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Prevalence of Pulmonary Tuberculosis and its co-infection with Fungal Pathogens in patients with lower respiratory tract infections referred to St. Peter's Specialized Hospital, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by **Zenawit Lakew**, entitled:

Prevalence of Pulmonary Tuberculosis and Its Co-infection with Fungal pathogens in patients with lower respiratory tract infections referred to St. Peter's Specialized Hospital, Addis Ababa, Ethiopia, and submitted in partial fulfilment of the requirements of Master of Science Degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards concerning originality and quality.

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Abbreviations

AAU	Addis Ababa University
AFB	Acid-Fast Bacilli
CPA	Chronic pulmonary aspergillosis
HIV	Human immunodeficiency virus
IFI	Invasive fungal infection
IRB	Internal Review Board
LRTI	Lower Respiratory Tract Infection
LPCB	Lactophenol Cotton Blue
MDR-TB	Multi-Drug Resistant TB
MTB	Mycobacterium Tuberculosis
PI	Principal Investigator
PTB	Pulmonary Tuberculosis
RERC	Research Ethics Review Committee
RIF	Rifampicin
RR	Rifampicin resistance
SPSS	Statistical Package for the Social Sciences
TB	Tuberculosis
WHO	World Health Organization

Abstract

Background: Tuberculosis remains a worldwide problem despite efforts to eliminate the disease and TB co-infection with fungal pathogens.Co-infection is the occurrence of concurrent infection of an individual with more than one disease-causing pathogen. The most frequent co-infection is that of PTB co-infected with HIV and that of PTB co-infected with *Candidiasi*s.

Objective: To determine the prevalence and etiologies of tuberculosis and TB fungal co-infection in patients with signs and symptoms of lower respiratory tract infections.

Materials and methods: A prospective hospital-based, cross-sectional study was conducted at the study site through November 2023 and May 2024. From 530 study participants, sputum samples were obtained. Using a GeneXpert Mycobacterium tuberculosis (MTB)/Resistance to rifampicin (RIF) assay equipment, a portion of the sputum was used for the diagnosis of tuberculosis. The remaining sample 0.5 ml was grown on BHI agar, and the fungal isolates were identified using conventional microbiological techniques; CROM agar media and germ tube formation for yeasts and lactophenol cotton blue staining technique for mycelial fungal differentiation. SPSS version 22 used for data analysis.

Results: A total of 530 sputum samples were collected and examined for lower respiratory tract infections. Number of Male participants were slightly less than females and middle-aged to elderly study subjects were dominant. The prevalence of sputum negative for Gene Xpert or fungal culture negative was 318 (60.0%). Gene Xpert-positive samples were 42 (7.9%) and that of fungal infections were 189(36.7%), respectively. Of 42 Xpert positive 23(4.3%) patients were infected with TB only and 19(3.9%) patients were TB co-infected with fungal pathogens. Similarly, 189 (36.7%) sputum samples were culture positive for fungal species in which 170 (32.1%) patients were infected with fungal pathogen only and 19 (3.9%) patients were TB co-infected with fungal pathogens. Of the Xpert positive tests, four were rifampicin resistance while two were MDR. Before presentation about 4.1% and 2.3% of the study subjects were treated with anti-TB and antibacterial drugs respectively.

Conclusions: Among patients with symptoms of respiratory infections tested for TB and fungal pathogens 42 subjects had tuberculosis of 42 Xpert-positive patients 23(4.3%) were infected with TB only and 19(3.9%) patients were TB co-infected with fungal pathogens. Screening for TB should be conducted concomitantly with fungal pathogens.

Key words- Tuberculosis, Co-infection, fungal pathogens, Lower track respiratory infections.

1. Introduction

1.1 Background

Pulmonary tuberculosis (PTB) is a serious chronic lung disease resulting from the Mycobacterium tuberculosis (MTB) complex. It ranks among the top 10 causes of death globally and is the primary cause of death from a single infectious pathogen [1]. According to the WHO 2019 global TB report, it has been estimated that about 10 million people had tuberculosis of which about 5.7 million were male, 3.2 million were female, and 1.1 million were children [1].

Pulmonary tuberculosis (TB) is one of the most common diseases in developing nations and is associated with low socioeconomic status. The infection remains a disease of poverty, with 95% of cases and 98% of deaths occurring in developing states [2]. Greater than 95% of PTB cases have been reported from resource-limited areas of the globe, chiefly from countries in, Africa, Latin America, Asia, and the Middle East [3]. Ethiopia places tenth out of the thirty countries with the highest TB burden, with an estimated incidence rate of 151/100,000 [1].

Despite the active disease's microbiological cure, 66% of pulmonary tuberculosis patients who survive treatment develop serious structural lung abnormalities [4]. Post-TB lung disease is the term used to describe these residual changes, and one common example of post-TB lung disease is infectious complications like chronic pulmonary aspergillosis (CPA) [4]. Post-TB lung disease reduces life expectancy, increases the risk of recurrent TB infection, and significantly increases the risk of other bacterial and fungal infections. It's estimated that 1.2 million people worldwide suffer from CPA as a result of PTB [5]. PTB patients' immune systems were eventually suppressed by the chronic nature of the disease and extended chemotherapy, either with or without corticosteroids, making them vulnerable to fungal infection [6].

Mycobacterium tuberculosis has developed resistance over time against the drugs used for its treatment hence drug resistance has become a serious problem around the world. Development of multi-drug resistant TB (MDR-TB) which is described as tuberculosis with resistance to both rifampicin and isoniazid drugs [7,8] has been a major health threat in the world. Data obtained from the latest anti-TB drug resistance investigation showed that 4.1% of new and 19% of previously treated TB cases in the world are found to be multidrug-resistant tuberculosis [9].

Lower respiratory tract infection (LRTI) is a disease of the windpipe predominantly of the bronchi and the lungs [10]. Infection has been recognized as one of the most significant health issues and a main cause of morbidity and mortality in many developing nations [11]. It is a worldwide problem accounting for more than 5 million deaths each year [12]. The principal etiological agents of LRTI are TB, bacterial, and fungal pathogens [13]. Coughing, shortness of breath, weakness, fever, and fatigue are the most common clinical presentation of LRTI [14].

Fungal infections in the lungs cause a disease known as pulmonary fungal infection. The ubiquity of fungi and/or their spores makes lung colonization or infection inevitable; nevertheless, the mechanisms distinguishing fungal colonization from fungal infection are not well understood, making this topic extremely difficult to address. This study investigated the possibility of a fungal infection based on the isolation of fungi either by themselves or in association with tuberculosis in suspected patients. Numerous fungal species have been recognized as etiological agents of lower tract infection. In many literatures, species of *Aspergillus* [15] *Candida*, *Cryptococcus* [16] *Pneumocystis* [17], and dimorphic fungi [18] are the most significant. Nonetheless, there has been a change in the epidemiology of the fungi that cause lower respiratory tract infections. Further to that, several molds, such as *zygomycetes*, *Penicillium* spp., *Fusarium* spp., and *Scedosporium* spp., have been identified as the causative agents of respiratory tract infections in addition to *Candida albicans*. Even though these fungi are rarely identified in immune-competent patients' respiratory tracts, they may spread to other systemic organs and infect people who are already seriously ill with potentially fatal invasive fungal infections [19].

Co-infection is the occurrence of concurrent infection of an individual with more than one disease-causing pathogen. The most frequent co-infection is that of PTB co-infected with HIV and that of PTB co-infected with *Candidiasis.* In some states up to about 80% of PTB patients are co-infected with HIV [20] and about 15% - 32% of PTB patients are also co-infected with Candidiasis [21]. With the widespread use of antibacterial drugs and an increasing number of immunocompromised patients, pulmonary fungal infections are becoming more common.

Because of the high likelihood of morbidity and death caused by the combination of the two illnesses, co-infection of pulmonary fungal infection with PTB increases the burden of PTB [22,23]. Furthermore, similarities in clinical and radiological presentation of pulmonary fungal infection and PTB have made definite diagnoses between these two infections difficult.

A typical sign of pulmonary illnesses caused by mycobacterium tuberculosis (MTB) and fungal pathogens is a persistent cough lasting longer than three weeks. In cases when A definitive diagnosis cannot be made between PTB and fungal lung infection, fungal infections may be treated empirically with anti-TB chemotherapy, which can have undesirable clinical effects. [19]. As in most developing countries, empirical treatment of PTB patients in Ethiopia is common. To this end, the spectrum of pulmonary fungal infections and their association with PTB in Ethiopia is lacking. Because of this, determining the spectrum of potential fungal pathogens from the sputum of patients presenting clinical and radiological characteristics like 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 to determine the Prevalence of pulmonary tuberculosis and PTB co-infection with fungal pathogens in patients with lower respiratory tract infections referred to St. Peter's Specialized Hospital.

1.2 Statement of the Problem

Pulmonary tuberculosis is a disease caused by an acid-fast bacillus (AFB) known as the MTB complex [10,11]. PTB is one of the top 10 aetiological agents causing human death and suffering worldwide, according to the WHO report [24]. Globally, between 9.0 and 11.1 million individuals (9.8 million males, 3.2 million women, and 1 million children) had active PTB illness in 2017 [13]. Globally in 2022, the estimated proportion of MDR/RR-TB cases with pre-XDR TB (i.e. resistance to any fluoroquinolone for which testing was done) was 18% [44]. More than 1.6 million people die from fungal diseases each year, affecting over a billion people worldwide. However, compared to tuberculosis, fungal infections continue to be poorly suspected by doctors [25]. This primarily relates to sub-Saharan African states with high rates of tuberculosis (TB), where a significant proportion of the population, including HIV/AIDS patients, is susceptible to fungal infections [26].

Due to similarities in the ways that fungal infections and tuberculosis manifest, misdiagnosis is frequently associated with longer hospital stays, financial losses, higher rates of morbidity, and poor outcomes in the clinic [26]. Patients in regions where tuberculosis is endemic, such as Asia and Africa, are frequently started on anti-TB medication even when microbiological test results are negative [27]. Furthermore, the lack of diagnostic methods has masked the true burden of fungal infections, especially in settings with low resources, giving the impression that they are uncommon compared to well-known conditions like tuberculosis [26]. The challenge of false-positive TB diagnosis as a result of the National TB program's efforts to lower the incidence and mortality rate from TB has also added to the story above [28].

Because of this, determining the spectrum of potential fungal pathogens from the sputum of patients giving a presentation of clinical as well as radiological features like pulmonary tuberculosis and the magnitude of the association between TB and potential fungal pathogens is an active area of research.

1.3 Significance of the study

Studies on the prevalence of pulmonary tuberculosis and co-infection of pulmonary fungal pathogens in patients with lower respiratory infections is deficient in Ethiopia as well as worldwide. Therefore, the present study may assist to have a baseline data for further study.

Resemblances in the clinical and radiological presentation of pulmonary fungal infection and PTB have made definite diagnoses between these two infections difficult. Lack of such a diagnosis problem between the two infections may result in empirical treatment with poor clinical outcomes, such as treating fungal infections with anti-TB chemotherapy.

This study may assist in the diagnosis and treatment of those patient. It may also contribute national guideline amendment and development for pulmonary fungal infection diagnosis and treatment.

