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The prevalence and Spectrum of Fungal Pathogens and among Confirmed Pulmonary Tuberculosis Patients at Saint Peter's Specialized Hospital, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Addis Abe be entitled: The prevalence and Spectrum of Fungal Pathogens and among Confirmed Pulmonary Tuberculosis Patients at Saint Peter's Specialized Hospital, Addis Ababa, Ethiopia. And submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology Track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

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Chairman of the Department or Graduate Program Coordinator

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Acronyms

AAU	Addis Ababa University
ABPA	Allergic Broncho pulmonary Aspergillosis
AFB	Acid Fast Bacilli
BHI	Brain Heart Infusion agar
CDC	Center of Disease Control and prevention
CAP	Chronic Pulmonary Aspergillosis
CAM	Chrome Agar media
EQA	External Quality Assessment
FCPTB	Fungal Co-Infection pulmonary TB
FIS	Fungal infections
HIV	Human Immune Deficiency Virus
LPB	Lacto Phenol Blue
MDR-TB	Multi Drug Resistant TB
MTB	Mycobacterium Tuberculosis
PCP	Pneumocystis Carinii Pneumonia
PTB	Pulmonary TB
PDA	Potato Dextrose Agar
QC	Quality Control
RTI	Respiratory Tract Infection
SDA	Sabourd Dextrose Agar
SPP	Species
TB	Tuberculosis

Abstract

Background: Fungal infections were a major cause of morbidity and mortality in confirmed patients with Pulmonary Tuberculosis. These infections had clinical and radiological characteristics of similar to tuberculosis which may be easily misdiagnosed as tuberculosis. Fungal pulmonary infection could be acquired in tuberculosis, immune deficiency patients, and other chronic diseases. Many physicians missed fungal pulmonary infection because it does not show specific clinical manifestations. The burden of sever fungal infections was not well addressed in Ethiopia. So, this study was undertaken to determine the profile of fungal pathogens and co-infections among pulmonary tuberculosis confirmed patients at Saint Peter's Specialized Hospital, Addis Ababa, Ethiopia.

Methods: A hospital-based cross-sectional study was conducted from April 2020 - June 2021. Five hundred sputum samples were collected from pulmonary tuberculosis confirmed patients who were screened by Xpert MTBRif/assay.All sputum samples were inoculated to Sabrouad dextrose agar, Brain Heart Infusion agar and Potato Dextrose agar. Fungi were identified following standard microbiological procedures. The inoculated Medias were incubated at 25^0C and 37 ^{0}C for 4 weeks respectively for each sputum samples. Mold identifications were performed by macroscopically; looking the growth form and rate of growth, surface and reversed coloration and by its microscopic appearance. Yeasts were identified by Chromo agar medium used for Candida species and urease used for Cryptococcus neoformans. Data analyses were carried out using SPSS version 20 software.

Result: In this study, out of 500 sputum samples taken from TB confirmed patients, 322(64.4%) of them were infected by pulmonary fungal isolates. Male patients accounted for220(68.3%)whereas female patients accounted for 102 (31.7%).Yeast isolates 89 (27.6%),mold isolates were 71(22%), and mixed infections showed in 162 (50.3%) patients.

Conclusion: High distribution of fungal isolation (64.4%) was obtained in patients with confirmed pulmonary tuberculosis. We recommend for policymaker's to conduct further

studies and consider the need of fungal screening in these patients. Pulmonary mycoses can be misdiagnosed and mis- treated as pulmonary tuberculosis diseases. Our study indicates that fungal etiology mustberequiredin100%theclinicallyconfirmed pulmonary tuberculosis patients.

Keywords: Fungal co-infection, pulmonary Tuberculosis, Ethiopia

1. Introduction

1.1. Background

The burden of fungal respiratory tract infection is not easily definable. The frequency of the infection had been increasing in the last few decades. The prevalence of fungal lung infections is largely related to pulmonary tuberculosis (PTB), HIV/AIDS, chronic obstructive pulmonary disease, and use of immunosuppressive drugs are implicated for such an increase of disease (1,2). Globally, it is predicted that 1.2 million individuals live with chronic pulmonary Aspergillosis (CPA) as a result of PTB, in which the occurrence of CPA is the highest in Africa, Western Pacific, and South-East Asia (3). The chronic nature of PTB along with prolonged chemotherapy with or without corticosteroids resulted in immune suppression in PTB patients. Finally, PTB confirmed patients affected by fungal infection (4).

Pulmonary fungal infection (pneumonic 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 their inhalation, the reactivation of dormant infection and via hematogenous dissemination. Fungi or their spores are abundant and exist virtually everywhere in the human environment (5, 6). The worldwide nature of fungi and their spore's colonization or disease 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 most literature, species of *Aspergillus*, *Candida*, *Cryptococcus*, *Pneumocystis*, and thermally dimorphic fungi are the most significant fungal species have been reported as etiological agents of fungal lung infection. Accordingly, many mycelial fungi, such as *Scedosporium SPP.*, *Fusarium SPP.*, P*enicillium SPP.*, dematiaceous filamentous fungi, zygomycetes, and yeasts other than *C. albicans* have developed as etiological agents of respiratory fungal disorders. (7, 8, 9, 10)

The incidence of invasive candidiasis has a bimodal distribution, peaking at both ends of the age spectrum(11). Pneumocystis has occurred only in patients who are allergic to sulfa drugs, in patients who do not adhere to the preventive treatment and, occasionally, in patients who become infected before prophylaxis (12). Aspergillosis occurs predominantly in immune compromise patients. Major risk factors include neutropenia, hematopoietic stem-cell and solid

-organ transplantation, prolonged and high-dose corticosteroid therapy, hematological malignancy, cytotoxic therapy, advanced AIDS, and chronic granulomatous diseases (13, 14, and 15).Cryptococcosis, caused by the encapsulated yeasts Cryptococcus neoformans and Cryptococcus gattii, is an opportunistic systemic disease of global distribution that mainly affects patients with medical conditions in their immune systems, with meningoencephalitis being the leading cause of death (16.17).

Pulmonary tuberculosis is principally a disease of poverty, with 95% of cases and 98% of deaths occurring in developing countries (18). Ethiopia stands 10^{th} among the 30 high TB burden countries with an estimated incidence rate of 151/ 10,0000 (3). 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. Therefore, proper diagnosis of fungal pathogen especially in PTB patients is critical (19).

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 (20). As in most developing countries treatment of PTB patients in Ethiopia is empirical and the spectrum of pulmonary fungal infections and their association with PTB in Ethiopia is lacking.

The higher prevalence of pulmonary fungal pathogens, especially in cases of sputum for M. tuberculosis positive 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 including Ethiopia.

So, in this study, the association of fungi infection with Pulmonary TB confirmed patients were

investigated, since the magnitude of fungal and PTB co-infection is an active field of research. Therefore, the objectives of the study were to determine the profile of fungal pathogens and co-infections among pulmonary tuberculosis confirmed patients referred to Saint Peter's Specialized Hospital, Addis Ababa, Ethiopia.

1.2. Statement of the problem

The major to Global burden of respiratory tract infection is responsibleforone-thirdofinfectiousdisease associated mortality, with an estimated 4.3 million annual deaths. Among these, fungal infections oftherespiratorytractarelargelyunrecognizedormisdiagnosedandthetrueburdenisdifficult to describe (21).Fungal lung infections increase significantly over the last two decades, which is largely attributed to the extensive use of broad-spectrum antibiotics, long-term use of immune suppressiveagents,and the increasing population of terminally ill, debilitated and immune compromised patients .so fungal disease commonly known as mycosis are on huge rise and range of greater concern.

The burden of severe fungal infections (FIs) is not well addressed in Ethiopia. From FIs with multiple demographic sources using modeling, CPA and allergic bronchopulmonary Aspergillosis (ABPA) were estimated by using the prevalence of asthma, chronic obstructive pulmonary disease, and the 105,000,000 incidence tuberculosis cases. (22) This study aimed to identify the co-infection of TB with pulmonary fungal infection Ethiopia and the problem was amplified here as other developing country due to majority of pulmonary tuberculosis and paucity of diagnostic mycology laboratories .Based on this fact we include in the research problem to determine prevalenceanddistributionoffungalspeciesandco-infectionwithpulmonarytuberculosisat high mycobacterium tuberculosis burden and excellence of tuberculosis hospital in the country.

