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Prevalence and Distribution of fungal species in sputum collected from pulmonary tuberculosis suspected Patients at Saint Peter's Specialized Hospital, Addis Ababa, Ethiopia.

BY: Solomon Bati (BSC, MSc candidate).

Advisor: Adane Bitew (MSc, PhD).

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This is to certify that the thesis prepared by **SOLOMON BATI**, entitled: **"Prevalence and Distribution of fungal species in sputum collected from pulmonary tuberculosis suspected 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) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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External Examiner_____ Signature _____Date _____

Internal Examiner_____ Signature _____Date _____

Advisor Adane Bitew (PHD) Signature _____Date _____

_____

Chairman of the Department or Graduate Program Coordinator

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Abbreviations

AAU	Addis Ababa University
AFB	Acid Fast Bacilli
AHB	Addis Ababa Health Bureau
BHI	Brain Heart Infusion Agar
CDC	Center for Disease Control and Prevention
CPA	Chronic Pulmonary Aspergillosis
HIV	Human Immune Deficiency Virus
MDR-TB	Multi Drug Resistant TB
MTB	Mycobacterium Tuberculosis
MTBC	Mycobacterium Tuberculosis Complex
NAA	Nucleic Acid Amplification
PCP	Pneumocystis Pneumonia
PTB	Pulmonary Tuberculosis
QC	Quality Control
RIF	Rifampicine
RTI	Respiratory Tract Infection
SDA	Sabouraud Dextrose Agar
Spp.	Species
TB	Tuberculosis
WHO	World Health organization

Abstract

Background: Pulmonary mycosis is a systemic fungal infection that occurs when fungi cause's disease of the lungs. Fungal pulmonary infection has been emerging recently due to widely used broad-spectrum antibiotics and steroids, immune deficiency or suppression in tuberculosis, patients are easily vulnerable to opportunistic fungal infections.

Objective: To determine the distribution of pulmonary fungal isolates among pulmonary tuberculosis presumptive patients in the sputum sample by conventional culture.

Methods: A cross sectional study was conducted at Saint Peters specialized hospital on 636 sputum samples from presumptive tuberculosis patients. Early morning sputum samples were collected aseptically and Presence of pulmonary Tuberculosis was screened by Xpert MTB-Rif/assay. All sputum samples were cultured using Sabrouad dextrose agar and Brain heart infusion agar medium incubated at 25^0C and 37^0C for 4 weeks respectively for each sputum samples. Identification was performed by noting; the growth form and rate of growth, surface and reversed coloration. Yeasts were identified by conventional biochemical tests and assimilation characteristics. Chromo agar medium used for Candida species and urease used for *Cryptococcus neoformance* and the rest were non-yeast species. Data analysis was carried out using SPSS version 23 software.

Results: The distribution of pulmonary fungal isolates among pulmonary tuberculosis presumptive patients was at 75.9% of the infected patients, male patients accounted for 51.4%) %, whereas female patients accounted for 48.6%.Yeast isolates (82%) and mold isolates were (18%). Among 163 tuberculosis (TB) cases 127(77.9%) were co-infected with pulmonary fungal pathogen.

Conclusion: High distribution of fungal isolation (75.9%) was obtained in patients with respiratory symptoms. We recommend for policy makers to conduct further studies and consider the need of fungal screening in these patients.

Keywords: Respiratory fungal infection, presumptive TB patients, Yeast species, Filamentous fungi, Co-infection

1. Introduction

1.2 Background

Pulmonary mycosis is a fungal infection of the lungs [1,40]. It occurs when fungi cause diseases of the lungs through direct infection of the pulmonary tissues, by invading pulmonary airspaces/lung cavities, or through their ability to trigger an immunological reaction when fungal material is inhaled [2]. It can be caused by either endemic or opportunistic fungi or a combination of both [3].

Opportunistic fungal infections involve ubiquitous fungi and occur predominantly in individuals whose immune systems are compromised. These infections do not follow any particular geographic distribution and are seen with increasing frequency worldwide.The opportunistic fungal pathogens includeCandida species, Aspergillus species, *Penicillium marneffei, Scedosporium apiospermum, Zygomycetes, Trichosporon beigelii,*Fusarium speciesand *Cryptococcus neoformans.*Most cases of primary mycoses are asymptomatic or clinically mild infectionsoccurring in normal patients living or traveling in endemic areas. However, patients exposed tohigh inoculums of organisms or those with altered host defenses may suffer life-threateningprogression or reactivation of latent foci of infection [4].