2. Literature Review

2.1 Lower respiratory tract infections

Lower respiratory tract infections (LRTIs) are the most common infections in humans. An estimated 2.74 million fatalities globally are related to LRTIs annually [30]. The most frequent LRTIs are pneumonia, acute trachea bronchitis, acute bronchitis, and chronic bronchitis. These conditions account for 4.4% of hospital admissions and are associated with high rates of morbidity and mortality as well as excessive medical expenses [31]. The most common microbial agents of LRTIs are *Mycobacterium tuberculosis*, yeasts and Molds, Gram-negative bacteria like *Pseudomonas* species, *Acinetobacter* species, *Klebsiella pneumoniae*, and *Haemophilus influenzae*, and Gram-positive bacteria like *Staphylococcus aureus* and *Enterococcus* spp [32-35].

2.2 Pulmonary Tuberculosis

2.2.1 The Burden of pulmonary tuberculosis and risk factors

The Mycobacterium tuberculosis (MTB) complex is a group of acid-fast bacilli (AFB) bacteria that cause pulmonary tuberculosis (PTB) [33,34]. PTB is among the top 10 etiological causes contributing to global mortality, per a World Health Organization report [35]. Millions of individuals suffer from PTB every year. In 2021, the reported number of people newly diagnosed with TB there was a partial recovery, to 6.4 million people worldwide which is compared to the report between the year 2016-2017; the estimated number of deaths from TB increased between 2019 and 2021, and total of 1.6 million in both HIV-negative and HIV positive individuals [39]. The majority of PTB cases occurred in adults (≥15 years old), and men were more likely than women to develop active PTB disease [40].

The most common clinical type of tuberculosis (TB), pulmonary TB is an infectious illness spread by air that is mostly resulted by members of the Mycobacterium tuberculosis (MTB) complex. It is also a greatest cause of death from a single pathogen. An estimated 10 million new cases and 1.5 million deaths worldwide were attributed to tuberculosis in 2018 (WHO, 2019). MTB-carrying droplets are transferred by coughing or sneezing. These droplets are the result of MTB bacilli that were inhaled and trapped in the alveoli at the lower airways. The bacilli then overcame peripheral immunity, multiplied inside an infected alveolar macrophage, and triggered an inflammation that resulted in damages to lungs. Those lung lesions may be asymptomatic/latent TB or they may develop into more extensive lung tissue destruction that results in cavities and/or consolidations along with symptoms and other

indicators of active TB. Weight loss, night sweating, fever, and productive cough are among the obvious symptoms and signs of tuberculosis (TB), which is needed for persistent tuberculosis transmission. In a wider range of results of infection and manifestations of diseases, latent and active tuberculosis represent the extremes [41].

The Tuberculosis incidence rate is counted among dividing number of new cases of a disease within one year in 100,000 populations [42]. The incidence varies greatly by geographic area, though. In sub-Saharan Africa and Asia, the yearly incidence may reach hundreds of cases per 100,000 people, but in the United States of America, it is lower than 5 cases per 100,000 individuals. The occurrence of TB is global. With 45% of new cases, Asia accounted for the majority of new tuberculosis infections in 2021. Africa came in second with 23% of new cases. Thirty high-TB-burden nations accounted for 87% of new cases of tuberculosis in 2021. Two-thirds of the global total came from eight countries: Bangladesh, Nigeria, Bangladesh, India, Pakistan, Indonesia, China, the Philippines, and the Democratic Republic of the Congo [39]. Generally, persons at high risk for developing TB disease fall into two categories: Persons who have been recently infected with TB bacteria (Close contacts, immigrants, age, homeless persons, injection drug users,) and Persons with medical conditions that weaken the immune system (HIV infection, Diabetes mellitus, Severe kidney disease, corticosteroids or organ transplant, etc…) [43].

There were an estimated 1.3 million incident cases of isoniazid-resistant TB in 2022, including people with both rifampicin-susceptible and rifampicin-resistant TB. Globally in 2022, the estimated proportion of MDR/RR-TB cases with pre-XDR TB (i.e. resistance to any fluoroquinolone for which testing was done) was 18% [44]. The prevalence of RR-TB is 1.1% among new and 7.5% among previously treated TB cases, respectively according to the preliminary report of the 2019 national TB Drug Resistance Surveys (DRS) and MDR prevalence was 1.03% among new and 6.52% among previously treated TB patients. Any INH resistance (6.16%) had the highest detected prevalence of all first-line drug resistance [45]. According to the systematic review conducted in Ethiopia in 2022, the overall prevalence of any anti-TB drug resistance was 14.25%, and INH and RIF resistance was found in 15.62% and 9.75% of patients with TB respectively. prevalence for INH and RIF-monoresistance was 6.23%) and 2.33% respectively. MDR-TB was detected in 2.64% of newly diagnosed cases and 11.54% of retreated patients with TB, while the overall pooled prevalence of MDR-TB was 10.78% [46], which indicates that there is a widespread drug resistance TB in our country which needs better attention and interventions.

2.3 Burden of pulmonary mycosis and its associated factors

Approximately 6.55 million patients develop life-threatening fungal infections annually, of which 3.75 million unfortunately do not survive. Among those Some fungal diseases are acute and severe (i.e., cryptococcal meningitis and fungal eye infection (keratitis), other recurrent (i.e., Candida vaginitis or oral candidiasis in AIDS), and other chronic (i.e., chronic pulmonary aspergillosis) [47]. Over 10 million patients in Europe, the USA, and Japan are at risk of invasive aspergillosis (IA) each year because of corticosteroids or other therapies. Over 50% of patients with IA die, even with treatment [48]. Several medical conditions, such as asthma, AIDS, cancer, organ transplantation, corticosteroid therapy, chronic obstructive pulmonary disease (COPD), pulmonary tuberculosis, post-surgical patients, and ICU admissions, can result in serious fungal infections [49]. Approximately 8% of Ethiopians suffer from fungi infections annually and among those, there was a prevalence of 15,200 estimated CPA cases and 11,500 invasive aspergillosis (IA) cases were total incidence [50].

2.4 Co-infection of tuberculosis with other lower respiratory tract infections.

The works of literature have shown that PTB and other lower respiratory tract infections (LRTIs) can co-exist, especially in regions where PTB is common [51,52]. The co-infection of PTB with other LRTI may be facilitated by active PTB disease. The primary cause of the co-infection may be the suppression of human immunity, which might happen as a result of a T lymphocyte deficiency during an active PTB disease. Early PTB disease is characterized by hormonal abnormalities that may impair the effectiveness of the immune system. These alterations include decreased pituitary function, elevated adrenal and pancreatic functional activity, elevated cortisol levels, and altered thyroid function [53]. In individuals with PTB, failing to detect LRTI may complicate PTB treatment, lead to worse health outcomes, and increase mortality rates. Shimazaki et al. reported a significantly greater death risk among HIV-negative PTB individuals due to a high incidence of bacterial co-infection [54].

A review article of Ekeng et al[55] demonstrated that, 80 cases in all that was misdiagnosed as tuberculosis: *Aspergillosis* (22.5%), *Histoplasmosis* (20%), *Blastomycosis* (17.5%), *Cryptococcosis* (13.8%), *Talaromycosis* (8.8%), *Coccidiomycosis* (6.3%), *Mucormycosis* (3.8%), *Phaeohyphomycosis* (1.3%) and *Chromoblastomycosis* (1.3%). More than one hundred cases of chronic bronchopulmonary aspergillosis were treated with anti-TB medication prior the proper diagnosis was obtained, according to case series from Pakistan and India. 25 cases (33.8%) died, and 45 (56.3%) had favourable results; the remaining patients' outcomes were unknown. 17 (21.3%) cases were infected with a human immunodeficiency

virus. The diagnostic techniques used were microscopy (12.5%), serology (22.5%), histopathology (57.5%), culture (52.5%), cytology (2.5%), gene sequencing methods (6.3%), and Gram stain (12.5%). We conclude that whenever an individual presents with symptoms provocative of unverified tuberculosis, the previously mentioned fungal infections should always be taken into consideration and ruled out. This will reduce the length of hospitalization and mortality connected with a delayed or inaccurate the identification of fungus infections.

Kali et.al [21] various techniques reported that *Candida* co-infection was observed in 30 (40%) of patients with pulmonary tuberculosis. *Candida albicans* was the most common isolate observed in 50% of the patients with co-infection, followed by *C. tropicalis* (20%) and *C. glabrata* (20%). Candida co-infection was found in 62.5% of female patients, while it was observed in only 29.4% of male patients. The mean ± SD age of the patients with *C. glabrata* infection was 65.83 ± 3.19, while the mean ± SD age of the patients with other *Candida* infections was 43.25 ± 20.44.

Candida Co-Infection with Mycobacterium tuberculosis in Tuberculosis Patients and Antifungal Susceptibility of the Isolates were investigated by Amala et al [56]. The study demonstrated that out of 400 sputum samples examined for TB 93 (23.3%) were positive and 32 (34.4%) out of 93 TB-positive cases were co-infected with *Candida* spp. *Candida albicans* was the most predominant species with a prevalence of 23 (67.6%), *C. tropicals* 4 (11.8%), *C. krusei* 4 (11.8%), and *C. parapsilosis* 3 (8.8%). One sample had a dual infection. Female subjects had a higher prevalence (19.4%) than the male (15.7%). Age group 31 - 40 years had a high prevalence of TB 32.3% and Candida 25.0%. Antifungal susceptibility testing showed that isolated *Candida* spp. was more susceptible to voriconazole and fluconazole compared to nystatin.

Profiling of potential pulmonary fungal pathogens and the prevalence of the association between pulmonary tuberculosis and potential fungal pathogens was done by Solomon et.al [57] in Ethiopia and the prevalence of co-infection of pulmonary tuberculosis and potential fungal pathogens was 20.0%. According to *Solomon et al*. a high prevalence of potential pulmonary fungal pathogens and the association of tuberculosis and potential fungal pathogens recorded which will enforce health personnel to pay due attention to these conditions and update laboratory and clinical diagnosis in health facilities.