1.3. Significance of the study

- The main merits to all patient suffering from over masked respiratory mycoses may get the chance of early diagnosis and treatment who otherwise be subjected to the inevitable consequences of undiagnosed or misdiagnosed respiratory mycosis complication and unwanted usage of antibiotics
- Isolation of fungal pathogens in sputum for which *M. tuberculosis* positive and study subjects confirmed with PTB applied microbiology tests of FI by differential diagnosis.Italsogives a clue to clinician confirmed the disease in parallel with pulmonary tuberculosis.
- To enhance the health care system based on actual data collection and analysis, interpreting the result will help the health professional better understanding of the difference between FCI &PTB
- Benefits patients affected with FCI&PTB alleviate the infection of pathogen appropriately and timely manner since there is a lack of research on this FCI&PTB, it will also add value for researchers, decision-makers and other stakeholders.
- Addressing the issue of TB and pulmonary mycosis assists in selecting appropriate drugs as the clinical and radiological pictures of both infections are similar.

2. Literature review

A number of studies had been under taken to identify pulmonary fungal infection among pulmonary tuberculosis patients globally.Theincidenceof systemic fungal infections remains far beyond treatment ,most invasive fungal infections are associated with high mortality rates of >50% (23,24). The Global burden of respiratory tract infection is responsibleforone-thirdofinfectiousdisease and associated mortality, with an estimated 4.3 million annual deaths. Among these, fungal infections of the respiratory tractarelargely unrecognized or misdiagnosed and the true burden is difficult to describe.Ithasbeenreportedthattheestimatedoverallincidenceofsystemic fungal infections up to 11.3%ofwhichrespiratory mycoses that involve the bronchi and lungs comprises 60%(25).

Worldwide in 2017, 6.4 million new cases of TB were officially notified to national authorities and then reported to WHO(26). The increasing number of immunocompromised patients, diseases caused by fungal infections remain a great threat in public health. Opportunistic fungi, including *Aspergillus* with invasive Aspergillosis(27,29). *Cryptococcus* with cryptococcosis (30,32). *Pneumocystis* with pneumonia (33) .and endemic fungi (34,35) are the main sources of fungal infections in the lungs of humans.

A deterministic model analysis was conducted by National Aspergillosis Centre about the global burden of chronic pulmonary Aspergillosis as a sequel to pulmonary tuberculosis rates were obtained from the World Health Organization and a scoping review of the literature was conducted to identify studies on residual pulmonary cavitations after PTB and estimate the global incidence of CPA after PTB. Having established that from 21% (United States of America) to 35% (Taiwan, China) of PTB patients developed pulmonary cavities and that about 22% of these patients developed Cain 2007, 7.7 million cases of PTB occurred globally, and of them, an estimated 372 000 developed CPA: from 11 400 in Europe to 145 372 in South-East Asia.

The prevalence rate ranged from < 1 case per 100 000 population in large western European countries and the United States of America to 42.9 per 100 000 in both the Democratic Republic of the Congo and Nigeria. China and India had intermediate five-year period prevalence rates of 16.2 and 23.1 per 100 000, respectively .Accordingly, the global burden of CPA as an outcome to PTB is substantial and warrants further investigation of CPA (36).

A cross sectional study conducted in China identified 197 cases of TB/cryptococcosis co-infection in 56 studies, which accounted for 62.9% (n=197/313) of all cases worldwide. A total of 49 (87.5%) studies were conducted at tertiary hospitals. Nearly all of the cases (99.5%, n=196/197) were collected from the monsoon region of China, particularly from southern and eastern China (71.1%, n=140/197). Before 2000, only 16 co-infection cases (8.1%) were reported, whereas 70 cases (35.5%) were reported during 2000–2009, and 111 cases (56.3%) were reported between 2010 and 2015(37).

Another cross-sectional study conducted in Taiwan stated that the prevalence of co-infection among the TB population was 0.6% (23 co-infection cases/4053 total cases). three co-infection cases from 31 tubercular meningitis patients (Three studies revealed that the rates of TB/cryptococcosis co-infection in TB/fungal co-infection ranged from 2.7% to 3.8% (n=1/31, Shandong; n=2/74, Guangdong; and n=1/26, Henan). The prevalence of TB/cryptococcosis co-infection among cryptococcosis was 5.4% (n=23/425, Taiwan), and higher rates (6.7%–26.7%) were reported in children with this infection (n=1/15, Henan; n=4/15, Chongqing). The co-infection rate in cryptococcal meningitis patients with and without HIV/AIDS was 42.9% and 15.0%, respectively (n=6/14, Yunnan; n=3/20, Henan) (37).

Based on cross-sectional study conducted by Mohammed R. et al.in Iran ,2018. Of 3577 patients, 10731 smears were prepared, 3.6% (n=130) of patients were identified as smear-positive pulmonary tuberculosis. Amongst the positive tuberculosis patients, 16/130 cases (12.3%) had the coinfection of TB with fungi microorganisms. 25 of this finding is concluded that coinfection of fungi agents in patients with tuberculosis that should be considered(38).

Across-sectional study by Arunava K, *etal .in* India ,2013 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 the male patients (P value 0.0133). 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 (P value 0.0138). Many patients with pulmonary tuberculosis have coinfection with Candida spp. The prevalence of non-albicans Candida species is increasing and may be associated with inadequate response to anti-tubercular drugs. C. glabrata infection has a strong association with old age (39).

A Cross-sectional study of one-year duration (January 2015-December 2015) by Mohammad Aadam*etal* in India was conducted on patients who were clinically diagnosed cases of Pulmonary Tuberculosis from A total of 100 patients suffering from Pulmonary Tuberculosis and Post-treated cases of Pulmonary-TB with respiratory symptoms were undertaken for the present study. The mean age group in the present study was 40 ± 10 years. Out of 50 participants in each group, in Group 1, 74%were males and 26% were females while in group 2, 72%were males and 28% were females. Of the 100 patients, 55% were positive for opportunistic fungal infection. Amongst them, 58% were in group 1 and 52% were in group 2. In group 1, 44% of males and 14% of females had opportunistic fungal infections. And in group 2, 38% males and 14% females showed opportunistic fungal infection. 31% isolates were yeasts (Candida, Cryptococcus and Rhodotorulaglutinis) and 24% isolates were molds (Aspergillus spp.) (40).

A Cross sectional study conducted Pal A, Nanda S. eta el at a tertiary care hospital in Western India in the title Filamentous Fungi causing Lower Respiratory Tract Co-Infections Fungal pathogens found in 14.37% cases, out of which, 9.6% were candida sp. and 4.2% were fungal molds while, Pneumocystis carinii cysts detected in 0.6%. The molds isolated were Fusarium sp.-- 1.8%, Aspergillus fumigatus-- 1.2%, Mucor sp.-- 1(0.6%) and Trichosporon sp.1 (0.6%). Bacterial pathogens found in 57.48% cases (44.3% mono-bacterial, 13.2% multifactorial coinfection), most common ones being, Klebsiella sp., and Pseudomonas sp. Bacterial and fungal coinfection found in 10.18% cases (41).

A cross -sectional study carried out in South India at a tertiary care hospital by Kahanpaa et al Out of the total 382 patients with suspected tuberculosis, 75 patients (19.6%) who were positive for acid fast bacilli by Ziehl-Neelsen stain were screened for Candida co-infection.

Among the 75 patients with pulmonary tuberculosis, Candida co-infection was observed in 30 (40%) patients. Candida albicans was the most common isolate observed in 50% of the patients with co-infection, followed by C. tropicalis (20%) and C. glabrata. The majority of these patients had persistence of pulmonary symptoms even after anti-tubercular treatment. Owing to the inherent anti-fungal resistance, non-albicans Candida species are often not amenable to anti-fungal treatment (42).

A cross sectional study conducted by Muni et al at Patna, Bihar in India. A total of 200 pulmonary tuberculosis patient's septum samples were taken, 70 patients (35%) whose sputum samples were positive for acid-fast bacilli were included in the study. Candida co-infection was observed in 26 (37.14%) patients which included 16 male and 10 female patients. The ratio of male to female in Candida infection group was 1.6:1. Candida albicans was the most common isolate among Candida species in pulmonary TB patients which was isolated in 50% of the patients (43).

A cross-sectional study was designed in an observational analytical method in Indonesia by Soedarsono S et al from 193 Sputum sample was collected and inoculated on SDA medium so there was a visible growth of colonies. Out of which 148 or 77% were PTB patients. Among the confirmed PTB patients 99% founded candida species positive. The proportion of fungal positive cultures in previously treated cases was higher than in new cases. C. albicans was the most common fungal species both in previously treated cases and new cases of pulmonary TB (44).

A study conducted Yahaya H, Taura D and et al In Africa with the objective Diversity of respiratory yeasts from suspected pulmonary tuberculosis and HIV of 195 samples 140(71.8%) test samples yielded fungal pathogens. Fungal organisms isolated were: Candida albicans (19.0%), Candida stellatoidea (9.7%), Cryptococcus neoformans (9.7%), Candida parapsilosis (9.7%), Torulopsis glabrata (5.6%), Mucorspp (7.2%), Penicillium marneffei (4.1%), Rhodotorula rubra (3.6%) and FusariumSpp. (3.1%) in that order (45).