Invasive pulmonary Aspergillosis and systemic candidiasis are the most prevalent opportunistic fungal infections. Mold infections that were once considered rare are now emerging as significant infectious complications of the severely immune compromised. The fungus Aspergillus is the leading cause of infection-related death in stem cell transplant recipients [4]. Allergic lung diseases can also develop in otherwise healthy subjects who are repeatedly exposed to environmental molds.

On the other hand, endemic fungal infections follow distinct geographic distributions that are determined by soil and climate conditions optimal for the fungi's growth. North America is home to three of the major endemic mycoses: The primary or endemic systemic fungal pathogens include*Coccidiodes immitis, Histoplasma capsulatum, Blastomyces dermatitidis,* and *paracoccidiodesbrasiliensis.*histoplasmosis, blastomycosis and coccidioidomycosis. Their prevalence varies by region [5].

These three endemic fungal diseases share many characteristics. Illness is acquired by inhaling aerosolized spores. Healthy individuals who contract these diseases generally experience few

symptoms or if they become ill, recover quickly on their own. In contrast, the infections can be life-threatening in patients with depressed immunity, especially those with acquired immune deficiency syndrome (AIDS) and those receiving immunosuppressive medications. The infections can persist and cause other lung diseases, such as emphysema, to worsen [5,6].

Tuberculosis (TB) is a chronic infectious disease. It is one of the top 10 causes of death and the leading cause from a single infectious agent. *Mycobacterium tuberculosis* (MTB*)* usually affects the lungs and causes pulmonary tuberculosis (PTB) [1].According to 2018 WHO report, Globally in 2017, there were an estimated 10.0 million incident cases of TB, equivalent to 133 cases per 100 000 population. Majority of estimated number of incident TB cases were PTB (85%).According to this report Ethiopia listed in the 30 high TB burden countries in the world with an estimated TB incidence of 150–400 per 100 000 individuals [7].

Pulmonary fungal infections have clinical and radiological characteristics are very similar to that of pulmonary tuberculosis thereby making the disease easily misdiagnosed and mistreated as tuberculosis (8).So, the absence of adequate study on pulmonary fungal infection in relation to presumptive pulmonary tuberculosis patients in Ethiopia, which inspire us to investigate the distribution of pulmonary mycoses in presumptive cases of pulmonary tuberculosis patients.

Therefore the objective of this study was to determine the distribution rate of pulmonary fungal infections in presumptive pulmonary tuberculosis patients and demographic characteristics in relation to systemic mycoses. The study was conducted on patients visiting Saint Peter Specialized Hospital, Addis Ababa, Ethiopia. It is the center of excellence in the treatment of TB and MDR_TB patients in the country.

1.3Statement of the Problem

Global burden of respiratory tract infection is responsible for one-third of infectious disease associated mortality, with an estimated 4.3 million annual deaths. Among these, fungal infections of the respiratory tract are largely unrecognized or misdiagnosed and the true burden is difficult to describe [9]. It has been reported that the estimated overall incidence of systemic fungal infection is up to 11.3% of which respiratory mycoses that involve the bronchi and lungs comprises 60% [10]. 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 immunosuppressive agents, and the increasing population of terminally ill, debilitated and immune compromised patients [2].

Case mortality in pulmonary mycosis can be as high as 90% in immune-compromised patients though immune-competent patients generally respond well to antifungal therapy [3]. Due to the lack of specific clinical manifestations and imaging feature, the diagnosis of pulmonary mycosis is difficult and often easily misdiagnosed and mistreated which also contribute to associated high mortality rate [3,10]. In most developing countries like Ethiopia, the problem is further amplified by the preponderance of pulmonary tuberculosis and paucity of diagnostic mycology laboratories. Consequently, patients with respiratory mycoses may be misdiagnosed astuberculosis, other bacterial diseases, flu-like infections, or any disease of obscure etiology [11]. The distribution of pulmonary fungal infections in Ethiopia and many other developing countries has remained largely unexplored and neglected. This has led to a widespread biased impression about their true public health significance. Since it imposes major public health threat equally like other respiratory infections, this neglect needs to come to end and has to be seen as a major call for concern [12].

Hence, Ethiopia is one of developing country and the problem was amplified here as other developing country due to preponderance of pulmonary tuberculosis and paucity of diagnostic mycology laboratories. Based on this fact we include in the research problem to determine prevalence and distribution of fungal species and co-infection with pulmonary tuberculosis at high mycobacterium tuberculosis burden and excellence of tuberculosis hospital in the country.