2.5 Conceptual framework of the study

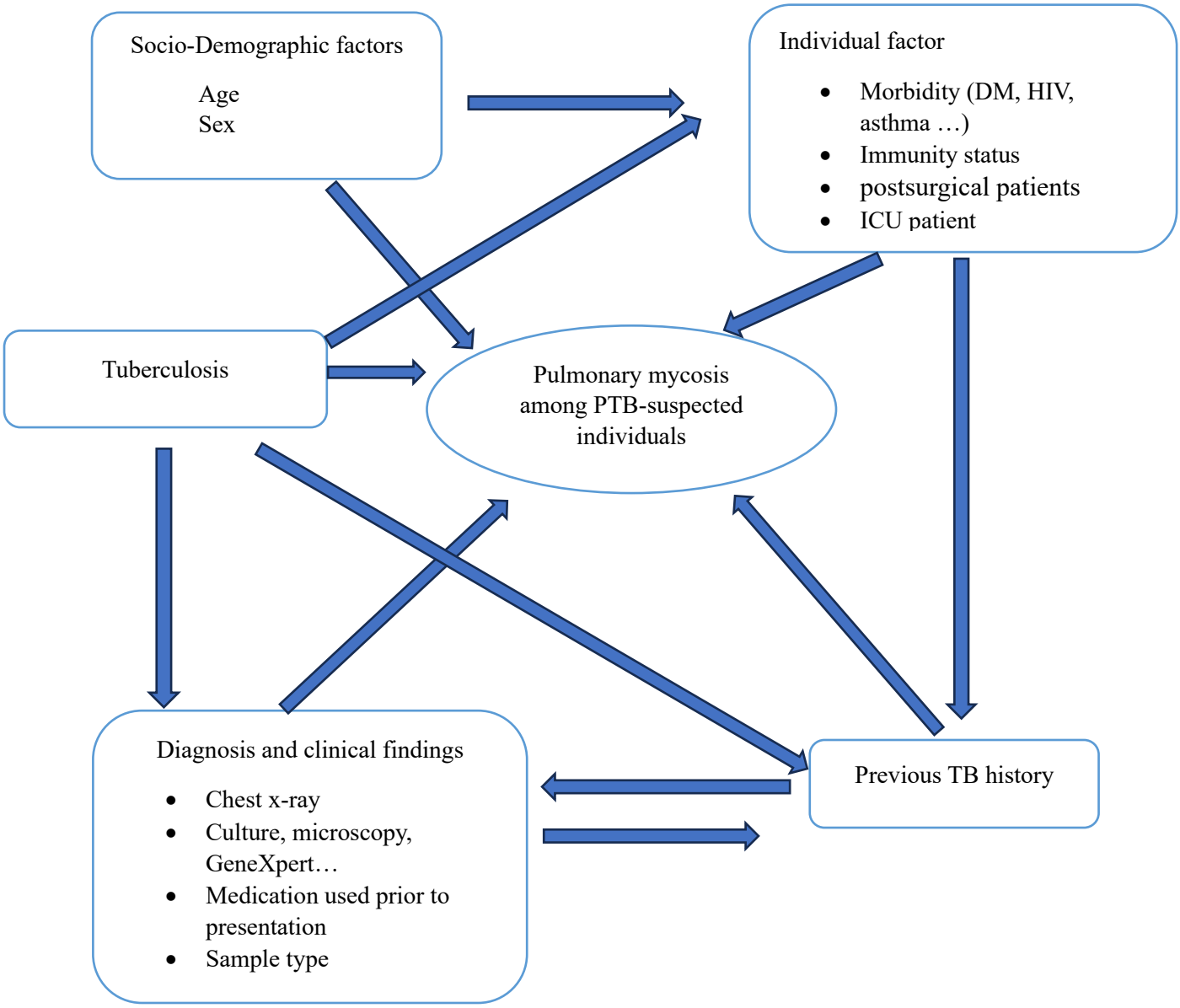

Figure 1 Conceptual framework of potential risk factors of pulmonary mycosis among pulmonary TB suspected individuals *[41,43,49,53,55]*

3. Objective

3.1 General objective

- To determine the Prevalence of pulmonary tuberculosis and its co-infection with fungal pathogens in patients with lower respiratory tract infections referred to St. Peter's Specialized Hospital, Addis Ababa, Ethiopia.

3.2 Specific objectives

- To determine the prevalence of pulmonary tuberculosis and
- To determine the prevalence of drug-resistant TB
- To determine pulmonary fungal infections, misdiagnose as tuberculosis.
- To determine TB co-infected with pulmonary fungal pathogens.

4. Materials and Methods

4.1. Study Area

The study was conducted at Saint Peter's Specialized Hospital located in the Gullele sub-city of Addis Ababa city administration, Ethiopia. It has established in 1953 E.C. The hospital was selected for this study because of the availability of a specialized referral laboratory for pulmonary tuberculosis. The hospital offers the best Clinic for initial triage and evaluation of patients presenting with symptoms of respiratory tract infection. The microbiology laboratory is well equipped and providing different referral services for Tuberculosis and MDR-related infection from every corner of the country and with an average turnover of 20-25 new TB suspects per day

4.2. Study Design and Period

A prospective hospital-based, cross-sectional study was conducted at the study site through November 2023 and May 2024.

4.3. Population

4.3.1. Source Population

The source of the population was those people referred to the TB clinic at the study site.

4.3.2. Study Population

Patients who were assessed clinically and radiologically for lower respiratory infection in the study site and met the inclusion criteria were the study population.

4.4. Inclusion and Exclusion Criteria

4.4.1. Inclusion Criteria

The study included adult out-patient patients who had clinical manifestations of lower respiratory tract infections, especially those who had additional symptoms and signs similar to PTB and had a persistent cough lasting more than three weeks.

4.4.2. Exclusion Criteria

Individuals who were under antifungal treatment for the last week before the data collection period were excluded.

4.5. Study Variables

4.5.1. Dependent Variables

- Incidence of tuberculosis
- Prevalence of Pulmonary fungal infections
- Prevalence of fungal and TB co-infection.

4.5.2. Independent Variables

- Age
- Sex
- Morbidities
- Smoking habit
- Previous TB treatment
- Clinical investigations(x-ray)
- Medications used before investigation etc…

4.6. Sample Size Calculation and Sampling Method

4.6.1. Sample size calculation

The sample size of this study was calculated based on a single population proportion formula,

$n= (Z\alpha/2)^2 P (1-P) /d^2$,

where; n=sample size

P=population proportion

Z=95% level of confidence (1.96), and

d=margin of error (d=0.05),

By taking the previous study, prevalence of pulmonary fungal infection was 42.3% [57].

The estimated minimum size of sample was (1.96)2×0.423(1-0.423)/(0.05)2=375. Consequently, 413 study participants were required as the minimum after adding a 10% contingency. Nonetheless, **530** study participants made up the sample size of this study.

4.6.2. Sampling Method

To determine the estimated sample size, a convenient sampling technique was applied. Within the research's designated time frame, all patients with lower respiratory tract infections that mimicked PTB who were referred to the study site's TB laboratory were enrolled.

4.7. Measurement and Data Collection

4.7.1. Socio-Demographic and clinical investigation Data

The socio-demographic data such as age, gender and other clinical characteristics like symptoms, morbidities was obtained from an electronic database completed by physicians and chest x-ray interpretations was taken from radiology department electronic backup data. In addition to that, patients were interviewed using a standard questionnaire. Before the actual data collection, a pre-test was conducted using demographic and clinical data collection formats and log books.

4.7.2. Sample Collection and Laboratory Procedure

Sputum collection

Study participants told for repeatedly clean their mouths carefully with clean water. A sputum sample used for microbiological investigation was collected and transported as follows. A sterile falcon tube was given to the patient and requested him or her to cough deeply to expectorate and produce a sputum specimen. The process of collecting samples was overseen by a qualified medical laboratory technologist. About half of the sputum was utilized for fungal culture, as indicated below, and the remaining portion was used for *Mycobacterium tuberculosis* detection.

Detection of M. tuberculosis

Detection of M. tuberculosis from sputum specimens was determined by using the GeneXpert Mycobacterium tuberculosis (MTB)/Resistance to rifampicin (RIF) assay machine (Cepheid, Sunnyvale, CA, USA) following Cepheid GeneXpert Dx System Users' manual; 2020. p. 2–13 [29]. Concisely, 4mL of sputum was mixed with 8mL of sample reagent. The mixture was, vortexed for about 15s, and then permitted to stand for 10 min at room temperature. The preparation was then vortexed again and allowed to stand for another 5 min. Then 2mL of the processed sample was transferred into a multichambered plastic Expert 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 completed the remaining assay steps. After 2h, result was collected from the GeneXpert computer.

Detection of drug resistance TB

Detection of drug resistance TB in this study performed by a rapid molecular DST is made possible by the Xpert MTB/XDR test. It assisted determination of MDR TB in this study. For use in peripheral and intermediate-level laboratories, expert MTB/XDR is a rapid nucleic acid amplification test that can identify both drug resistance and tuberculosis in one test. In specimens where tuberculosis is detected by Xpert MTB/XDR, Xpert MTB/XDR can also detect resistance to isoniazid, fluoroquinolones, ethionamide, and also amikacin. The Xpert MTB/XDR assay has been approved for reflex testing of sputum samples after a positive result for *M. tuberculosis* complex on the Xpert MTB/RIF or Ultra assays [59].

Fungal isolation and characterization.

Unprocessed sputum was inoculated directly onto duplicate Brain Heart Infusion agar in a screw cup test tubes supplemented with chloramphenicol (Oxoid, Basingstoke, UK) under safety cabinet level II at the study. All inoculated tubes were then transported to the Department of Medical Laboratory Science, College of Health Science, Addis Ababa University. One of the tubes were incubated at 25°C while the other one was incubated at 37°C aerobically in an inverted position (agar side up) for up to 4 weeks. Culture plates were checked twice a week for any fungal growth. The significant fungal isolates on culture were identified to the species level, using standard mycological procedures.

Brain Heart Infusion Agar with Chloramphenicol- The medium contains protease peptone and infusions from calf brain and beef heart which serve as sources of carbon, nitrogen, essential growth factors, amino acids, and vitamins. Dextrose is used as a source of energy. Di sodium phosphate helps in maintaining the buffering action of the medium whereas sodium chloride maintains the osmotic equilibrium of the medium [78].

Identification

Mold identification

Mycelia fungi were identified by studying their microscopic and macroscopic characteristics. Pigmentation of the front and the reverse side the culture, texture, topography, and rate of growth of each culture were considered for macroscopic identification. 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 was compared with established characteristics of fungal features using mycology atlases.

Yeast identification

Yeasts were identified by employing an array of standard biochemical and assimilation test procedures. Germ tube production test and CHROM agar Candida culture medium (Becton Dickinson, Paris, France) as per the instruction of the manufacturer were also used in yeast identification.

CHROMadar Candida culture medium (Becton Dickinson, Paris, France)

CHROMagar Candida, that can be used for the isolation and presumptive identification of C. albicans, C. krusei, and C. tropicalis and the differentiation of these species from other yeasts on the basis of strongly contrasted colony colors produced by reactions of species-specific enzymes with a proprietary chromogenic substrate. The medium greatly facilitates the detection of specimens containing mixtures of yeast species.

4.8. Data Quality Assurance

Pre-analytical

A sterile sputum collection cup was issued to patients. Patients were asked to produce sputum from deep-by-deep breathing three times and coughing to expectorate purulent sputum. Collected sputum samples were inspected for eligibility. Reagents used for GeneXpert MTB/RIF assay, CHROM agar, and BHI media preparation were checked for expiry date and any abnormal color change. Preventive maintenance of equipment was inspected.

Analytical

Test procedures for each test were strictly followed according to the standard operation procedures of the tests. Media preparation was performed according to the manufacturer's manual. Media was checked for sterility by incubating at 25-37^{o}c for weeks. If there is any kind of microbial growth, the prepared batch is discarded. The growth support of each batch of prepared media was checked with known representative yeast species.

Post analytical

The results generated were appropriately documented electronically. To prevent loss of data backup data were also documented on the result registration logbook. And also isolates were

kept. An appropriate disposal system by incineration was utilized after the disinfection of Specimens and cultures of organisms by autoclaving.

4.9. Data Analysis and Interpretation

SPSS version 22 was used to code, enter, and analyse all of the investigation's data. The chi-square test ($\chi 2$) is used to compare descriptive statistics. p-values lower than 0.05 were considered important. Finally, the results were presented in words and tables.

4.10. Operational Definitions

- **Multi-drug resistant TB** - TB that does not respond to at least isoniazid and rifampicin, of the two most powerful anti-TB drugs [29].
- **XDR-TB**- A rare type of MDR TB that is resistant to isoniazid and rifampicin, plus any fluoroquinolone and one of injectable second line drugs (that is: kanamycin, capreomycin, amikacin) [60].
- **Co-infection**: The presence TB and fungal infection on the same host.
- **Presumptive TB patient**; Tuberculosis suspected patient.
- **Recurrent TB (relapse/re-infection)**- Recurrent TB disease is defined as the diagnosis of a subsequent episode of TB following treatment completion or cure at the end of the most recent course of treatment [77].