A study conducted to examined pulmonary tuberculosis and co-infection with Candida such as C. albicans, C. tropicalis, C. kruezi and C. paraspsilosis which were initially thought to be normal floras of the oral cavity. The percentage of tuberculosis patients co-infected with Candida is

becoming a concern and might complicate the treatment of tuberculosis. A total of 400 sputum samples were collected and subjected to Ziehl- Neelsen staining technique and GeneXpert system, Gram's stain, Germ tube test and examination in KOH preparation were conducted. Culture on Sabouraud Dextrose Agar with gentamicin, and cultured on CHROMagar Candida and sugar fermentation were carried out for Candida. Out of the 400 sputum samples examined 93(23.3%) had TB and 32(8.0%) were positive for Candida. By gender the prevalence of tuberculosis were females 51(22.4%), males 42(24.4%) while the prevalence of Candida were females 18(35.3%) and males 14(33.3%). The percentage occurrences of *Candida* sp. isolated were *C. albicans* which was the predominant species 10 (21.5%), *C. topicals* 5(5.4%), *C. krusei* 4(4.3%) and *C. Para psilosis* 3(3.2%) respectively (46).

A cross-sectional study conducted in Kenya by Elizabeth Ns et al. Pulmonary fungal pathogens were isolated as co-pathogens with Mycobacterium tuberculosis in 76 (44.18%). Pathogenic fungi and other bacterial pathogens may be significant co-infecting pathogens complicating the management of TB. Clinicians in Kenya were not aware of co-infection of Mycobacterium tuberculosis with opportunistic pulmonary fungal and bacterial pathogens. HIV infection is a significant pre-disposition to pulmonary tuberculosis. The two conditions were present in severe immune suppression. The patient was spent a prolonged TB treatment, so immune suppressed patient was a high risk for acquiring opportunistic fungal pathogens. (47).

3. Objective

3.1. General objective

- To determine the prevalence and Spectrum of Fungal Pathogens and among Confirmed Pulmonary Tuberculosis Patients at Saint Peter's Specialized Hospital, Addis Ababa, Ethiopia.

3.2. Specific objectives

- Todetermineco-infectionofpulmonarytuberculosisandrespiratoryfungal infection.

- To assess the distribution of yeasts species and mold species causing pulmonaryf u n g a l infection among confirmed pulmonary TB patients.

4. Materials and methods

4.1. Study area

The study was conducted in Saint Peter Specialized Hospital Gullele sub-city Addis Ababa Ethiopia. Saint peter PTB specialized Hospital has 620 health professionals giving service about 86,624 patients per year and serving more than 5.6 million, more than 720 inpatients and 150-200outpatients has been visiting this hospital every day. The microbiology laboratory is well equipped and providing different referral services for Tuberculosis and MDR-related infection from every corner of the country. This lab is continuously assessed through external

quality assessment (EQA) by Ethiopian Public Health Institution (EPHI). Fungal co-infections with pulmonary tuberculosis are examined in the microbiology department. More than twenty-five patients are visiting the microbiology laboratory concerning PTB. So, it was possible to collect the samples aseptically and appropriate sputum from confirmed patients for the study.

4.2. Study design and period

A hospital-based cross-sectional study was conducted from April 2020 - June 2021.

4.3. Population

4.3.1 Source of population

All patient specimens which were referred to Saint Peter's specialized hospital for routine laboratory test during the study period.

4.3.2 Study population

All confirmed tuberculosis patients of new cases, follow up, and MDR referral cases confirmed by Genxpert were the study population.

4.4 Inclusion and Exclusion criteria

4.4.1 Inclusion Criteria

All samples collected from confirmed pulmonary tuberculosis patients which were sent to the microbiology department of Saint Peter Specialized hospital laboratory.

4.4.2 Exclusion criteria

Previously suspected pulmonary fungal infection cases and those who were on anti-fungal treatment were excluded from the study. In addition, patients who have incomplete information were excluded.

4.5. Study variables

4.5.1. Dependent variable

- All fungi isolates identified from those confirmed pulmonary tuberculosis patients.
- Distribution of yeast and mold species and causing pulmonary fungal infection.

4.5.2. Independent variables

- The demographic data’s and pulmonary tuberculosis result were used as independent variables

4.6. Measurement and data collection

The socio-demographic data, previous pulmonary fungal infection, and treatment history of the

study participants were obtained from the laboratory request brought by the patient assent and consent forms are filled during sample collection. Data was collected by laboratory personnel. Before the actual data collection, a pre-test on the calculated sample size for the pre-test was conducted using demographic and clinical data collection formats and logbooks. The purpose of the study, as well as any related harm and benefit, was explained to the study participants accordingly.

4.6.1 Sample size Determination

The sample size was calculated based on p-value = 0.5. Because we couldn't find the other study conducted in Ethiopia regarding this title. At 95% confidence interval, Margin of error tolerated is 5 % (0.05).

Study participants: n = Z^2 P (1- P)/ d^2 Where n = sample size z = 95% statistic for level of confidence (1.96) d = margin of error.

The sample size was estimated to be: = $(1.96)^2 \times 0.5 (1 - 0.5)/ (0.05)^2$ = 384.16 Therefore, by adding 10% contingency, a minimum of 423 participants must be included in the study. Finally, the sample size was increased to 500 in order to enhance the research finding and to make it significance to generalize the result.

4.6.2 Sampling method

A convenient sampling method is applied to achieve the estimated sample size. All pulmonary tuberculosis confirmed patients visiting Saint Peter's specialized hospital microbiology department laboratory within the specified time of the study.

4.6.3 Sample collection producer

Patients was asked to wash their mouth gently with tap water prepared for this purpose then, produce the samples in a room that aid patients made as open-air space away from other people to avoid aerosol spread and advised to give duplicate sputum sample using two wide mouthed, clean, 50ml capacity, sterile, disposable containers, polypropylene centrifuge tubes, translucent, screw capped container. Sputum in one of the containers was used for the investigation of tuberculosis while sputum in the second container was used for mycological investigation.

The patients were trained to inhale deeply 3 to 4 times before coughing out from the chest. The

sputum produced was done carefully kept in the sterile falcon tube without contaminating the outside of the tube. The lid of the container was done secured tightly

4 .6.4. 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 were filled during sample collection.

4 .6.5 Informed consent

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.

4.6.6 Principle of Laboratory analysis for Tb detection

The presence of pulmonary Tuberculosis was screened on the same day of sample collection using the country's current guideline, GeneXpert MTB/RIF assay is a rapid diagnostic test of Tuberculosis and drug resistance (MDR). It is based on a principle of nucleic acid amplification (NAA) test which simultaneously detects DNA of Mycobacterium tuberculosis complex (MTBC) and resistance to Rifampicin (RIF). (i.e., mutation of the rpoBgene) in less than 2 hours. The primers in the XpertMTB/RIF assay amplify a portion of the polyene containing the 81-base pair "core" region. The probes can differentiate between the conserved wild-type sequence and mutations in the core region that is associated with Rifampicin resistance. The Centers for Disease Control and Prevention recommends that NAA testing be performed on at least one respiratory specimen from patients who have a moderate or high suspicion of having pulmonary TB.

4.6.7 Fungal identification

Unprocessed sputum inoculated directly onto duplicated Brain Heart Infusion agar tubes supplemented with chloramphenicol (Oxoid, Basingstoke, UK) under safety cabinet level II at

Ethiopia Public Health Institution. All inoculated tubes were then be transported to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University. One of the tubes was incubated at 25 ^{0}cwhile the other one remained incubated at 37 ^{0}caerobically for up to four weeks. Culture plates were examined twice a week for any fungal growth.

4 .4.8 Identification

4 .4.8.1. 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 was complete considered for macroscopic identification. Diagnostic microscopic features of mycelia fungi were finished determined by using a lacto phenol cotton blue staining procedure. Briefly, a drop of lacto phenol cotton blue (LPCB) stain was placed on a clean glass slide. A piece of fungal culture was ended placed on clean glass slides containing LPCB for the staining process. A stained preparation was then be covered by a cover slide and was examined for microscopic characteristics such as macro and micro-conidia, chlamydo spores, the morphology of reproductive structures, and the nature of hyphae by using 10X and 40X objectives of the microscope. Features seen in the stained slide were compared with established characteristics of fungal features using mycology atlases.

4 .4.8.2 Yeast Identification

Yeasts were identified by employing an array of standard assimilation test germ tube production, and using CHROM agar culture medium, Candida Species isolate as per the instruction of the manufacturer.

4.9. Data Quality Assurance

Pre-analytical; Appropriate labeled falcon tubs with patient name and Id number was provided and instructed to wash their mouth gently by tap water prepared for this purpose then they produce sputum from deep by deep breathing three times and coughing so as to expectorate purulent sputum. Collected sputum samples were confirmed for eligibility. Reagents used for Xpert MTB/RIF assay, SDA, Chromo agar and BHI media preparation was checked for expiry date and any abnormal color change. Preventive maintenance of equipment was inspected.