1.4 Significance of the study

The results of the study can be merit to different groups. The main merit is 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. It also give a clue to clinician to suspect the disease in parallel with pulmonary tuberculosis. This study can also be used as a baseline data for epidemiological studies of respiratory fungal infection in the country, and may indicate the need of fungal diagnosis as part of routine respiratory tract infection diagnosis.

2. Literature Review

A number of studies had been undertaken to identify pulmonary fungal infection among pulmonary tuberculosis suspected patients globally. The incidence of systemic fungal infections remains far beyond hoped and despite treatment, most invasive fungal infections are associated with high mortality rates of >50% [9,11]. But in Ethiopia studies related to pulmonary mycosis is limited. In this section, we review a few of literature especially the ones that are closely related to the objectives of this study in addition to going through other studies.

A study conducted in India on prevalence of mycotic flora with pulmonary tuberculosis patient by Babita, *et al.*obtained that 18(24%) culture showed fungal growth among sputum of 75 diagnosed pulmonary tuberculosis patients were taken and cultured on SDA. *Candida albicans* was isolated in 8 cases (44.4%) *Aspergillus niger* was isolated in 6 cases (33.3%), *Aspergillus fumigatus* in 3 cases (16.5%) and *Aspergillus flavus* in only 1 case (5.5%) [13], another study conducted by Kali A, *et al*. showed that *Candida* co-infection was observed in 30 (40%) of 75 patients with pulmonary tuberculosis and *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 [14].

Across sectional study, carried out by Tshering O, *et al.* 200 clinically suspected pulmonary tuberculosis cases and in 54 (27%) patients various types of pathogens were detected. Fourteen (7%) patients were positive only for AFB, while fungus as a primary etiological agent was detected in 16(8%) patients. Fungus as a secondary etiological agent was detected in 4 (2%) patients [AFB with fungus in 2 (1%)], AFB with fungus and bacteria in 1 (0.5%) and bacteria with fungus in 1 (0.5%) patient [3].

Sunita B, *et al.* carried out a study on emerging of mycotic infection in patients infected with *Mycobacterium tuberculosis* in India got from highly pulmonary tuberculosis patients 500 out of the 203 patients were suffering from mycotic infection. The percentage of mycotic infections in pulmonary tuberculosis patients was 46%. Mainly four types of fungi, i.e. *Aspergillus niger, A. fumigatus, Histoplasma capsulatum* and *Cryptococcus neoformans* were recorded, which causes severe infection in lungs in patients suffering from pulmonary tuberculosis.The increasing

presence of overlapping opportunistic infections in tuberculosis, identification of such fungi that are present in pulmonary tuberculosis patients. Which may help in correct diagnosis of these diseases. So that cure rate will be increase [2,15].

A study carried out in India by S.Mathavi, *et al.* in 107 pulmonary tuberculosis patients about 38% of patients had fungal co-infection. The most common fungi were Candida species (18%) followed by Aspergillus species (15%). Most of the times these fungal infections are not diagnosed and often mistaken for recurrence of tuberculosis. Hence adequate measure must be taken for the early diagnosis and treatment of these opportunistic infections, which are associated with high rate of morbidity and mortality. More than90% of all reported fungal-related deaths result from species that belong to one of four genera: Cryptococcus, Candida, Aspergillus, and Pneumocystis[16,17].

Study conducted in China shows that invasive filamentous fungal infections of lung remain important causes of death in immune compromised patients especially Aspergillusspecies and other non-Aspergillus molds [18]. At Chang, China a study carried out on 68 patients the result shows that the main pathogens of pulmonary mycosis are Aspergillus(55.9%), followed by Cryptococcosis(27.9%) [10]. According to Gaurishanker P, *et al.*in 100 sputum samples 61% were culture positive and 39% were negative . 29% of 100 samples are aspergillus species while 32% of positive cultures are other than aspergillus species. Out of 61 positive culture 18 culture shows *Aspergillus fumigates*, 7 are *Aspergillus niger*, 3 culture shows *Aspergillus flavus* and 1 case show *Aspergillus nidulans*[19].

According to study conduct in Thailand show fungal isolates from 861 patients in which 245 were considered to be causative agents of invasive fungal infections. *Candidaalbicans* was the most common fungus isolated (46%, 396/861) but the most invasive fungal infection causing yeast obtained from this study was *Cryptococcus neoformans* (34.7%, 85/245) while *Penicillium marneffei* is the most invasive fungal infection causing mould(10.6%, 26/245) [20].A literature reviewed by Das R, *et al.* shows that in developing countries, the burden of invasive fungal infection is increasing largely. Instead in developed countries infections with *Candida albicans* may be decreasing in frequency, the number of persons at risk for them continues to grow. Prolong and deep neutropenia and treatments neutralizing macrophage inflammatory cytokines

have increased the likelihood of opportunistic infections. Extensive use of fluconazole in neutropenic patients has resulted in marked decrease in the incidence of invasive candidosis but it has resulted into a shift from highly susceptible to less susceptible Candida Spp.[21].

