasombut O. Prevalence of oral Candida carriage in Thai adolescents, J. Investig. Clin. Dent. 3 (1) (2012) 51–55
31. Latha R, Sasikala R, Muruganandam N, Venkatesh Babu R. Study on the shifting patterns of Non Albicans Candida in lower respiratory tract infections and evaluation of the CHROMagar in identification of the Candida species. J Microbiol Biotech Res; 2011:1:4–9.
32. Jain SK, Agrawal RL, Sharma DA, Agrawal M. Candida in pulmonary tuberculosis. J Postgrad Med. 1982 Jan;28(1):24–9.
33. Baradkar VP, Mathur M, Wanjari K, Kumar S. Candida in pulmonary tuberculosis. Bombay Hospital Journal. 2009; 51:52–3.
34. Lyon GM, Karatela S, Sunay S, Adiri Y. Antifungal susceptibility testing of Candida isolates from the Candida surveillance study. J Clin Microbiol. 2010; 48:1270–5.
35. Bitew and Abebaw. Vulvovaginal candidiasis: species distribution of Candida and their antifungal susceptibility pattern. BMC Women's Health. 2018; 18:94 https://doi.org/10.1186/s12905-018-0607-z
36. Lucena P, Barberán J, Eroles G, GranizoJ-J, Giménez M-J, Mir N et l. Significance of lower respiratory tract cultures yielding Aspergillus spp. Growth in a hospital without transplant patients. Rev Esp Quimioter 2010; 23:190–5.
37. Mwaura NE, Elizabeth VM, Christine B. Mycological findings of sputum sample from PTB patients attending TB clinic Nairobi, Kenya. Virol Mycol 2013; 2:119
38. Ved P, Prem M, Shashi V, Shivani S, Mahendra S. Prevalence and fungal profile of pulmonary aspergillosis in immunocompromised and immunocompetent patients of a tertiary care hospital. Int J Med Res Health Sci 2014; 3:92–7.
39. Smith JA, Kauffman CA. Pulmonary fungal infections. Respirology
40. Park SJ, Chung CR, Rhee YK, et al. Chronic pulmonary aspergillosis due to Aspergillus niger. Am J Respir Crit Care Med 2012;186: e16–7.
41. Vanittanakom N, Cooper CR, Fisher MC, Isanthana T. *Penicillium marneffei* Infection and Recent Advances in the Epidemiology and Molecular Biology Aspects. Clinical microbiology reviews. 2006; 19: 95–110 doi:10.1128/CMR.19.1.95–110.2
42. Ramırez I, Hidron A, Cardona R. Cardona Successful treatment of pulmonary invasive fungal infection by *Penicillium* non-*marneffei* in lymphoblastic lymphoma: case report and literature review. Clinical Case Reports 2018; 6(6): 1153–1157
43. Cimon B, Carrere J, Chazalette JP. Fungal colonization and immune response to fungi in cystic fibrosis. J Mycol Me´d. 1995; 5: 53_56.

44. Nucci M, Anaissie E. Cutaneous infection by Fusarium species in healthy and immunocompromised hosts: implications for diagnosis and management. Clin. Infect. Dis. 2002:**35**:909–920
45. Bush RK, Prochnau JJ. Alternaria-induced asthma. J Allergy Clin Immunol 2004; 113:227–234. PMID: 14767434
46. Chowdhary A, Agarwal K, Kathuria S, Gaur SN, Randhawa HS, Meis J. Allergic bronchopulmonary mycosis due to fungi other than Aspergillus: a global overview. Crit Rev Microbiol 2014; 40:30–48. doi: 10. 3109/1040841X.2012.754401 PMID: 23383677