Analytical; Media preparation was performed according to media manufacturers' instruction on the media itself. Prepared Chromo agar and BHI media checked for growth support using standard strains. Sterility of each batch of prepared Medias was checked by incubating un inoculated media at 250c – 30oc and the Expiration date of the medias were checked. Daily preventive maintenance procedure for Expert analyzer was done inspected before testing.

Post-analytical; All captured results including dependent variables, independent variables were checked at a time of enrolment for their completeness during data entry and during result analysis. The double entrance was used to check the correctness of the information.

4.10. Data analysis and interpretation

Data entries were by excel and analyses by using Statistical Package for Social Sciences (SPSS) software version 20. Descriptive statistics was completed used to describe the study participants concerning relevant variables and statistically significant difference in the association between demographical characteristics (i.e., age, sex), tuberculosis confirmation, and fungal positivity using at a 95% confidence interval with the aid of SPSS. Finally, the results were done analyzed and presented on words, and tables.

4.11. Ethical Consideration

This study was conducted after the approval of ethical clearance obtained from the Departmental Research and Ethics Review Committee of Addis Ababa University College of Health Sciences, Department t of Laboratory Sciences (DREDC605/21/MLS)and permission was obtained from Saint Peter's specialized hospital research directorate Personal information about the participants was treated confidentially.

4.12. Dissemination of Results

The final result of the study was submitted and presented to Addis Ababa University College of Health Sciences, Department of Laboratory Sciences. The test result was reported to the clinician and the host of the study, Saint Peter's specialized hospital research directorate. In addition, the research will be presentedtootherconcernedbodieslikeprofessionalassociationsanditwillbesubmittedto peer reviewed journals for publicati

5. Results

5.1. Clinical sign and symptoms of study participants

The most common presenting symptom was cough (95%) followed by expectoration (76.7%) and weight loss (45.1%).

Table1. Clinical sing symptoms of study participants.

Symptom	Number	Percent
Cough	475	95
Expectoration	384	76.7
Weight loss	226	45.1
Fever	213	42.6
Shortness of breathing	182	36.3
Anorexia	176	35.1
Chest pain	136	27.2
Fatigue	80	15.9
Night sweats	69	13.8

5.2. Demographic Data Analysis

A total of Five hundred (n=500) study participants were enrolled in the present study, of which 347(69.4%) were males and 153 (30.6%) were females. The maximum numbers of patients were taken from 25-34 age groups which was 100 (31%) as showed in table2.

Age	Confirmed PTB cases		Confirmed Fungal cases		The sum of confection
	Male	Female	Male	Female	
≤ 15	3	4	3	3	6 (2%)
15 - 24	40	21	25	15	40 (12.4%)
25 - 34	72	56	62	38	100 (31%)
35 – 44	116	38	69	26	95 (29%)
45 - 54	57	21	33	11	44 (14%)
55 - 64	35	7	19	6	25 (8%)
≥65	24	6	8	3	11 (3.4%)
Total	**347**	**153**	**220**	**102**	**322**

Table 2: Socio-demographic, character tics of study subjects (n=500)

5.3. Fungal pathogens isolated

In this study, a total of 500 sputum samples from confirmed PTB patients were cultured, of which 322 (64.4%) fungal isolates were identified as a co–infection with MTB. Whereas from the remaining 178 (35.6 %) patients' fungal pathogen were not identified, of those fungal infected patients 162 (50.3%) of them were infected by more than one types of fungal isolates the remaining 160 (49.69%) isolates were only one fungal pathogen. Of the 322 fungal isolates, yeast isolates were account 182 (56.5%) and molds account 140 (43.5%). Of 182 yeast isolates, 89 of them were isolated singly, 33 of them isolated double, and 60 of them identified triple.

Table 3. Spectrum of Fungal pathogens isolated.

Sn	Fungal Isolation	Frequency	Percentage (%)
1	*Candida albcans*	64	19.88
2	*Candida krusei*	16	4.97
3	*Candida tropicalis*	9	2.80
	Yeast total	**89**	**27.64**
1	Aspergillus .fumgatus	10	3.11
2	Aspergillus. niger	14	4.35
3	*Pencillium spps*	3	0.93
4	*Scopularis*	1	0.31
5	*Alternaria spps*	1	0.31
6	*Pen. Marnnefi*	6	1.86
7	*Scopularisis Spps*	2	0.62
8	*Clodosporium Spps*	2	0.62
9	*Other* Aspergillus *Spps*	10	3.11
10	*Acromoniume Spps*	2	0.62
11	Aspergillus.flavu	7	2.17
12	*Mucor spp*	13	4.04
	Mold total	**71**	**22.05**
1	Mix *Candida albcans&C. tropicalis*	5	1.55
2	Mix *Candida albcans&Candida krusei*	11	3.42
3	Mix *Candida albcans, C. tropicalis & Candida krusei*	17	5.28
	Mixed Yeast total	**33**	**10.25**
1	Aspergillus. niger &*Mucor spps*	6	1.86
2	Aspergillus *Spps* &*S.Apospo*	2	0.62
	Mixed Mold total	**8**	**2.48**
1	*Candida albcans&* Aspergillus . niger	27	8.39
2	*Candida albcans&* Aspergillus .fumgatus	10	3.11
3	*Candida albcans &* Aspergillus.flavu	19	5.90
4	*Candida albcans, Candida krusei &* Aspergillus .fumgatus	17	5.28
5	*Candida albcans&Penic.spps*	15	4.66
6	*Candida albcans&Mucor spps*	6	1.86
7	*Candida krusei,Asp. Nig&Pylomycosis*	8	2.48
8	*Candida krusei&* Aspergillus niger	19	5.90

	Mixed Yeast plus Mold total	162.00	50.3
	Total fungal isolation	322.00	100.00

5.4 Fungal spp Isolated from confirmed PTB patients in relation to socio-demography data.

Pulmonary fungal isolates were distributed in all age groups of confirmed PTB patients. However, in the age group of (25-44) years of age were the one containing the highest number 347 (96.4%) were identified in the study. The least 24 (4.5%) were recorded. in age group < 15 and > 65 age group as shown in table 4

Among 500 PTB positive cases 347 (69.4%) were male and 153 (30.6 %) were female. Male subjects presented more positive identification than as compared to their female counterparts 220 (44%) and 102 (20.4%) respectively as stated in table 1. However, no association was found between gender and fungal isolates in our study (P=0.929) table 5. Fungal isolate from the sputum of both female and male except Alternaria Spp and Cladosporium isolated from only in males but.71 (22.05 %) of fungal isolates from mold species were Asp.spps found in every age groups of the subject in addition to and C.albicnas from Yeast family which constitute 89 (27.64 %). However, there was no association found between age group and pulmonary fungal infection.

From our study conducted Aspergillus Spps is the most dominant Spp. which amounted 10 (3.11) from which 99 were male found to be positive and the remaining were 50 females which the mode is 35-44 years of age. Table 4

Regarding the yeast species C. albicans is the highest prevalence that constitutes 99 male and 50 female which dominates 45-54 age group than other candida species.

Table4: Detection and isolation Yeast species in sputum in a confirmed PTB cases in relation to socio-demographic data, at Saint Peter's Specialized Hospital micro biology laboratory, Addis Ababa, Ethiopia, from April to June, 2021

yeast isolated	Age group	Frequency	Total
Candida albicans	15-24	12	12
	25-34	14	14
	35-44	21	21
	45-54	8	8
	55-64	6	6
	>=65	3	3
	total	64	64
Candida krusei	15-24	5	5
	25-34	2	2
	35-44	2	2
	45-54	4	4
	55-64	1	1
	>=65	2	2
	total	16	16
Candida tropicalis	15-24	1	1
	25-34	2	2
	35-44	3	3
	45-54	2	2
	55-64	1	1
	total	9	9

Yeast isolated total from co-infected cases89 (27.6%) and C.*albicans 64(20%) the age group (35-44)* was the highest yeast species in co-infected cases. another yeast infection C.krusei 16(4.96%) the age group (45-54) and C.tropicals 9(2.8%) age group (35- 44) about the total isolate yeast co – infection C.albican the most predominant infection the age group (35-44) as indicated on table 4.