4.11. Ethical Considerations

Ethical considerations and obligations were duly addressed, and the study was conducted after received approval letter from a department research and ethical review committee (DRERC) of the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. All procedures performed in studies involving human participants were under the ethical standards of the department research and ethical review committe of the Department of Medical Laboratory Sciences and with the 1964 Helsinki Declaration and its later amendments. Before the collection of data, the participants provided written informed consent. Every responder was granted the freedom to decline participation in the study and to leave at any point while it was being conducted. To ensure confidentiality, every piece of information collected from the research participants was coded. The hospital doctor notified the participants and ensured they received appropriate therapy upon confirmation of a fungal infection test result.

4.12. Dissemination of the Result

The result was submitted to the Addis Ababa University, College of Health Science, Department of Medical Laboratory Science. The findings will be presented at national and international scientific conferences. It will also be sent/ submitted to different peer-reviewed journals for publication.

5. Result

5.1 Characteristics of study participants with mycobacterial and fungal sputum sample

Five hundred thirty (530) subjects with signs and symptoms of lower respiratory tract infections underwent both tuberculosis and fungal infections of which 263 (49.6%) were male. The participant's mean age was 48.9 years, with minimum and maximum ages of 16 and 94 years, respectively. Of a total of 530 sputum samples collected and examined for lower respiratory tract infections 42 (7.9%) were GeneXpert positive in which 23 patients were infected with TB only and 19(3.9%) patients were TB coin-infected with fungal pathogens. Similarly, out of 530 sputum samples collected and examined for lower respiratory tract infections 189 (36.7%) were culture-positive for fungal species,170 (32.1%) patients were infected with fungal pathogen only and 19 (3.9%) patients were TB co-infected with fungal pathogens. Our study demonstrated that the prevalence of lower respiratory infection was 318 (60.0%) while that of tuberculosis and fungal infections was 42 (7.9%) and that of fungal infections was 189(36.7%), respectively (Table 1). Of the GeneXpert positive tests, four were rifampicin resistance while two were MDR (Data not shown).

Table 1 Gender and age profile of study participants in relation to identified pathogens of sputum samples

Variables	Frequency (%) (n=530)	Not identified TB or fungi	TB-positive only	Fungal-positive only	Co-infection of TB & fungal infection	*P-value*
Gender						
Male	263(49.6)	162	15	71	15	0.219
Female	267 (50.4)	156	8	99	4	
Total	530	318	23	170	19	
Age groups in years						
15-24	42 (7.9)	19	3	18	2	0.003
25-34	87(16.4)	50	6	25	6	
35-44	83 (15.7)	49	5	26	3	
45-54	85 (16.0)	51	7	25	2	
55-64	105(19.8)	66	0	36	3	
>65	128(24.2)	83	2	40	3	

Signs and Symptoms of study participants at the presentation are shown in Table 2. At the time of presentation, 527 (99.2%) patients had productive cough. Other common signs and symptoms were night sweating (49.9%) chest pain (47.8%), loss of appetite (28.8%), and weight loss (28.8%). Haemoptysis (6.0%) and fever (5.1%) were the least.

Table 2 Signs and Symptoms of the study participants at presentation in relation with identified pathogens of sputum samples

Variables	Frequency (%) (n=530)	Not identified TB or fungi	TB-positive only	Fungal-positive only	Co-infection of TB & fungal infection	*P- value*
Productive cough	527 (99.2)	84	23	169	19	0.671
Shortness of breath	91 (17.1)	38	4	38	11	0.000
Chest pain	254 (47.8)	149	14	81	10	0.340
Haemoptysis	32 (6.0)	14	3	12	3	0.010
Fever	27 (5.1)0	12	3	9	3	0.007
Weight loss	131 (24.7)	63	11	50	7	0.000
Loss of appetite	153 (28.8)	83	10	51	9	0.013
Night sweating	265 (49.9	160	16	78	11	0.448
Easily fatigued	125 (25.5)	60	10	47	8	0.001

Clinical chest x-ray interpretation of study participants is shown in Table 3. All (100%) study subjects had chest radiographs. Chest radiographs at presentation were normal in 80.6%; 8.5% had cavity lesions, 4.2% had infiltrates and/or consolidations, 4.5% had fibrosis, and 2.1% had bronchitis. The distribution of organisms identified in sputum differed across categories of chest radiographs ($p<0.001$). Among the subsets of study subjects with normal chest radiographs, 10 patients were identified with TB only,130 patients with fungi only, and 3 patients with TB coinfected with fungi. The presence of a cavity lesion was the most common radiographic abnormality among all patients (8.5%).

Table 3. Chest radiographs at presentation in relation with identified pathogens of sputum samples.

Variables	Frequency (%) (n=530)	Not identified TB or fungi	TB-positive only	Fungal-positive only	Co-infection of TB & fungal infection	*P- value*
Normal	427 (80.6)	284	10	130	3	<0.001
Fibrosis	24 (4.5)	10	2	11	1	
Cavity lesion	45 (8.5)	10	9	13	13	
Bronchitis	11 (2.1)	5	0	5	1	
Infiltrate /consolidation	23 (4.2)	9	2	11	1	

Risk factors recorded at presentation are shown in Table 4. About 14 (2.6%) study subjects had known HIV infection and 21 (4.0%) patients had diabetes mellitus. Nine (1.7%) were current cigarette smokers and 6.8% and 1.7% had prior TB treatment once and more than once, respectively. Reported medication use at presentation is shown in the table below, and about 4.1% and 2.3% of study subjects used Anti-TB and antibacterial treatment prior to presentation. other medication used accounts for 60(11.3%) which includes medication for HIV, Diabetes, Hypertension, Cardiac disease, chronic kidney disease, and so on.

Table 4 Potential risk factors for PTB and pulmonary mycosis of study participants recorded at presentation

Variables	Frequency (%) (n=530)	Not identified TB or fungi	TB-positive only	Fungal-positive only	Co-infection of TB & fungal infection	*P-value*
Morbidities						
Hypertension	51(9.6)	31	-	20	-	0.678
Diabetes	21(4.0)	7	-	12	2	
HIV	14(2.6)	9	1	3	1	
Asthma	21(4)	13	-	7	1	
Sinusitis	2(4)	0	-	2	-	
Other chronic infection	8(1.5)	6	-	2	-	
Current smoking						

Yes	9(1.7)	2	-	6	1	0.048
No	521(98.3)	316	23	164	18	
Previous pulmonary TB						
Recurrent TB	45(8.6)	17	4	18	6	< 0.001
Number of times TB treated						
Once	36(6.8)	16	4	12	4	0.381
More than once	9(1.7)	2	-	5	2	
Antimicrobials prior to presentation						
Anti-TB	22(4.1)	12	3	6	1	0.293
Anti-bacteria	12(2.3)	7	-	4	1	
Other medications	60(11.3)	31	1	27	1	

5.2 The spectrum of fungal isolates

Of the 530 sputum cultures used in this investigation, 189 had potential fungal pathogens, and 24 samples produced more than one species of fungal. A total of 241 fungal isolates in all were found. Among the isolates, 183 (75.9%) contained yeasts, including *Cryptococcus neoformans* 4 (2.2%), *Candida albicans* 92 (50.3%), *Candida tropicalalis* 43 (23.4%), and *Candida krusei* 36 (19.7%). The most common species, *C. albicans*, accounted for 50.3% of the yeast isolates. *Aspergillus* spp. (33; 56.9%), *Penicillium* spp. (7; 12.1%), and *Scedosporium apiospermum* (15; 28.9%) were the most common isolates among the remaining 58 (24.1%) mycelial fungus isolates (Table 5).

Table 5- Spectrum of fungal isolates in presumptive Pulmonary Tuberculosis patients.

Fungal species	Single (pure) isolates	Mixed with other fungi isolates	Total isolates (n=530)
Molds			
Aspergillus niger	24	2	26
Aspergillus fumigatus	3	-	3
Aspergillus terreus	3	1	4
Penicillium marneffei	1	-	1
Penicillium spp.	4	2	6
Scedosporium apiospermum	11	3	15
Acremonium spp.	-	1	1
Bipolaris spp.	1	-	1
Alternaria spp.	1	-	1
Mold sub-total	48	10	58
Yeasts			
Candida albicans	59	33	92
Candida krusei	23	13	36
Candida tropicalis	23	20	43
Other NAC spp.	8	-	8
Cryptococcus neoformans	4	-	4
Yeast sub-total	117	66	183

NAC*:* Non albican candida.

Table 5-mixed isolates (continued)

A. niger+Aspergillus terreus	-	-	-
A. niger+Fusarium spp.	-	1	1
A. niger+P. marneffei	-	-	-
A. niger+Penicillium spp.	-	3	3
A. niger+Scedosporium spp.	-	2	2
A. niger+C. neoformans	-	-	-
A. niger+Acremonium spp.	-	2	2
Penicillium spp+Scedosporium spp.	-	1	1

Penicillium spp+Collectricum +Acrimonium spp.	-	1	1
C.albicans+Scedosporium spp.	-	1	1
C.albicans+C.tropicalis+A.niger	-	4	4
C.albicans+C.krusi+A.niger	-	1	1
C.albicans+C.krusi+Pencillium spp.	-	1	1
C.tropicalis+A.niger	-	1	1
C.tropicalis +Scedosporium spp.	-	2	2
C.tropicalis+Scedosporium spp.*+A.niger*	-	1	1
C.krusi+Scedosporium spp.	-	1	1
Other NAC+Alternia spp.	-	1	1
Other NAC +Scedosporium spp	-	1	1
Total mixed culture		24	24

As shown in the above table, out of the total 241 fungal isolates,24 cultures were with mixed isolates. This includes Mold isolates with more than one mycelial species and yeast isolates with more than one species. Or else isolation of Molds and yeast spp from a single culture. *A. niger with* Fusarium spp, *Penicillium* spp, *Scedosporium* spp. *Acremonium* spp were among the common mixed isolates found.

5.3 Pulmonary Tuberculosis and Pulmonary Fungal Co-infection

The co-infection of pulmonary tuberculosis and pulmonary fungal infection in our study was 19(3.7%). As shown in Table 6,17(89.5%) of the fungal pathogens among PTB patients were yeasts while 2(10.5%) were mycelial fungi. Among the yeasts, *Candida albicans* was the most common.

Table 6 Spectrum of fungi in fungal–tuberculosis co-infection patients.

Fungal species	Number of MTB positive samples(n=530)	Percentage (%)
C. albicans	12	63.2
C. krusei	2	10.5
C. tropicalis	2	10.5
Other NAC spp.	-	-
Cryptococcus neoformans	1	5.3
Total yeast species	**17**	**89.5**
Aspergillus spp.	2	10.5
Total mycelial fungi	**2**	**10.5**
Total	**19**	**100**

6. Discussion

An estimated 2.74 million people globally die from lower respiratory tract infections (LRTIs), the most frequent infection in humans. [29]. It comprises a wide range of diseases of which TB and pulmonary fungal infections are the most significant [60]. Among the etiological agents of LRTs *Mycobacterium tuberculosis*, the etiological agent TB is the most significant. Despite all global efforts to eliminate TB, tuberculosis as an infectious disease has remained a worldwide threat. Co-infection of the respiratory tract by fungi and TB has been recognized for its wide range of clinical spectrum and chronicity.