A study conducted in central India on prevalence of opportunistic fungal infection in patients with pulmonary tuberculosis result revealed that out of 100 samples which 49% of total were positive for fungal infections and 65.31% infections were due to yeasts while 37.69% infections were due to filamentous fungi [22]. Gaurishanker P*et al*. carried out a study to isolate of aspergillus species from sputum samples at tertiary care hospital and revealed that out of 100 sputum samples 61% were culture positive and 39% were negative among cases of chronic respiratory diseases. 29% of 100 samples are aspergillus Spp. while 32% of positive cultures are other than aspergillus Spp. Highest number of male & female patients were from 31-40 years of age and then gradually decrease in number in 21-30 and 41-50 years of age group [23].

In Africa a study conducted to determine prevalence of pulmonary mycosis from acquired immune deficiency syndrome patients and obtained that among 195 samples in 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. The study finally concludes that pulmonary opportunistic mycoses in AIDS patients should include treatment for Candidiasis and Cryptococcosis for all age groups as well as additional antifungal agents if patients fall within 21-45 age groups [24].

A study conducted in Nigeria by Yahaya H, *et al.* to identify diversity of respiratory yeasts from suspected pulmonary tuberculosis patients and obtained result 111 (37%) yeast out of 300 samples. Yeast mainly belonging to the genus candida. *Candidakrusei* was 36(12%),*Candida albicans* 28(9.3%), *Candida tropicalis* 22(7.3%) and *Candida glabrata* with the prevalence of 14(4.7%).Candida co – infection with the TBwas 7(2.3%) in male samples and 4(1.3%) in female samples. The prevalence of non – albicans species is increasing possibly due to their apparently greater capacity than *C. albicans* to invade deep tissues of immune compromised host and inadequate response to anti – tuberculosis drugs [25].

A study carried out in Kenya by Mwaura N *et al.,* mycological findings of sputum samples from pulmonary tuberculosis patients and report that pulmonary fungal pathogens were isolated as co-pathogens with MTB in 76(44.18%). Yeasts accounted for 46/172 (26.7%). Finally, the study concludes that pathogenic fungi and other bacterial pathogens may be significant co-infecting pathogens complicating the management of TB (26).Ekenna O*et al.,* conducted a study in Nigeria showed that of the 274 patients seen, 41(15%) had positive sputum for AFB, while 61(22.3%) had positive sputum for fungal isolates. The most common fungal isolates were Aspergillus spp (42.9%), Scopulariopsis (14.3%), Chrysosporium (8.9%), Penicillium (7.1%), Fusarium (7.1%) and Acremonium (5.4%) [27].

A 2017 review of literature on fungal infections in Ethiopia shows that there is scarcity of evidences on this problem and existing ones focused mainly on superficial and mucosal mycosis while there is almost none on an invasive or systemic infection. The study revealed that the existing literature on any fungi in Ethiopia over a span of two decades revealed only 91publications, out of which only 45 original articles were on human fungal infections. Out of the45 publications, 17 were on superficial infections mainly on Dermatophyte infections, 14 onmucosal Candidiasis and Candiduria, 9 on Cryptococcal meningitis, 4 on Pneumocystispneumonia, and one on fungal keratitis. On the other hand, there was no study on invasivefungal infections such as blood stream infections with Candidaor chronic pulmonaryAspergillosis [28]. Tafese B et al,. reported that around 9% of Ethiopians suffer from fungal infections annually, mostly school children with Tinea capitis. Cryptococcosis and PCP are the major causes of mycoses-related deaths. Upgrading both mycosis and systemic mycosis diagnosis capacity and national surveillance and study of fungal infections isneeded to know exact problem of those infections.

3. Objectives

3.1 General Objectives

To determine prevalence and distribution fungal species in sputum collected from pulmonary tuberculosis presumptive patients at Saint Peters Specialized Hospital Addis Ababa, Ethiopia, from January to June 2109.

3.2 Specific Objectives

- To assess the distribution of filamentous fungal species causing pulmonary fungal infection among pulmonary tuberculosis presumptive patients.
- To assess the distribution of yeasts species causing pulmonary fungal infection among pulmonary TB presumptive patients.
- To determine co-infection of pulmonary tuberculosis and respiratory fungal infection.