Table 5. Detection and isolation of on culture sputum in confirmed PTB in Mixed infection Mold and Yeast in relation socio demographic data at Saint Peter's specialized Hospital Micro biology Laboratory, Addis Ababa Ethiopia from April to June, 2021

mixed isolated	age group	frequency	Total
Mix Candida albicans & Candida tropicalis	25-34	4	4
	35-44	1	1
	total	5	5
Mix Candida albicans & Candida krusei	15-24	2	2
	25-34	4	4
	35-44	2	2
	45-54	1	1
	55-64	1	1
	>=65	1	1
	total	11	11
Mix Candida albicans, Candida tropicalis & Candida krusei	<15	1	1
	15-24	2	2
	25-34	6	6
	35-44	5	5
	45-54	2	2
	55-64	1	1
	total	17	17
Aspergillus niger & Mucor spp	25-34	1	1
	35-44	2	2
	45-54	2	2
	>=65	1	1
	total	6	6
Aspergillus spp & Pseudo appressorium	15-24	1	1
	35-44	1	1
	total	2	2
Candida albicans &Aspergillus niger	<15	2	2
	15-24	5	5
	25-34	2	2
	35-44	6	6
	45-54	5	5
	55-64	3	3
	>=65	3	3
	total	26	26
Candida albicans& Aspergillus fumigatus	25-34	2	2
	35-44	5	5

	45-54	2	2
	>=65	1	1
	total	10	10
Candida albicans & Aspergillus flavus	<15	1	1
	15-24	1	1
	25-34	8	8
	35-44	6	6
	55-64	3	3
	total	19	19
Candida albicans, Candida krusei & Aspergillus fumigatus	15-24	1	1
	25-34	3	3
	35-44	7	7
	45-54	3	3
	55-64	2	2
	>=65	1	1
	total	17	17
Candida albicans & Penicillium spp.	15-24	2	2
	25-34	4	4
	35-44	3	3
	45-54	4	4
	55-64	1	1
	>=65	1	1
	total	15	15
Candida albicans & Mucor spp	<15	1	1
	15-24	1	1
	25-34	2	2
	35-44	2	2
	total	6	6
Candida krusei, Aspergillus niger & Paecilomycosis	25-34	3	3
	35-44	3	3
	55-64	1	1
	>=65	1	1
	total	8	8
Candida krusei & Aspergillus niger	15-24	1	1
	25-34	7	7
	35-44	4	4
	45-54	3	3
	55-64	2	2
	>=65	3	3
	total	20	20

Total isolated mixed infection 162 (50.3%) Candida albicans & Candida tropicalis 5(1.55%),Candida albicans&Candida krusei 11(3.41%),Candida albicans, Candida tropicalis&Candida krusei 17 (5.27%), Aspergillus spp &Pseudo appressorium 2(0.62%),Candida albicans&Aspergillus niger 26(8%),Candida albicans&Aspergillus fumigatus 10 (3.1%),Candida albicans,Candida albicans & Aspergillus flavus 19 (5.9) Candida krusei and Aspergillus fumigatus 17(5.27%),Candida albicans&Penicillumspp 15(4.65%),Candida albicans&Mucor spp 6 (1.86%),Candida krusei Aspergillus niger& Paecilomycosis 8(2.48%) and Candida albicans&Aspergillus niger 19 (5.9%) the most predominant mixed infection Candida albicans&Aspergillus niger, other Aspergillus spp and Candida spp the age group more affected mixed infection 25-34 and 35-44 on this indicated on table 5.

Table 6. Number of mold isolates detected confirmed PTB relation socio demographic data at Saint Peter's specialized Hospital Micro biology Laboratory, Addis Ababa Ethiopia from April to June, 2021

Mold isolated	Age group	Frequency	Total
Aspergillus fumigatus	25-34	2	2
	35-44	4	4
	45-54	2	2
	55-64	2	2
	TOTAL	10	10
Aspergillus niger	15-24	1	1
	25-34	4	4
	35-44	5	5
	45-54	2	2
	55-64	2	2
	TOTAL	14	14
Penicillium spp.	15-24	1	1
	25-34	2	2
	TOTAL	3	3
Scopulariopsis	55-64	1	1
	TOTAL	1	1
Alternaria spp	25-34	1	1
	TOTAL	1	1
Penicillium marneffei	25-34	3	3
	35-44	1	1
	45-54	1	1
	>=65	1	1
	TOTAL	6	6
Scopullariopsis Spp	25-34	1	1
	45-54	1	1
	TOTAL	2	2
Cladosporium spp	25-34	1	1
	55-64	1	1
	TOTAL	2	2
Aspergillus spp	25-34	4	4
	35-44	4	4
	55-64	2	2
	TOTAL	10	10
Acremonium spp	35-44	2	2
	TOTAL	2	2
Aspergillus flavus	15-24	1	1
	25-34	1	1
	35-44	1	1
	45-54	3	3
	55-64	1	1
	TOTAL	7	7
Mucor spp	15-24	2	2
	25-34	4	4
	35-44	2	2

	45-54	2	2
	55-64	2	2
	>=65	1	1
	TOTAL	13	13

Total mold isolate cases mold consists of 71(22%) and *A.niger* was highest mold isolated fromco-infectedcases14(4.34%). and mucor spp 13(4%), A.fumigatus 10 (3.1%)*Aspergilus spp10(3.1%) ,A.flavus 7 (2.1%) and penicillum spp 3(0.93%), Cleodosporium spp1 (0.3%),Alternaria spp1(0.3%) and Scopullariopsis spp1(0.3%)* co-infected cases, isolated in mold spp age group infection most affected (35-44) the predominant mold infected cases Aspergilus spp and mucor spp and so on this indicated on table 5predominat mold infected cases Aspergilus spp and mucor spp and so on this indicated on table 5*spp1(0.3%) co-*infected cases ,isolated in mold spp age group infection most affected (35-44) the predominant mold infected cases Aspergilus spp and mucor spp and so on this indicated on table 6.

Table 7. The prevalence of fungal pathogen among confirmed PTB sputum samples of(n=500)

prevalence of fungal pathogen	Prevalence
Culture positive Fungal pathogen	322
Tuberculosis infection	500
Yeast infection	89
Mold infection	71
Mixed infection (mold& yeast)	162
Culture negative samples	178

The prevalence of fungal pathogen among confirmed PTB sputum samples of(n=500). From confirmed PTB patient's culture 322 were positive from which fungal- tuberculosis co-infection was constituted Mold 71, yeast 89 and Mixed infection 162.

Table 8. Prevalence of fungal pathogen by sex in tuberculosis patients (n= 500 TB positive patients)

Group	Number tested	Fungal-confection	
		Yes	No
Less Than 15	7	15	2
15-24	61	59	23
25-34	128	169	48
35-44	154	158	66
45-54	78	65	21
55-64	42	41	11
>65	30	20	7
total	500	547	178

6.Discussion

Themainobjectiveofthepresentstudywastodeterminethedistributionoffungalisolates among pulmonary tuberculosis confirmed patients in the sputum sample. This review emphasizes the reality that to determine the distribution of fungal pathogen isolate patients by collected 500 patients among affirmed PTB sputum tests and found 70% I male person positive and 30% female were isolated which agreed with the study conducted in IA. et al. reported the prevalence of fungal infection among the patents accounted for 68%. Of the infected patients, male patients accounted for 59.8%, while female patients accounted for 40.1%. (50)

In our present study the fungi distribution in a confirmed PTB 500 or (64%) fungi positive pathogens were isolated. Out of the pathogen's isolated *Yeast* accounted*89*(27.64%), and *Molds* were71(22.05%) and mixed infection 162 (50.3%) which represents PTB patients were more susceptible to yeast than Molds which our study Suits with the study conducted and come across of the 340 samples, 14.4 ***et al.*** in keneya university of Nirobi "out % (n=49) and 15.6% (n=53) were positive for yeasts and molds respectively. *Candida albicans* and *C. krusei* were the most prominent isolates constituting 49.0% (n=24) and 20.4% (n=10) of the total yeasts respectively."*(49)*

At the species level, however, *C. albicans* were the most prevalent species amounting for 64(19.88%) were yeast isolates. In the case of mold culture 71(22.04 %) isolates were mycelia fungi, other mixed infection 162 (50.3%)whereas *Aspergillus* SPP. (10;3.11%)Aspergilu niger (14;4.35%), Aspergilus flavu (7;2.17%)*Penicillium SPP.* (3,0.93%), Penmarnnefi (6 1.86 %) *Scopularisis Spps* (3,0.934%),Alternaria spps *(1;0.31%)Clodosporium Spps (2 0.62%)*, and *Mucor* SPP. (13; 4.04 %) being the major isolates with regards to mold isolates were laboratorial analysis which is in line with Chakrabarti A, Shivaprakash MR presentation of "7% patients dying in teaching hospitals had invasive which accounted *Candida* spp. accounts for 8-15% . Specific patient groups have very high frequencies of fungal infections: about 20% of lung transplant recipients are colonized and infected; and *Penicillium marneffei*. [2]

From the present study conducted a confirmed PTB patients 322 were isolated with respiratory mycosis infection of fungi from whichof confirmed PTB persistent 322 licenses were confined with efficient contamination of