The detection (prevalence) rate of TB in Ethiopia from subjects with signs and symptoms of lower respiratory tract infection was variable. In 2014 the magnitude and incidence of TB in Ethiopia were 211 and 214 per 100,000 populations respectively [61] with a prevalence rate of multidrug resistance TB of 2% among new cases in 2006 and 4.5% among new cases in 2016 [61,62]. Our present study demonstrated a 7.9% prevalence rate of TB which is three times less than the prevalence rate of 27.2% reported by Adane and Solomon in 2021[57]. Further of the Xpert positive tests, 4 were rifampicin resistance while 2 and 1 were 1 MDR respectively. These differences in prevalence status among Ethiopian studies may be explained by the fact that Ethiopia has achieved most of the Millennium Development Goals targets related to TB. Assefa et al [63] reported that TB incidence rate and TB mortality rate declined by 54% and by 72% respectively during the Millennium Development Goals. Furthermore, the prevalence rate of TB in our study was low compared to studies conducted in Nigeria (45.2%) by Uzoamaka et al [64] and Tunisia (24%) by Fekih et al [65].

As a result of the extensive use of antibacterial drugs and an increasing number of immunocompromised patients, pulmonary fungal infections are becoming more common. However, the occurrence of pulmonary fungal infections and TB co-infection is rarely reported. The most frequently found species among our patients in the current investigation were *Candida albicans* and *Aspergillus niger*. Our result was consistent with published data in Ethiopia [57]. In this study out, of 530 sputum samples collected and examined 43 (7.9%) were Xpert positive in which 19(3.9%) patients were TB co-infected with fungal pathogens. The result of our study disagreed with the findings of Adane and Solomon [57] who reported a 20.0% prevalence rate of TB co-infections with fungal infection. Our findings about the prevalence of fungal co-infection with tuberculosis were in line with those of Sani et al. [66].

Similar investigations by Shome et al. [67] and Bansods et al. [68] found a prevalence of TB fungal infections in the range of 18% to 40%.

Assessment of signs and symptoms at presentation depicted that cough was the commonest sign and symptom accounting for 99.2% and this was consistent with the reports of Ekenna et al. [71] and Attia et al. [69]. Among Chest radiograph abnormality at the presentation 45(8.5%%) patients had cavity lesions where 9 were with TB infections,13 with fungal infections, and, 13 were with TB co-infected with fungal pathogens. Four hundred twenty-seven study participants (80.6%) were normal. Like our result, TB fungal co-infection was the commonest among patients with cavitary lesions on chest radiography [69]. The distribution of organisms identified in sputum differed across categories of chest radiographs ($p<0.001$).

In our study, samples collected from males were slightly less than that of females, and middle-aged to an elderly study subject were dominant. About 4.0% and 2.6% of our study subjects were with diabetes where 12 were with fungal infection, 2 were TB co-infected with fungal pathogens and 7 were with no identified TB or fungi. About 2.6% of our study subjects were with HIV where 1 was TB infected, 1 was TB co-infected with the fungal pathogen, 3 were infected with fungal pathogens and 9 were with no identified TB or fungi.

Tuberculosis co-infected with HIV and TB co-infected with Candidiasis have been the most recurrent co-infections. In some situations, up to about 80% of PTB patients are co-infected with HIV [20], and about 15% - 32% of PTB patients are also co-infected with *Candidiasis* [21]. Our work revealed that among 180 yeast isolates 17 yeast isolates were co-infected with TB. Similarly, out of 58 Mold isolates only two *Aspergillus* spp were co-infected with TB. Regarding *Aspergillus* spp., the result was contrary as many other studies have documented *Aspergillus* species as the major fungus reported in patients with PTB [70,71]. Among the 19 patients with pulmonary tuberculosis, *Candida* co-infection was observed in 16 (84.2%) of our patients. *Candida albicans* was the most common isolate observed in 63.2% of the patients with co-infection, *C. tropicalis* came next. (10.5%) and *C. krusei* (10.5%). Numerous writers from around the globe have reported that *Candida* species is the most common fungus identified in TB patients' sputum. Amala et al [55] demonstrated that among 93 TB patients, 32 (34.4%) patients were co-infected with *C. albicans* as the predominant species with the isolation of 61.8%. Ndukwu et al [72] reported a prevalence rate of 25.3% TB Candida co-infection and *Candida albicans* were the most prevalent species. Kali et al [20]

depicted that among 75 patients with TB, Candida co-infection was seen in 30 (40%) of patients with pulmonary tuberculosis. *Candida albicans* is the most frequent species seen in 50% of the patients. The prevalence of TB/Candida co-infection is extremely high ranging from 45% - 92% in several Indian studies [73]. Several *non-Albicans Candida* species are also reported more frequently, even though Candida albicans remain the predominant species in pulmonary candidiasis [57,55,75].

Although several researchers have identified *Candida* species as the most often occurring fungus species found in TB patients' sputum, there has long been debate on the significance of this species given that up to 32.5% of healthy people have *Candida* in their respiratory tracts. This may contaminate the specimens of sputum during the sample collection process. [74]. Bronchoscopy has been suggested as one of the different approaches to reduce this problem [75]. Yet in developing countries such as Ethiopia bronchoscopy has not been always effective.

7. Strengths and Limitations of the Study

7.1 Strength of the study

This study addressed the status of tuberculosis and fungal co-infection of the study participants at a time. The study also determines the drug resistance tuberculosis (RR, INH monoresistance, MDR, and pre-XDR) of our study participants. In addition, this paper tried to assess potential risk factors of pulmonary mycosis which is the most essential thing to be considered.

7.2 Limitations of the study

The limitation of the study was that we couldn't ascertain the anti-fungal susceptibility test of the fungal isolates due to a lack of equipment and resources. The limitation of our study was we couldn't discuss our findings well with other studies since studies in this area are limited.

8. Conclusion and Recommendation

8.1 Conclusion

Lower respiratory tract infection (LRTI) is a disease of the respiratory tract predominantly of the bronchi and the lungs pulmonary tuberculosis is one of the most common diseases in developing nations and is associated with low socioeconomic status. Since the major etiological agents of LRTI are TB, bacterial and fungal pathogens, our study tried to investigate the prevalence of PTB and its co-infection with fungal pathogens from a sputum sample collected from PTB perceptive individuals visited St. Peters' specialized hospital's TB laboratory during the data collection period.

Among patients with symptoms of respiratory infections tested for TB and fungal pathogens 42 subjects had tuberculosis, 23(4.3%) were infected with TB only and 19 (3.9%) patients were TB co-infected with fungal pathogens. Among fungal isolates, 183 (75.9%) were yeasts while 58 (24.1%) isolates were molds. Among yest isolates, *C. albicans* were predominantly isolated Age of the study participants, smoking habit, chest radiograph interpretation and previous TB history had a significant association with pulmonary TB and pulmonary fungal infection. And there were no statistical association with PTB and pulmonary mycosis in this study. The existence of fungal pathogens in TB patients poses a significant risk of making the patients more devastated due to the pathogenic synergism existing among the infections thereby escalating TB morbidity and mortality.

8.2 Recommendation

Although the prevalence rate of the co-infections was low, it is recommended if TB patients should be concomitantly tested for fungal pathogens. And further studies should be done in this area for a better understanding.

9. References

1. World Health Organization (WHO). Global Tuberculosis Report 2019. Geneva: WHO, 2019.
2. Sharma SK, Mohan A. Multi-drug resistant tuberculosis. Indian J Med Res 2004; 120: 354–376.
3. World Health Organization (WHO). Global tuberculosis report 2013: Geneva, 2013.
4. Khan R, Malik NI, Razaque A. Imaging of pulmonary post-tuberculosis sequelae. Pakistan J Med Sci. 2020; 36: S75–S82. https://doi.org/10.12669/pjms.36.ICON-Suppl.1722 PMID: 31933611.
5. Smith I. Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence. Clin Microbial Rev 2003 ;16(3):463–496.
6. Loddenkemper R, Lipman M, Zumla A. Clinical aspects of adult tuberculosis. Cold Spring Harb Perspect Med 2016 ;6(1): a01784
7. Tadolini M, Lingtsang RD, Tiberi S, Enwerem M, D'Ambrosio L, Sadutshang TD, et al. The first case of extensively drug-resistant tuberculosis was treated with both delamanid and bedaquiline. Europe Resp J. 2016;48(3):935.
8. Tavanaee Sani A, Ashna H, Kaffash A, Khaledi A, Ghazvini K. Mutations of *rpob* Gene Associated with Rifampin Resistance among *Mycobacterium Tuberculosis* Isolated in Tuberculosis Regional Reference Laboratory in Northeast of Iran during 2015-2016. Ethiop J Health Sci. 2018 ;28(3):299-304. doi: 10.4314/ejhs.v28i3.7. PMID: 29983529; PMCID: PMC6016358.
9. World Health Organization (WHO). Guidelines for the treatment of drug-susceptible tuberculosis and patient care, 2017 update. 2017.
10. Ghanei M, Peyman M, Aslani J, Zamel N. Mounier-Kuhn syndrome: a rare cause of severe bronchial dilatation with normal pulmonary function test: a case report. Respir Med. 2007;101(8): 1836-1839. PubMed| Google Scholar
11. Maitland K, Kiguli S, Opoka RO, Olupot-Olupot P, Engoru C, Njuguna P et al. Children's Oxygen Administration Strategies Trial (COAST): a randomized controlled trial of high flow versus oxygen versus control in African children with severe pneumonia. Wellcome Open Res. 2017;2: 100. PubMed| Google Scholar
12. WHO: Forum of International Respiratory Societies. The global impact of respiratory disease - second edition. Sheffi eld, European Respiratory Society. 2017;2.

13. Dube FS, Kaba M, Robberts FJ, Ah Tow L, Lubbe S, Zar HJ et al. Respiratory microbes present in the nasopharynx of children hospitalized with suspected pulmonary tuberculosis in Cape Town, South Africa. BMC Infect Dis. 2016;16(1): 597. PubMed| Google Scholar
14. Crampton J. Why nurses should use clinical reasoning to diagnose a cough? Primary Health Care. 2013;23(7): 18-24. Google Scholar
15. Yao Z and Liao W. Fungal respiratory disease. Curr Opin Pulm Med 2006; 12: 222–227.
16. Kronstad JW, Attarian R, Cadieux B. Expanding fungal pathogenesis: Cryptococcus breaks out of the opportunistic box. Nat Rev Microbiol 2011; 9: 193–203.
17. Kronstad ZF, Doshi AV and Bhaduri AS. Pneumocystis pneumonia. N Engl J Med 2004; 351: 12623.
18. Lortholary O, Denning DW, and Dupont B. Endemic mycoses: a treatment update. J Antimicrob Chemother 1999; 43: 321–331.
19. Nucci M. Emerging fungal diseases. Clin Infect Dis 2005; 41: 521–526.
20. WHO Multidrug-Resistant Tuberculosis (MDR-TB) Indicators (2010) A Minimum Set of Indicators for the Programmatic Management of MDR-TB in National Tuberculosis Control Program. WHO/HTM/TB/2010.11, World Health Organization, Geneva.
21. Kali, A., Charles, M.P., Noyal, M.J., Sivaraman, U., Kumar, S. and Easow, J.M. (2013) Prevalence of Candida Co-Infection in Patients with Pulmonary Tuberculosis. Australasian Medical Journal, 6, 387-391. https://doi.org/10.4066/AMJ.2013.1709
22. Denning DW, Pleuvry A and Cole DC. Global burden of chronic pulmonary aspergillosis as a sequel to pulmonary tuberculosis. Bull World Health Organ 2011; 89: 864–872.
23. Kaur R, Dhakad MS, Goyal 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.
24. WHO: Forum of International Respiratory Societies. The global impact of respiratory disease - second edition. Sheffi eld, European Respiratory Society. 2017;2 24
25. Bongomin F, Gago S, Oladele RO, Denning DW. Global and Multi-National Prevalence of Fungal Diseases-Estimate Precision. J. Fungi 2017, 18, 57. 25
26. Mandengue CE, Ekeng BE, Oladele RO. Disseminated Histoplasmosis; A threat in advanced HIV Disease population in sub-Saharan Africa. J. Adv. Med. Med. Res. 2021, 33, 115–144.

27. Ekeng B.E, Edem K, Akintan P., Oladele R. Histoplasmosis in African children: Clinical features, Diagnosis and Treatment. Ther. Adv. Infect. Dis. 2022, 9, 20499361211068592. 27
28. Houben RMGJ, Lalli M, Kranzer K, Menzies NA, Schumacher SG, Dowdy DW. What if they don't have tuberculosis? The consequences and trade-offs involved in false-positive diagnoses of tuberculosis. Clin. Infect. Dis. 2019, 68, 150–156. 28
29. Xpert® MTB/RIF 1 301-1404, Rev. G July 2020
30. Collaborators GL. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: A systematic analysis for the Global Burden of Disease Study 2015. Lancet Infect. Dis. 2017, 17, 1133–1161
31. Nowicki, J.; Murray, M.T. Bronchitis and Pneumonia. Textbook of Natural Medicine; Elsevier: Amsterdam, The Netherlands, 2020; pp. 1196–1201.e1. [CrossRef]
32. Gebre AB, Begashaw TA, Ormago MD. Bacterial profile and drug susceptibility among adult patients with community-acquired lower respiratory tract infection at tertiary hospital, Southern Ethiopia. BMC Infect. Dis. 2021, 21, 440
33. Mishra S, Kattel H, Acharya J, Shah N, Shah A, Sherchand J, et.al. Recent trend of bacterial etiology of lower respiratory tract infection in a tertiary care center of Nepal. Int. J. Infect. Microbiol. 2012, 1, 3–8
34. Behera B, Sahu K, Bhoi P, Mohanty J. Prevalence and antimicrobial susceptibility patterns of bacteria in ICU patients with lower respiratory tract infection: A cross-sectional study. J. Acute Dis. 2020, 9, 157–160.
35. Singh S Sharma A, Nag V. Bacterial pathogens from lower respiratory tract infections: A study from Western Rajasthan. J. Fam. Med. Prim. Care 2020, 9, 1407–1412.
36. Gagneux S. Ecology and evolution of Mycobacterium tuberculosis. Nat Rev Microbiol 2018; 16:202-13
37. Achtman M. Evolution, population structure, and phylogeography of genetically monomorphic bacterial pathogens. Annu Rev Microbiol 2008; 62:53-70
38. World Health Organization. Global Tuberculosis Report 2013
39. World Health Organization. Global Tuberculosis Report 2022; ISBN 978-92-4-0061729
40. MacNeil A, Glaziou P, Sismanidis C, Maloney S, Floyd K. Global epidemiology of tuberculosis and progress toward achieving global targets – 2017. MMWR Morb Mortal Wkly Rep 2019; 68:263-6.

41. Lin P L, and Flynn J L. The end of the binary era: revisiting the spectrum of tuberculosis. J. Immunol. 2018; 201, 2541–2548. doi: 10.4049/jimmunol.1800993
42. Nasehi M, Mirhaghani L. National tuberculosis control guide. 2nd Tehran: Andishmand Publisher. 2009:5-21.
43. CDC-TB risk factors. (2024) https://www.cdc.gov/tb/risk-factors/index.html
44. World Health Organization, Global Tuberculosis Report 2023; Drug resistance TB.
45. Guidelines for Clinical and Programmatic Management of TB, TB/HIV, DR-TB and Leprosy in Ethiopia,2021.
46. Reta, M.A.; Tamene, B.A.; Abate, B.B.; Mensah, E.; Maningi, N.E.; Fourie, P.B. Mycobacterium tuberculosis Drug Resistance in Ethiopia: An Updated Systematic Review and Meta-Analysis. Trop. Med. Infect. Dis. 2022, 7, 300. https://doi.org/ 10.3390/tropicalmed7100300
47. Global action for fungal infection (GAFF), annual report 2022.
48. The Fungal Infection Trust. How common are fungal diseases? Fungal Research Trust 20th Anniversary meeting. London June 18th, 2011, updated August 2017.
49. Felix Bongomin, Sara Gago, Rita O. Oladele and David W. Denning.Global and Multi-National Prevalence of Fungal Diseases—Estimate Precision, *J. Fungi* 2017, 3, 57; doi:10.3390/jof3040057
50. Weldemhret T. Burden of pulmonary tuberculosis and challenges related to tuberculosis detection in Ethiopia, J Infect Dev Ctries 2023; 17(5):578-582. doi:10.3855/jidc.13169
51. Iliyasu G, Mohammad AB, Yakasai AM, Dayyab FM, Oduh J, Habib AG. Gram-negative bacilli are a major cause of secondary pneumonia in patients with pulmonary tuberculosis: Evidence from a cross-sectional study in a tertiary hospital in Nigeria. Trans R Soc Trop Med Hyg 2018; 112:252-4.
52. Moore DP, Klugman KP, Madhi SA. Role of Streptococcus pneumoniae in hospitalization for acute community-acquired pneumonia associated with culture-confirmed Mycobacterium tuberculosis in children: A pneumococcal conjugate vaccine probe study. Pediatr Infect Dis J 2010; 29:1099-4
53. Langbang A, Deka N, Rahman H, Kalita D A study on bacterial pathogens causing secondary infections in patients suffering from tuberculosis and their pattern of antibiotic sensitivity. Int J Curr Microbiol Appl Sci 2016; 5:197-203.
54. Shimazaki T, Taniguchi T, Saludar NR, Gustilo LM, Kato T, Furumoto A, et al. Bacterial co-infection and early mortality among pulmonary tuberculosis patients in Manila, The Philippines. Int J Tuberc Lung Dis 2018; 22:65-72.

55. Ekeng B E, Davies AA, Osaigbovo II, Warris A. Oladele R.O and D.W. Pulmonary and Extrapulmonary Manifestations of Fungal Infections Misdiagnosed as Tuberculosis: The Need for Prompt Diagnosis and Management. J. Fungi 2022, 8, 460. https://doi.org/10.3390/ jof8050460
56. Amala SE, Hanson A, Wokem GN. Candida Co-Infection with Mycobacterium tuberculosis in Tuberculosis Patients and Antifungal Susceptibility of the Isolates. Journal of Tuberculosis Research. 2020; 8: 53-65. [C 11123]
57. Muluneh T. Spectrum of fungal pathogens in sputum samples from pulmonary tuberculosis suspected patients at Kotebe Primary Hospital Addis Ababa Ethiopia. Master Thesis, http:// www.etd.aau.edu.et/
58. Bitew A, and Bati S. 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. SAGE Open Medicine .2021; Volume 9: 1–9
59. Cao Y, Parmar H, Gaur RL, Lieu D, Raghunath S, Via N, Battaglia S, Cirillo DM, Denkinger C, Georghiou S, Kwiatkowski R, Persing D, Alland D, Chakravorty S. Xpert MTB/XDR: a 10-Color Reflex Assay Suitable for Point-of-Care Settings To Detect Isoniazid, Fluoroquinolone, and Second-Line-Injectable-Drug Resistance Directly from Mycobacterium tuberculosis-Positive Sputum. J Clin Microbiol. 2021 Feb 18;59(3):e02314-20. doi: 10.1128/JCM.02314-20. PMID: 33298611; PMCID: PMC8106700.
60. Xpert® MTB/XDR 1 302-3514, Rev. C April 2021
61. Mahashur A. Management of lower respiratory tract infection in outpatient settings: focus on clarithromycin. Lung India. 2018;35(2): 143. PubMed| Google Scholar
62. WHO. Ethiopia Tuberculosis Progress in 2014, World Health Organization (WHO). http://www.afro.who.int/en/ethiopia/countryprogrammes/topics/4481tuberculosis.htm 2015
63. Eshetie S, Gizachew M, Dagnew M, Kumera G, Woldie H, Ambaw F, et al. Multidrug-resistant tuberculosis in Ethiopian settings and its association with previous history of anti-tuberculosis treatment: a systematic review and meta-analysis. BMC infectious diseases. 2017;17(1):219.

64. Assefa Y, Damme WV, Williams OD, Hill PS. Successes and challenges of the millennium development goals in Ethiopia: lessons for the sustainable development goals. BMJ global health. 2017;2(2):e000318.
65. Uzoamaka M, Ngozi O, Johnbull OS, Martin O. Bacterial etiology of lower respiratory tract infections and their antimicrobial susceptibility. Am J Med Sci. 2017;354(5): 471 475. PubMed| Google Scholar
66. Fekih Hassen M, Ben Haj Khalifa A, Tilouche N, Ben Sik Ali H, Ayed S, Kheder M, et al. Severe community-acquired pneumonia admitted at the intensive care unit: Main clinical and bacteriological features and prognostic factors: a Tunisian experience. Rev Pneumol Clin. 2014;70(5): 253-259. PubMed| Google Scholar
67. Sani FM, Uba A, Tahir F, Abdulahi IN, Akedola HA, Mustaph Jet al. The spectrum of pulmonary fungal pathogens, associated risk factors, and anti-fungal susceptible pattern among persons with presumptive tuberculosis at Gombe, Nigeria. Int J of Mycobacteriol 2020; 9: 144–149. 22.
68. Shome SK, Upreti HB, Singh MM, Pamra SP. Mycoses associated with pulmonary tuberculosis. Int J Tuberc. 1976; 23:64-8.
69. Bansods RM. Emerging of mycotic infection in patients infected with mycobacterium tuberculosis world. J Med Sci 2008; 3: 74–80.
70. Attia EF, Pho Y, Nhem S, Sok C, By B, Phann D et al. Tuberculosis and other bacterial co-infection in Cambodia: a single center retrospective cross-sectional study. BMC Pulm Med. 2019;19(1): 60.
71. Kurhade AM, Deshmukh JM, Fule RP, Chande C, Akulwar S: Mycological and serological study of pulmonary aspergillosis in central India. Indian J Med Microbiol. 2002, 20:141-4. 18.
72. Ekenna O, Uba A, Chikwem JO, Mambula S, Aliyu MB, Mohammed I: Relevance of moldy fungi as agents of chronic lower respiratory tract infection in patients seen in Maiduguri, Nigeria. West Afr J Med. 2007, 26:117-20.
73. Ndukwu, C.B., Mbakwem-Aniebo, C. and Frank-Peterside, N. (2016) Prevalence of Candida Co-Infection among Patients with Pulmonary Tuberculosis in Emuoha, Rivers State, Nigeria. Journal of Pharmacy and Biological Sciences, 11, 60-63.
74. Baradkar, V.P., Mathur, M., Wanjari, K. and Kumar, S. (2009) Candida in Pulmonary Tuberculosis. Bombay Hospital Journal, 51, 52-53.
75. Santiwongkarn P, Kachonboon S, Thanyasrisung P, Matangkasombut O. Prevalence of oral Candida carriage in Thai adolescents. J Investig Clin Dent. 2012;3: 51-5. 13.