Organisms from which 22.04% (71/322) were Aspergillosis which exactly prove the study of clinical laboratory profile of chronic pulmonary Aspergillosis by Ramakrishna Pai Jakribettua, Thomas Georgeb “A total of 30 patients were included in the study of 50% (14/30) of the patients had a history of pulmonary tuberculosis. In all the four dead patients, the cause of death was respiratory failure and all patients were already treated for pulmonary tuberculosis. Conclusion When a patient with pre-existing lung disease like chronic obstructive pulmonary disease or old tuberculosis cavity presents with cough with expectoration, breathlessness, and hemoptysis, CPA should be considered as the primary differential diagnosis.[3]

Respiratory tract infection is globally responsible for one-third of infectious disease–associated mortality, accounting for 4.3million annual deaths. Among these, fungal diseases of the respiratory tract are generally unrecognized, and the true burden is elusive. In spite of treatment, most invasive fungal infection is associated with high mortality rates of >50%]. In common, contagious diseases of the respiratory tract are considered synonymous with invasive pulmonary infection caused by Aspergillus spp. However, over the last decay a number of uncommon filamentous fungi, such as Pseudopodium, Fusarium, Penicillium, melanized molds, and basidiomycetes, have risen as etiological agents of well-characterized respiratory disorder. It is hence that the term “respiratory mycosis” has presently broadened to include not just invasive disease but also lesser-recognized. Similar to our study Yeast finding 89 (27.64%) C.albicans 64 (19.88%), C.krusi 16 (4.97%) and C.tropicals 9(2.80%) molds finding 71(22.04%) and other mixed infection 162(50.31%) The over finding of Chowdhary A, Agarwal K, Meis JF.is too comparative to our consider conducted in confirmed PTB (6).

Of five hundred samples comprised a number of males was 347 (69.4%) and females was 153 (30.6%). So, from this male isolate were fungal infection counted 220 (68.3%) and female fungal infection were counted 102 (32. 7%) the result is closely related to diagnosis the fungal element by aid of radiographic characterization and pathological examination.(51).
report almost pneumonic clutters and inspected for fungal disease of 200 tests 116 (58%) patients were male though 84 (42%) where female fungal species with the highest incidence was found to be Aspergillus species (36.94%) and the least being sepedonium species (0.90%).

In our study *C.albicans*, *C.krusei, C.tropicalis, A.niger, A.fumigatus* those isolate were dominate theagegroupwhichwascorrelatewiththefindingsofMuhammadTetal.thelowestisolated

wasshowninagegroupwasbelow15yearscouldbebecauseveryfewindividualsparticipated in this study.

Findings from this study revealed that 70% prevalence of co-infection, which in line with those reported by TauraD*etal.*andYadu*etal.* (52) MuhammadT*etal.*90.2%prevalenceof opportunistic fungal infectionsincoinfectedpatients. That finding was highest than our due to apartfromtheidealopportunisticrelationshipbetweenfungusandTBinfectionsofthelung, infection also provides ideal immune suppression that allows these fungi thrive favorably (52)

7. Strengths and Limitations of the Study

7.1Strengths

The study had addressed both yeast and mold species that mainly responsible for causing respiratory mycosis in pulmonary tuberculosis patients at confirmed PTB high TB burden of confirmed hospital.

7.2 Limitations

Due to financial constraints and manpower, Immune status, risk factors for fungal infection and drug susceptibility testwerenotperformedinthisstudy.Culturalisolationforthepresenceof MTB couldn't be performed due to some resource limitation at study period. Due to lack of facilities and resources, unable to determine the antifungal susceptibility fungal isolates another limitation of our study

8. Conclusion and Recommendations

8.1 Conclusion

Pulmonary mycoses can be simply misdiagnosed and mistreated as pulmonary tuberculosis .Our study indicates that fungal etiology must be required in100%theclinicallyconfirmed pulmonary tuberculosis patients; otherwise, the case may be missed or misdiagnosed*. albicans, C. krusei, C. tropical's,* other yeast Spp.,*A. Niger*and*A. Fumigatus Spp* dominates both PTB positive patients.

Of 500 patients, 500 sputum cultured were prepared, 64.4% (n=322) of patients were identified as smear-positive fungus from which males were 220 and 210 were female respectively. The mean age of patients was 39±years. With increasing age, the prevalence of TB has increased which was statistically significant Prevalence of fungi co infection in the age group 25 - 44 over in males and females had an ascending trend. Amongst the positive tuberculosis patients, Yeast species were the most dominant than molds respectively however the at species level C albican and Aspergillus had the co-infection of TB with fungi microorganisms.

Co-infection of fungal agents was also seen in (100. %) of MTB positive subjects.*C.albican* was the predominant fungi in PTB positive e subjects.Aspergillus species and Candida species were the predominant species among the positive subjects and therefore found to specially cause or help in complicating broncho pulmonary disorders hence ,in the absence of specific predictionswithregardstopulmonarysymptoms,thepossibilityoffungalcolonizationneedsto be explored

8.2 Recommendations

As there is a high prevalence of fungal infection in both TB positive patients, routine screening for fungal infection is recommended for proper diagnosis and early treatment.

- We recommend the health care system guide lines should be reviewed for the diagnosis of fungal pathogens.
- Based on our results, the prevalence of fungal infections was very high among confirmed PTB Patient's So health care we recommend professionals capacity building conducted &also laboratory equipment& reagent must be equipped.

- The importance of accurate diagnoses should be emphasized, and the various procedures must be outlined in specialized health care system of immune suppressed patients.
- From our study conducted we recommend PTB patients were not treated for fungal path igins.so it is very important to diagnosis immune suppressed patient with fungal pathogens.

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10. Annexes

Annex I: Participant Information sheet, Consent/Assent.

My name is Addis Abe be I am a laboratory technologist postgraduate student at Addis Ababa University. Now I am conducting a study entitled study Fungal Co Infection and Profile of Fungal Pathogens Among Patient with Confirmed Pulmonary Tuberculosis Patients at Saint Peter's Specialized Hospital, Addis Ababa, Ethiopia. You are invited to participate in this study. Please read the following statements and ask any unclear points before you agree to participate. If you agree to be included in this study, I would like to ask you to sign on a document to show your agreement; participate accordingly, and give clinical specimen.

Introduction The topic of this study is Since pulmonary fungal infection (pulmonary mycosis) is one of the major health problems but it is neglected and masked by MTB in our country, the result of the study can be helpful in planning and intervention to solve the problem. Participation in this study is exclusively voluntarily. If you are not interested to participate or if you once decide to participate and withdraw yourself at any time, there will be no consequences and you will get all the services provided in the Laboratory. If you decide to participate, you have to sign on the consent form and you may obtain a copy of this information sheet.

Confidentiality The information in your records is strictly confidential. All information that you give and the results from your specimen will be used for treatment of the participant and this study only. Only limited numbers of professional will have access to the information. The information will be encoded in a computer and saved with password protection.

Benefits of participation- by participating, you will get no financial benefits. The finding of the study is useful for better understanding of the problems of pulmonary mycosis (pulmonary fungal infection). You will also obtain all the results of the analysis and communicated to your physician for the appropriate management.

Rights of participants -Your participation is completely voluntary, and you can refuse to participate or withdraw from the study at any time. Refusal to participate will not result in loss of medical care provided or any other benefits. You can get your results of the analysis.

Communication In case if you have any questions, unclear ideas and doubt about the project,

contact addresses are: Investigator:

Addis Abebe (BSc, MSc student), DMLS; AAU: +251982268831

Email- dilnaddu 6@gmail.com

Advisor: Adane Bitew (PhD), DMLT, AAU. +251911039162

For additional information, please contact Addis Ababa University, College of Health Sciences, Department of Medical Laboratory Sciences at: Telephone. +251112755170 Your signature below indicates that you have read /or listened, and understand the information provided for you about the study. Before you sign, please understand 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 question.

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: Consent form for participants older than 18 years old (adult)

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 36 researchers 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: Assent form for the age 12-17 years old

The objective and the application of the study were briefly explained to me. I am also informed that all information contained within the laboratory request is to be 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 assent form voluntarily to the researcher to give my specimen for the mentioned study and agreed to use the sample for further study in my signature.

Guardians 'name_____ Signature/fingerprint:____ _____Date _____
Participant Name_____ Signature/fingerprint:____ _____Date _____
Witness's Name_____ Signature/fingerprint:____ _____Date _____
Investigator's name_____ Signature/fingerprint: _____

III: Pare _____Date _____

Annex IV-natal/guardian consent form (for ages less than 11 years old)

I was informed take whatever time I need to discuss the study with my family and friends, or anyone else I wish to. The decision to let my child join, or not to join, is up to me, and will take him/her about 10 minutes, it is not painful and my child can stop participating at any time and will not lose any benefits as thereof. As parent or legal guardian, I assure in my signature to become my child a participant in the research study described in this form. Guardian's

Name _____Signature/fingerprint: _____Date _____

Witness's Name _____ Signature/fingerprint: _____Date _____

Investigator's Name ----------Signature/fingerprint: _____Date _____

Annex V: Amharic Versions of Patient Information Sheet.

እኔ አዲስ አበበ በአዲስ አበባ ዩኒቨርሲቲ፣ጤና ሳይንስ ኮሌጅ፣የህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍልየሁለተኛ ዲግሪ ተማሪ ስሆን የምርምር ሰራን በመሰራት ላይ እገኛለሁ።እርስዎም በዚህ ጥናት ላይ እንዲሳተፉ ተጋብዘዋል።በጥናቱ ለመሳተፍ ፈቃደኛ ሆነዉ ከተሰማሙ መስማማትዎን የሚያሳይ ዶክመንት ላይ እንዲፈርሙ እጠይቃሁ።

መግቢያ

The prevalence and Spectrum of Fungal Pathogens and among Confirmed Pulmonary Tuberculosis Patients at Saint Peter's Specialized Hospital, Addis Ababa, Ethiopia.

.ማለትም በመተንፈሻ አካላት ላይ በሽታን የሚያመጡ ፈንገሱች ሰርጭት በሚል ርዕሰ እያጠናሁ እገኛለሁ።ይህጥናትምበ ተሳታፊ ሙሉ ፈቃደኝነት ላይ የተመሠረተነዉ።ከጥናቱተሳታፊየሚጠበቁ በዚህ ጥናት ለመሳተፍ የሚሰማሙ ከሆነ የእክታ ናሙና እንዲወሰድ መሰማማት ይጠበቅቦታል።የጤና ባልሙያ ከእርስዎ ናሙናዉን ይሰበሰባል።ከተወሰደዉ ምናሙና ላይ የሚገኙ መረጃዎች የእርሶን ማንነት የማይገልጹ ማሰረጃዎችን ማለትም ስም፣አድራሻና የመሳሰለት መረጃዎች ሳይጨምርና ለዚህ ጥናት አገልግሎት ብቻ የሚዉል መሊያቁጥር በመጠቀም ከዚህሆስፒታል ዉጭ ለሚገኙ ለሥራ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መሰማማት ይጠበቅቦታል።ናሙና ስጡማለት በሽታው ይገኝብዎታል ማለት አይደለም።በእርስዎ ናሙና ውሰጥ የበሽታ አምጭ ተህዋሰ ቢገኝ ከጤና ባለሙያዉ አሰፈሊጊውን ህክምና ያገኛሉ።የመረጃዉ ሚሰጥራዊነት ማንኛዉም የሰጡት መረጃ እና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማብቻ ነዉ።ይህን ማህደር ሊያገኙ የሚችሉ የተወሰኑ የጥናቱ ተባባሪ ሠራተኞ ችብቻ ናቸዉ።ከዚህም በላይ ሰለእርስዎ ያለዉን ማንኛዉንም መረጃ የይለፍ-ቃል ባለዉ የኮምፒዉተር የመረጃማህደር ዉሰጥ እንዲቀመጥ ይደረጋል።ተሳታፊዉ የሚያጠፋዉ ጊዜየተዘጋጀዉን የሰምምነት ቅጽ ለመፈረምና ናሙና ለመሰጠት 3-7 ደቂቃ ያሰፈልጋል።በጥናቱ በመሳተፍ የሚሰከትላቸዉ ችግሮች ናሙና በሚሰበሰብበት ወቅት ምነም አይነት ችግር አያሰከትልቦትም።

በጥናቱ በመሳተፍ የሚያሰከትላቸዉ ጥቅሞች ይህ ጥናት የማሰተርሰ ዲግር መመረቂያ እንደመሆኑ መጠን በመሳተፍዎ የሚያገኙት የገንዘብ ጥቅማጥቅም የለም። ሆኖም በቀኑ በሚደረገዉ የላቦራቶሪምረመራ ለህኪምዎ በመሰጠት አሰፈላጊዉን ህክምና እንዲያገኙ ይደረጋል እንደአሰፈላጊነቱም የካልቸር ዉጤቶትንም ጠብቆ አሰፈላጊዉን ምርመራ ይደረግሎታል።ለወደፊት በተመሳሳይ ሁኔታ ዉሰጥ ላሉ በሽተኞች በመረጃ ላይ የተመሰረተ ህክምና ለመሰጠት ያግዛል ከፈለጉ የላቦራቶሪ ዉጤቶችን በነፃ ያገኛሉ እንዲሁም ሰለ አሰፈላጊዉ ህክምና ከህኪምዎጋርይነጋገራሉ።የጥናቱ ተሳታፊዎችመብት ትብብርዎ ሙሉ በሙሉ በፍቃደኝነት ላይየተመሠረተናተሳትፎዎንመተውናበማንኛውምሰዓት ጥናቱን ማቆም ይችላሉ።በጥናቱ ዉሰጥ ያሎትን ተሳትፎ በማንኛዉም ጊዜ የማቐረጥ ሙሉ መብትዎ የተጠበቀ ከመሆኑም በላይ ራሶን ከጥናቱ በማግለል ዎምክንያ ትየሚቀርብዎት ምንም ዓይነት የሆስፒታል አገልግሎት አይኖርም።ከዚህም

በተጨማሪ ጥናቱን በተመለከተ ማንኛዉንም ዓይነትጥያቄየ መጠቅና ገለፃ የማግኘት መብት አለዎት።የላቦራቶሪ ምርመራ ዉጤቱንም በነፃ ማግኘት ይቻላል።ግንኙነትና ጥያቄ ይህን ጥናት በተመለከተ ወይም ከዚህጋራ በተዛመደ መልኩ ሰለሚያጋጥዉ ድንገተኛ ችግር ወይም ጥያቄ ካሎት በሚከተሰዉ አድራሻይጠቀሙ፡

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አዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል! አማካሪ፣አዳነ ቢተዉ (ፒ.ኤች.ዲ) ሞባይል+251911039162, ኢ-ሜይል፤bitewadane@gmail.comአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ላብራቶሪ ሳይንስ ትምህርትክፍል! ለተጨማሪ መረጃ አዲስ አበባ ዩኒቨርሲቲ፤የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል ይጠይቁ; ሰልክ-+251112755170 ከዚህበታ ችየሚገኘው ፊርማዎ ለእርስዎ የተሰጠውን መረጃ ማንበብዎን፣ መሰማትዎን እና መገንዘብዎን የሚያሳይነው።ከመፈረምዎበፊትእባክዎትንየጥናቱንዓላማ፣የተሳትፎጉዳትናጥቅሙ፣የመተው፣የማቋረጥ፣መብትናነፃነትእ ንዳለዎትይረዱ።

ተሰማምተዋል? የጥናቱንመግለጫእንብብያለሁ/ ሰምቻለሁ እናም ተረድቻለሁ።መመሪያውምን እንደሆነ ና በእኔም ንሊከሰት እንደሚችል ተረድቻለሁ።በጥናቱላይለመሳተፍ፤እሰማማለሁ_____ አልሰማማም_____

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Annex VI: Amharic Versions of Consent Form for Participants Older Than 18 Years of Age

የተሳታፊ ስምምነት ቅጽ ይህ ጽstudy Fungal Co Infection and Profile of Fungal Pathogens Among Patient with Confirmed Pulmonary Tuberculosis Patients at Saint Peter's Specialized Hospital, Addis Ababa, Ethiopia.ማለትም በመተንፈሻ አካላት ላይ በሽታን የሚያመጡ ፈንገሱችስርጭትበቅዱስኢትዮጵያ" በሚል ሆነዉ ከተሰማሙ መስማማትዎን የሚያሳይ ፊርማ

1. እኔ በመተንፈሻ አካላት ላይ በሽታን የሚያመጡፈንገሱችስጭት
2. At Saint Peter's specialized hospital, Addis Ababa Ethiopia".የሚለዉ ጥናት አላማ በደንብ ተገንዝቤአለሁ።
3. ከእኔየ ሚወስደዉ ናሙና ለጥናቱ አላማ ብቻ እንደሚዉል ተረድቻለሁ።
4. ሁለም መረጃዎች እና የናሙና ዉጤቱ ምስጢራዊመሆኑንተገንዝቤአለሁ።
5. በጥናቱ ላይበ መሳተፌምንም የገንዘብ ክፍያ እንደ ማላገኝ ተረድቻለሁ።
6. በጥናቱ ያለመሳተፍ እንዲሁም በማንኛዉም ጊዜየማቃረጥ መብትእንዳለኝአዉቄአለሁ።
7. ሁለም መረጃዎች በአስተባባሪዉ /ዎች ተገልጾልኝ በደንብተረድቻለሁ።

የተሳታፊ ፊርማ፡----------

የተሳታፊ አድራሻ፡----------

ቀን፡----------

በሰምምነቱ ወቅት የነበሩምስክሮች

1._____

2._____

ይህንንጥናትበተመለከተጥያቄቢኖርዎትወይምከዚህጋራበተዛመደመልኩሰለሚያጋጥመዎትድንገተኛችግርበሚከተለዉአ ድራሻይጠቀሙ።

ሞባይል፡+251982268831 የሕክምና ላብራቶሪ ሳይንስትምህርትክፍል

የጤናሳይንስኮሌጅ፤አዲሰአበባዩኒቨርሲቲኢ-ሜይሌ፤delenad6@gmail.comለተጨማሪ መረጃ፡አዲሰ አበባ ዩኒቨርሲቲ፤ የሕክምና ላብራቶሪ ሳይንስት/ ክፍል ይጠይቁ።ሰልክ፡+251112755170

Annex VII: Amharic versions of ascent form for participants less than 18 years of age

The prevalence and Spectrum of Fungal Pathogens and among Confirmed Pulmonary Tuberculosis Patients at Saint Peter's Specialized Hospital, Addis Ababa, Ethiopia.