76. El-Ebiary M, Torres A, Fàbregas N, de la Bellacasa JP, González J, Ramirez J, et al. Significance of the isolation of Candida species from respiratory samples in critically ill, non-neutropenic patients: An immediate postmortem histologic study. Am J Respir Crit Care Med. 1997 Aug;156::583-90.
77. Naidoo K, Dookie N. Insights into Recurrent Tuberculosis: Relapse Versus Reinfection and Related Risk Factors [Internet]. Tuberculosis. InTech; 2018. Available from: http://dx.doi.org/10.5772/intechopen.73601
78. Sagar Aryal.Brain Heart Infusion (BHI) Agar- Composition, Principle, Preparation, Results, Uses [Internet]. *Microbe Notes;*2022, Available from: https://microbenotes.com/brain-heart-infusion-bhi-agar/

10. Annex

Annex I. Participant Information Sheet

Participants' Information Sheet in English Version

Title of the Research Project: Prevalence of Pulmonary Tuberculosis and Its Co-infection with Fungal Pathogens in Patients with Lower Respiratory Tract Infections referred to St. Peter's Specialized Hospital, Addis Ababa, Ethiopia.

Principal Investigator: Zenawit Lakew (BSc, MSc candidate)

Name of the Organization: Addis Ababa University, College of Health Sciences School of Medical Laboratory.

Introduction: 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. The similarity in the clinical and radiological presentation of pulmonary fungal infection and PTB has made definite diagnoses between these two infections difficult. So, the result of the study can be helpful in planning and intervention to solve such diagnosis and treatment problems.

Purpose of the Research Project: To determine the status of pulmonary tuberculosis, drug-resistant TB, TB coinfected with pulmonary fungal pathogens, and fungal infections misdiagnosed as tuberculosis in the sputum of patients with lower respiratory infections St. Peter's Specialized Hospital…

Duration: The study was carried out at the Saint Peter's Specialized Hospital located in the Addis Ababa Administrative region between November 2023 and May 2024.

Confidentiality: We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be protected by a password on the computer only accessible to personnel involved in the study. There is no sensitive issue that you will be asked related to your social interest but any information that is obtained in connection with this study will remain confidential.

Potential benefits to subjects and society: - You will not receive any payment for your participation in this research. The finding of the study is useful for creating a better understanding of the misdiagnosis of pulmonary mycosis with PTB You will also obtain all the

results of the analysis and communicate to your physician for the appropriate treatment. Participation and Withdrawal from the Study: Participation is voluntary and you have the right not to participate in this study. Refusal to participate will not result in loss of medical care provided or any other benefits.

Contact information

If you have any questions about this study, you can contact the following principal investigator and advisor for further information.

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For additional information, please contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences by Telephone at .+251112755170 Your signature below indicates that you have read/or listened, to and understand the information provided for you about the study. Before you sign, please understand the purpose of the study, procedure, risks and benefits of participation, right to refuse or withdraw, confidentiality and privacy, and who to contact if you have any questions.

I have read/ or listened to the description of the study and I understand what procedures are

and what will happen to me in the study.

Agree to participate? Yes----------No----------

Annex II. Informed Consent Form for Adults (in English Version)

Participant code: _____

The objective and the application of the study were briefly explained to me. I am also informed that my demographic and clinical data was used for this research purpose from the laboratory request form and they were kept confidential. Moreover, I have been well informed of my right to refuse information, decline to cooperate, and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care. It is therefore with full understanding of the situation that I agreed to give the informed consent voluntarily to the researcher to give my specimen for the mentioned study.

Participant name_____ Signature/fingerprint: _____Date_____

Witness's name _____Signature/fingerprint: _____ Date_____

Investigator's name_____ Signature/fingerprint: _____Date_____

Annex III. Amharic Versions of Patient Information Sheet

እኔ ተማሪ ዜናዊት ላቀዉ በአዲስ አበባ ዩኒቨርሲቲ ፣ጤና ሳይንስ ኮሌጅ ፣የህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል የሁለተኛ ዲግሪ ተማሪ ስሆን የምርምር ስራን በመስራት ላይ እገኛለሁ።እርስዎም በዚህ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል ።በጥናቱ ለመሳተፍ ፈቃደኛ ሆነዉ ከተስማሙ መስማማትዎን የሚያሳይ ወረቀት ላይ እንዲፈርሙ እጠይቃሁ።

መግቢያ

Prevalence of Pulmonary Tuberculosis and its co-infection with Fungal pathogens in patients with the lower respiratory tract infections referred to St. Peter's Specialized Hospital, Addis Ababa, Ethiopia.፣ ማለትም የመተንፈሻ አካል የሚያጠቃ የሳምባ ነቀርሳ እና በመተንፈሻ አካላት ላይ በሽታን የሚያመጡ ፈንገሱች ስርጭት በታችኛው የመተንፈሻ አካል በሽታ የተጠቁ ግለሰቦች ላይ በሚል ርዕስ እያጠናሁ እገኛለሁ ።ይህ ጥናትም በተሳታፊ ሙሉ ፈቃደኝነት ላይ የተመሠረተ ነዉ። ከጥናቱ ተሳታፊ የሚጠበቁ በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ የአክታ ናሙና እንዲወሰድ መስማማት ይጠበቅቦታል።የጤና ባለሙያ ከእርሰዎ ናሙናዉን ይሰበስባል።ከተወሰደዉም ናሙና ላይ የሚገኙ መረጃዎች የእርሶን ማንነት የማይገልጹ ማስረጃዎችን ማለትም ስም፣ አድራሻና የመሳሰለት መረጃዎች ሳይጨምርና ለዚህ ጥናት አገልግሎት ብቻ የሚዉል መለያ ቁጥር በመጠቀም ከዚህ ሆስፒታል ዉጭ ለሚገኙ ለሥራ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅቦታል ።ናሙና ሰጡ ማለት በሽታው ይገኝብዎታል ማለት አይደለም።በእርሰዎ ናሙና ውስጥ የበሽታ አምጭ ተህዋስ ቢገኝ ከጤና ባለሙያዉ አስፈሊጊውን ህክምና ያገኛሉ።የመረጃዉ ሚስጥራዊነት ማንኛዉም የሰጡት መረጃ እና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ ።ይህን ማህደር ሊያገኙ የሚችሉ የተወሰኑ የጥናቱ ተባባሪ ሠራተኞች ብቻ ናቸዉ።ከዚህም በላይ ስለ እርስዎ ያለዉን ማንኛዉንም መረጃ የይለፍቃል ባለዉ የኮምፒዉተር የመረጃ ማህደር ዉስጥ እንዲቀመጥ ይደረጋል ።ተሳታፊዉ የሚያጠፋዉ ጊዜ የተዘጋጀዉን የስምምነት ቅጽ ለመፈረምና ናሙና ለመስጠት 3-7 ደቂቃ ያስፈልጋል ። በጥናቱ በመሳተፍ የሚስከትላቸዉ ችግሮች ፡- ናሙና በሚሰበሰብበት ወቅትምነም አይነት ችግር አያስከትልቦትም።

በጥናቱ በመሳተፍ የሚያስከትላቸዉ ጥቅሞች፡- ይህ ጥናት የማስተርስ ዲግር መመረቂያ እንደ መሆኑ መጠን በመሳተፍዎ የሚያገኙት የገንዘብ ጥቅማጥቅም የለም።ሆኖም በቀኑ በሚደረገዉ የላቦራቶሪ

ምረመራ ለሀኪምዎ በመስጠት አስፈላጊዉን ህክምና እንዲያገኙ ይደረጋል እንደ አስፈላጊነቱም የካልቸር ዉጤቶትንም ጠብቆ አስፈላጊዉን ምርመራ ይደረግሎታል።ለወደፊት በተመሳሳይ ሁኔታ ዉስጥ ላሉ በሽተኞች በመረጃ ላይ የተመሰረተ ህክምና ለመስጠት ያግዛል ከፈለጉ የላቦራቶሪ ዉጤቶችን በነፃ ያገኛሉ እንዲሁም ስለ አስፈላጊዉ ህክምና ከሀኪምዎ ጋር ይነጋገራሉ ።የጥናቱ ተሳታፊዎች መብት ትብብርዎ ሙሉ በሙሉ በፍቃደኝነት ላይ የተመሠረተና ተሳትፎዎን መተውና በማንኛውም ሰዓት ጥናቱን ማቆም ይችላሉ ።በጥናቱ ዉስጥ ያሎትን ተሳትፎ በማንኛዉም ጊዜ የማቋረጥ ሙሉ መብትዎ የተጠበቀ ከመሆኑም በላይ ራሶን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም ዓይነት የሆስፒታል አገልግሎት አይኖርም።ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛዉንም ዓይነት ጥያቄ የመጠየቅና ገለፃ የማግኘት መብት አለዎት።የላቦራቶሪ ምርመራ ዉጤቱንም በነፃ ማግኘት ይቻላል። ግንኙነትና ጥያቄ ይህን ጥናት በተመለከተ ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥዉ ድንገተኛ ችግር ወይም ጥያቄ ካሎት በሚከተሰዉ አድራሻ ይጠቀሙ።

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ለተጨማሪ መረጃ አዲስ አበባ ዩኒቨርሲቲ ፤የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል ይጠይቁ፥ ስልክ-+251112755170 ከዚህ በታች የሚገኘው ፊርማዎ ለእርስዎ የተሰጠውን መረጃ ማንበብዎን፣ መስማትዎን እና መገንዘብዎን የሚያሳይ ነው ።ከመፈረምዎ በፊት እባክዎትን የጥናቱን ዓላማ ፣ የተሳትፎ ጉዳትና ጥቅሙ ፣የመተው ፣የማቋረጥ ፣መብትና ነፃነት እንዳለዎት ይረዱ። ተስማምተዋል?የጥናቱን መግለጫ አንብብያለሁ / ሰምቻለሁ እናም ተረድቻለሁ።መመሪያው ምን እንደሆነና በእኔ ምን ሊከሰት እንደሚችል ተረድቻለሁ ።በጥናቱ ላይ ለመሳተፍ

እስማማለሁ _____ አልስማማም_____

Annex IV. Amharic Versions of Consent Form for Adult Participants

የተሳታፊ ስምምነት ቅጽ

ይህ ‘Prevalence of Pulmonary Tuberculosis and Its Co-infection with Fungal pathogens in patients with the lower respiratory tract infections referred to St. Peter’s Specialized Hospital, Addis Ababa, Ethiopia` ማለትም የመተንፈሻ ኣካል የሚያጠቃ የሳምባ ነቀርሳ እና በመተንፈሻ አካላት ላይ በሽታን የሚያመጡ ፈንገሱች ስርጭት በታችኛው የመተንፈሻ ኣካል በሽታ የተጠቁ ግለሰቦች ላይ በቅዱስ …. ኢትዮጵያ” በሚል ጥናት ላይ ለመሳተፍ ፍቃደኛ ሆነዉ ከተስማሙ መስማማትዎን የሚያሳይ ፊርማ ዶክመንት ላይ እንዲፈርሙ እጠይቃሁ።

1. እኔ የመተንፈሻ ኣካል የሚያጠቃ የሳምባ ነቀርሳ እና በመተንፈሻ አካላት ላይ በሽታን የሚያመጡ ፈንገሱች ስርጭት በታችኛው የመተንፈሻ ኣካል በሽታ የተጠቁ ግለሰቦች ላይ በቅዱስ ….ኢትዮጵያ” የሚለዉ ጥናት አላማ በደንብ ተገንዝቤአለሁ።