ይህ ገጽ ማለትም በመተንፈሻ አካላት ላይ በሽታን የሚያመጡ ፈንገሱችስጭት" በሚልርዕሰእድሜያቸውከ18ኣመትበታችለሆናቸውተሳታፊዎችሰምምነትየመጠየቂያቅጽነው።ናሙኖየምንወሰድበትመሳሪ ያንጽህናውሙሌበሙሌአሰተማማኝናከዚህበፊትጥቅምሊይያልዋለነው።ናሙኖበምንወሰድበትጊዜየሚኖርህመምየሚፈ ጥርሰሜትምሆነአደጋየሚያሰከትልሂደትየለውም።ለጥናቱየሚወሰደውናሙኖለጥናቱአላማብቻይውላል።የናሙኖውውጤ ትምሰጥራዊነትየተጠበቀሲሆንበናሙኖውውሰጥየበሽታአምጭተህዋሰቢገኝከጤናባለሙያዉአሰፈላጊውንህክምናያገኛ ሉ።በጥናቱላይበመሳተፍዎምንምየገንዘብክፍያአያገኙም።በጥናቱለመሳተፍየመፍቀድምሆነያለመፍቀድእንዱሁምበማን ኛዉምጊዜየማቋረጥመብትአለዎት።

(ሰም) በጥናቱ እንዲሳተፍ/ እንድትሳተፍ ይፈቅዳሉ? ፈቃዮኛ ከሆኑ፤የተሳታፊ

ፊርማ፣---------- የፈቀደው ግለሰብ ፊርማ፣----------

እድራሻ፣---------- ቀን፣--------በሰምምነቱ ወቅት የነበሩምሰክሮች

1._____

2._____

ይህንጥናትበተመለከተጥያቄቢኖርዎትወይምከዚህጋራበተዛመደመልኩሰለሚያጋጥመዎትድንገተኛችግርበሚከተለዉአ ድራሻይጠቀሙ።

ሞባይል፡+2519 82 26 8831 የሕክምና ላብራቶሪ ሳይንስ ትምህርት ክፍል የጤና ሳይንስኮሌጅ፤ 41 አዲስ አበባ ዩኒቨርሲቲኢ-ሜይሌ፤dilnaddu 6@gmail.com ለተጨማሪ መረጃ:አዲስ አበባ ዩኒቨርሲቲ፤የሕክምና ላብራቶሪሳይንስት/ ክፍል ይጠይቁ።

ሰልክ፡+25111275517

Annex VIII: Reagent Preparation (Stains and media) and Quality Control Quality Control

Sabouraud Dextrose Agar with Chloramphenicol

Pancreatic Digest of Casein	5.0 g
Peptic Digest of Animal Tissue	5.0 g
Dextrose	40.0 g
Agar	15.0 g
Chloramphenicol	0.05 g

Storage Instructions: store plates in the dark at 2 – 8°C ready for use. For slopes: Dispense 10ml. amounts into bottles.

PRINCIPLES OF THE PROCEDURE Sabouraud Dextrose Agar is a peptone medium supplemented with dextrose to support the growth of fungi. The peptones are sources of nitrogenous growth factors. Dextrose provides an energy source for the growth of microorganisms. Chloramphenicol is a broad-spectrum antibiotic which is inhibitory to a wide range of gram-negative and gram-positive bacteria. For slopes: Dispense 10 ml. amounts into UGB bottles. Autoclave 12°C/15 minutes. Store at room temperature and Final pH7.0 at 25° C.

B. Brain Heart Infusion Agar with Chloramphenicol

Calf brain, infusion from	200g
Beef heart, infusion from	250g
Proteose peptone	10g
Dextrose	2g
Disodium phosphate	2.5g
Sodium chloride	2.5g
Agar	15g
Chloramphenicol	0.05g
Final pH (at 25°C)	7.4±0.2

Storage Instructions: store plates in the dark at 2 – 8°C ready for use. For slopes: Dispense 10mlamounts into bottles in slant position

Annex VIIII Principle

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. Disodium 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. Suspend52g in 1000ml, cold freshly distilled water

2. Heat to boiling to dissolve the medium completely

3. Sterilize in autoclave for 15 minute at 15 pounds pressure (1210 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 neofo mans. The former is urease negative while the latter is urease positive.

Composition Ingredients per liter of deionized water:

Ingredients per liter of deionized water:

Urea	20.0 g
Sodium Chloride	5.0 g
Monopotassium Phosphate	2.0 g
Peptone	1.0 g
Dextrose	1.0 g
Phenol Red	0.012 g
Agar	15.0 g

Agar 15 Final pH 6.7 +/- 0.2 at 250 C.

Principle of Urease Test

Urea is the product of 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.

Preparation

1. Dissolve the ingredients in 100 ml of distilled water and filter sterilizes (0.45-mm pore size).

2. Suspend the agar in 900 ml of distilled water, boil to dissolve completely.

3. Autoclave at 1210 C and 15 psi for 15 minutes.

4. Cool the agar to 50 to 550 C.

5. Aseptically add 100 ml of filter-sterilized urea base to the cooled agar solution and mix thoroughly.

6. Distribute 4 to 5 ml per sterile tube (13 x 100 mm) and slant the tubes during cooling until solidified

Quality Control of Urease Test

Positive Proteus vulgaris (ATCC13315)

Weak positive: Klebsiella pneumoniae (ATCC13883)

Negative: Escherichia coli (ATCC25922)

Test procedures Gene xpert, MTB/rif assay (principle) 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 is able to 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 (MDR-TB). 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 do not bind, this indicates the presence of a mutation and therefore 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

Test procedure

1. 4ml of sputum sample was mixed with 8ml of sample reagent (1:2). Close the lid of the container tightly and vortex for 15 seconds
2. After 15 min mixed specimen to stand for 15 minutes at room temperature
3. Shake the container once during the 15-minute incubation i.e., after 10 minutes
4. After 5min 2ml of the prepared sample was transferred to a cartridge for MTB/Rif assay
5. The cartridge was put in GeneXpert closed system real-time PCR analyzer.
6. After 2hr the result was ready

Quality Control

Pre-analytical

A sterile sputum collection cup was issued to patients. Patients are asked to produce sputum from deep-by-deep breathing three times and coughing so as to expectorate purulent sputum. Collected sputum samples were inspected for eligibility. Reagents used for Xpert MTB/RIF assay, SDA, BHI media preparation and KOH 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 standard operation system of the tests. Media preparation was performed according to manufacturers' manual Prepared SDA Medias was checked for sterility by incubating at 25-370 C for weeks sterility of each batch of prepared media was checked by incubating un inoculated media at 25-370 C for weeks if there is any kind of microbial growth the prepared batch was discarded. Growth support of each batch of prepared Medias was checked with known representative yeast species C. albicans and mold of Aspergillus species

Post analytical • Results generated was appropriately documented electronically • To prevent loss of data backup data will also be documented on result registration logbook. • Appropriate disposal system by incineration was utilized after disinfection of Specimens and cultures of organisms by autoclaving.

Annex IX: Demographic and Clinical Data Record Format Addis Ababa University, collage of health sciences,

Department of medical laboratory science. Demographic and clinical record format for the prevalence and distribution of fungal species in sputum from pulmonary TB confirmed patients at Saint Peter's specialized hospital, Addis Ababa Ethiopia.

I. Sample ID no. _____ MRN_____ Age_____

II. Gender 1 Female 2 Male

III. Previously treated by anti-pulmonary fungal drug

IV. Gene Xpert MTB Rif assay Positive Negative

11. 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:**Addis Abebe (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor:

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.