2. ከእኔ የሚወሰደዉ ናሙና ለጥናቱ አላማ ብቻ እንደሚዉል ተረድቻለሁ።

3. ሁለም መረጃዎች እና የናሙና ዉጤቱ ምስጢራዊ መሆኑን ተገንዝቤአለሁ ።

4. በጥናቱ ላይ በመሳተፌ ምንም የገንዘብ ክፍያ እንደማላገኝ ተረድቻለሁ።

5. በጥናቱ ያለመሳተፍ እንዱሁም በማንኛዉም ጊዜ የማቋረጥ መብት እንዳለኝ አዉቄአለሁ።

6. ሁለም መረጃዎች በአስተባባሪዉ/ዎች ተገልጾልኝ በደንብ ተረድቻለሁ።

የተሳታፊ ፊርማ፡---------- የተሳታፊ አድራሻ፡----------ቀን፡----

በስምምነቱ ወቅት የነበሩ ምስክሮች

1._____

2._____

ይህንን ጥናት በተመለከተ ጥያቄ ቢኖርዎት ወይም ከዚህ ጋራ በተዛመደ መልኩ ስለሚያጋጥመዎት ድንገተኛ ችግር በሚከተለዉ አድራሻ ይጠቀሙ። ሞባይል፡+251927348648 የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል የጤና ሳይንስ ኮሌጅ ፤ አዲስ አበባ ዩኒቨርሲቲ ኢ-ሜይሌ፤zenawitlakew@gmail.com ለተጨማሪ መረጃ ፡አዲስ አበባ ዩኒቨርሲቲ ፤የሕክምና ላብራቶሪ ሳይንስ ት/ክፍል ይጠይቁ። ስልክ፡+251112755170

Annex V: Demographic and Clinical Data Record Format

Data extraction Questioner

Variables	Mark (X)	Number
Sex		
Male		
Female		
Age		
Morbidities		
Current smoking		
Previous pulmonary TB		
Yes		
No		
Number of times TB treatment		
One		
More than one		
Symptoms at presentation		
Cough		
Shortness of breathing		
Anorexia		
Chest pain		
Fatigue		
Night sweat		

Haemoptysis		
Weight loss		
Fever		
Reported medication used at the presentation		
Antimicrobials prior to presentation		
Antibacterial/ antifungal		
Anti-TB		
Unknown		
Other medications prior to presentation		
Clinical chest X-ray interpretation		
Normal chest x-ray		
Cavitary lesion(s)		
Infiltrates and/or consolidation		
Fibrosis		
Bronchiectasis and/or bronchitis		
Pathogenic organisms identified in sputum sample		
None		
TB only		
Fungal only		
TB and fungal		

Annex VI. Laboratory Standard Operating Procedures and Quality Control

A. Test Principle and Procedure of GeneXpert MTB/Rif Assay

The GeneXpert (Cepheid) is a closed, self-contained platform for the extraction, amplification, and detection of Mycobacterium tuberculosis (MTB) complex from unprocessed samples. The GeneXpert system can generate a result within 2 hours. The Xpert MTB/RIF assay allows for the rapid detection of MTB and rifampicin (RIF) resistance by combining automated extraction, amplification, and detection on a single system. RIF is one of the first-line anti-TB drugs and is also a surrogate marker for multi-drug resistant TB (MDRTB). The assay amplifies a portion of the "rifampicin resistance determining region" of the rpoB gene, the most common site for RIF mutations, in real-time, using two sets of primers. Fluorescent probes are then used to differentiate between wild-type and mutant strains so that if one or more probes don't bind, this indicates the presence of a mutation and RIF resistance. A sample processing control (SPC) consisting of spores from *Bacillus globigii*, is included in the assay as an internal control to ensure adequate processing of the sample as well as to monitor the presence of PCR inhibitors. A probe check control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

Report could be

- MTB DETECTED
- Rif Resistance NOT DETECTED
- Rif Resistance DETECTED
- Rif Resistance INDETERMINATE
- MTB NOT DETECTED

Reagents and Instruments

- Xpert MTB/RIF Assay Cartridges with Integrated Reaction Tubes
- Sample Reagent (Sodium Hydroxide & Isopropanol)
- Disposable Transfer Pipettes

Procedure for raw sputum specimens

1. Label each Xpert MTB/RIF Assay cartridge with the sample ID

2. Carefully open the lid of the sputum collection container.

3. Pour or pipette (pipette not provided) approximately 2 times the volume of the Sample Reagent into the sputum (2:1 dilution, Sample Reagent: sputum).

4. Replace and secure the lid.

5. Shake vigorously 10 to 20 times or vortex for at least 10 seconds

6. Incubate the sample for a total of 15 minutes at 20–30 °C.

7. Between 5 and 10 minutes into the incubation period, shake vigorously 10 to 20 times or vortex for at least 10 seconds.

8. Preparing the Cartridge

9. Open the cartridge lid, and then open the sample container

10. Using the provided transfer pipette, aspirate the liquefied sample close to the line on the pipette.

11. Dispensing the sample slowly to minimize the risk of aerosol formation, transfer the Sample Reagent-treated sample into the sample chamber of the Xpert MTB/RIF cartridge.

12. Close the cartridge lid firmly. The remaining Sample Reagent-treated sample can be kept for up to four hours at 2–8 ºC.

13. Starting the Test

14. Turn on the GeneXpert Instrument System

15. Log on to the GeneXpert Instrument System software using your username and password.
16. In the GeneXpert System window, click Create Test (GeneXpert Dx) or Orders and Order Test (Infinity).

17. Scan or type in the Patient ID (optional). If typing the Patient ID, make sure the Patient ID is typed correctly. The Patient ID is associated with the test results and is shown in the View Results window.

18. Scan the barcode on the Xpert MTB/RIF Assay cartridge. The Create Test window appears. Using the barcode information, the software automatically fills the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date.

19. Click Start Test (GeneXpert Dx) or Submit (Infinity).

20. Viewing and Printing Results.

B. A test principle of GeneXpert MTB/XDR Assay

The Xpert MTB/XDR assay, performed on the GeneXpert Instrument Systems, is a nested real-time polymerase chain reaction (PCR) in vitro diagnostic test for the detection of extensively drug-resistant (XDR) Mycobacterium tuberculosis (MTB) complex DNA in unprocessed sputum samples, concentrated sediments prepared from sputum, or BD™ Mycobacterial Growth Indicator Tube (MGIT™) culture. In specimens where MTB is detected, the Xpert MTB/XDR assay can also detect isoniazid (INH) resistance-associated mutations in the katG and fabG1 genes, oxyR-ahpC intergenic region and inhA promoter; ethionamide (ETH) resistance associated with inhA promoter mutations only; fluoroquinolone (FLQ) resistance-associated mutations in the gyrA and gyrB quinolone resistance determining regions (QRDR); and second-line injectable drug (SLID) associated mutations in the rrs gene and the eis promoter region. The Xpert MTB/XDR assay is intended for use as a reflex test for a specimen (unprocessed sputum, concentrated sputum sediments, or MGIT culture) that is determined to be MTB positive. This test is intended as an aid in the diagnosis of XDR tuberculosis (TB) when used in conjunction with clinical and other laboratory findings.[60].

B. Fungal Identification Sample-

Sputum Collection

- 5-10 ml; early morning before eating
- Use mouth rinse and brush before collection
- Collected in sterile wide-mouthed container Unacceptable sample • Saliva, nasal secretion, throat swab, 24-hour collection

Transport of specimens

1. Specimens should be transported in sterile, humidified, leak-proof containers. Dermatological specimens, however, should be transported in a dry container. Transport medium should not be used unless the specimen can be easily and completely retrieved from

the medium. Although fungi can be recovered at times from specimens submitted in anaerobic transport media, such media should be avoided.

2. Specimens should be processed and inoculated to primary isolation media as soon as possible after collection, ideally within a few hours. It should not be presumed that successful methods for the storage of fungal cultures are suitable for the temporary storage of clinical specimens that harbor relatively few fungal cells.

3. The effect of refrigeration on fungal specimens has not been well studied, but if processing is to be delayed for more than several hours, it is recommended that specimens be stored under refrigeration at 4°C.

Brain Heart Infusion Agar with Chloramphenicol- The medium contains protease peptone and infusions from calf brain and beef heart which serve as sources of carbon, nitrogen, essential growth factors, amino acids, and vitamins. Dextrose is used as a source of energy. Di sodium phosphate helps in maintaining the buffering action of the medium whereas sodium chloride maintains the osmotic equilibrium of the medium.

Directions To rehydrate the medium

1. Suspend 52g in1000 ml, cold freshly distilled water

2. Heat to boiling to dissolve the medium completely

3. Sterilize in an autoclave for 15 minutes at 15 pounds pressure (121o c)

4. Finally add 0.05g chloramphenicol

Urease test Media used in Urease Test: Christensen's Urea Agar It is used to differentiate between *Candida albicans* and *Cryptococcus neoformans*. *C.albican* is urease negative while the *Cryptococcus neoformans* is urease positive.

Principle of Urease Test: Urea is the product of the decarboxylation of amino acids. Hydrolysis of urea produces ammonia and CO2. The formation of ammonia alkalinizes the medium, and the pH shift is detected by the color change of phenol red from light orange at pH 6.8 to magenta (pink) at pH 8.1. Rapid urease-positive organisms turn the entire medium pink within 24 hours. Weakly positive organisms may take several days, and negative organisms produce no color change or yellow as a result of acid production.

Lactophenol cotton blue staining

Lactophenol cotton blue was used for microscopic identification and characterisation of fruiting bodies such AS conidia, sporangia, rhizoids and hypha or mycelia of cultivated fungi on SDA. A drop of lactophenol cotton blue stain was placed on a clean grease-free glass slide. A small fragment of cottony, woolly or powdery colony was picked from the midpoint of the culture using a sterile straight wire and placed on a clean glass slide for the staining process. A clean coverslip was applied avoiding air bubbles. Excess stain was removed with blotting paper and the preparation examined using ×10 and ×40 objectives of the microscope. Fungal element features such as microconidia, macroconidia, chlamydospores and hyphae with spiral, pertinate and antler-like structures were investigated. These features seen on the stained slide were compared with established characteristic fungal features using mycology atlases.

Quality Control

Pre-analytical

A sterile sputum collection cup was issued to patients. Patients were asked to produce sputum from deep-by-deep breathing three times and coughing to produce purulent sputum. Collected sputum samples were inspected for eligibility. Reagents used for GeneXpert MTB/RIF assay, SDA, BHI media preparation, and other reagents were checked for expiry date and any abnormal color change. Preventive maintenance of equipment was inspected.

Analytical

Test procedures for each test were strictly followed according to the standard operation procedures of the tests. Media preparation was performed according to the manufacturer's manual. Medias were checked for sterility by incubating at 25-37 c for weeks. If there is any kind of microbial growth, the prepared batch is discarded. The growth support of each batch of prepared media was checked with known representative yeast species.

Post-analytical

The results generated were appropriately documented electronically. To prevent loss of data backup data were also documented on the result registration logbook. And also isolates were kept. An appropriate disposal system by incineration was utilized after the disinfection of Specimens and cultures of organisms by autoclaving.

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university, and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Zenawit Lakew (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Dr. Adane Bitew (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.