4. Methods and Material

4.1 Study Area

The study was conducted in Addis Ababa, Ethiopia at Saint Peter's specialized hospital. Addis Ababa is the capital city of Ethiopia which has an estimated population of 3.6 million in the city proper and a metro population of more than 4.6 million [28]. Saint Peter Specialized hospital is one of the specialized health facilities in Addis Ababa with 455 health professionals giving service to more than 70,600peoples dwelling around it. The facility has an average new outpatient turnover of 150-200 per day. The laboratory now acting as a referral laboratory for MTB related cases for its catchment health facilities and specialized center for all referred cases from different parts of the country. The Microbiology department was accredited by Ethiopian national accrediting organization (ENAO) and continuously assessed by (national accrediting organization (ENAO). Even though there is no mycology laboratory in the facility, the facility has well organized TB clinic and TB laboratory with an average turnover of 10-25 new TB suspects per day. All tuberculosis referral cases sent to the hospital as a criteria genxpert test can be done for all them.

4.2Study Design and Period

A cross sectional study was conducted from February 2019 to May, 2019 at Saint Peter's Specialized Hospital, Addis Ababa, Ethiopia.

4.3 Population

4.3.1 Source Population

The source populations were patients who attend tuberculosis clinic and rehabilitation center of Saint Peter's specialized hospital and its referring sites during study period.

4.3.2 Study Population

The study populations were those patients attending PTB department and rehabilitation center of Saint Peter's specialized hospital during the study period.

4.4 Inclusion and Exclusion Criteria

4.4.1 Inclusion Criteria

All sputum samples from patients clinically suspected of pulmonary tuberculosis those who were sent to the microbiology department of Saint Peter Specialized hospital laboratory willing to participate in the study were included in the specified period of the study.

4.4.2 Exclusion Criteria

Previously confirmed pulmonary fungal infection cases and who were on anti-fungal treatment were excluded from the study. Insufficient and inappropriate sputum samples and sputum from patients lacking full clinical data was excluded.

4. 4Study Variables

4. 4.1Dependent Variables

The prevalence of pulmonary fungal infection.

4.4.2Independent Variables

The demographic data and pulmonary tuberculosis result were used as independent variables.

4.6Measurement and Data Collection

4.6 .1Sample Size Calculation

Sample size was calculated based on a single population proportion formula. The expected prevalence, p=42.3 % (p=0.423) taken from Muluneh T. astudy in Addis Ababa [47].
At 95% confidence interval,
Margin of error tolerated is 5 % (0.05)

$$\text{Study participants: } n = Z^2 P (1- P)/ d^2$$

Where n = sample size
z = 95% statistic for level of confidence (1.96)
P = population proportion (42.3%)

d = margin of error (degree of accuracy desired
(d=0.05) and 95% level of confidence (z=1.96)

The sample size was estimated to be: = $(1.96)^2 \times 0.423\ (1 - 0.423)/\ (0.05)^2 = 375$
Therefore, by adding 10% contingency, a minimum of **413** participants should be included in the study. We included 636 different participants in order to get more representative findings, power and strength of the study.

4.6.2Sampling Method

Convenient sampling method was utilized to achieve the estimated sample size. All pulmonary tuberculosis presumptive patients visiting Saint Peter's specialized hospital microbiology department laboratory within the specified time of the study.

4.6.3 Data Collection Procedure

4.6.3.1Demographic data

The socio-demographic data, previous pulmonary fungal infection and treatment history of the study participants was obtained from laboratory request form 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 calculated sample size for pre- test was conducted using demographic and clinical data collection formats and log books. The purpose of the study as well as any related harm and benefit were explained to the study participants accordingly.

4.6.3.2Sample collection and processing

Collection Procedure

Patients were asked to wash their mouth gently by 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. The patients were instructed to inhale deeply 3 to 4 times before coughing out from the chest. The sputum produced was carefully spit into the sterile falcon tube without contaminating the outside of the tube. The lid of the container was screwed tightly before being processed, with utmost care not wrapping the container with the laboratory request form.

4.6.4Laboratory Analysis

4.6.4 .1MTB Screening Using MTB/RIF Assay

Presence of pulmonary Tuberculosis was screened on the same day of sample collection using the country's current guideline, GeneXpert MTB-Rif/assay. GeneXpert MTB/RIF assay is a rapid diagnosis test of Tuberculosis (TB) and drug resistance (MDR). It is based on a principle of nucleicacid 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 rpoBgene containing the 81 base pair "core" region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with Rifampicin resistance. The Centers for Disease Control and Prevention (CDC) 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 [32].

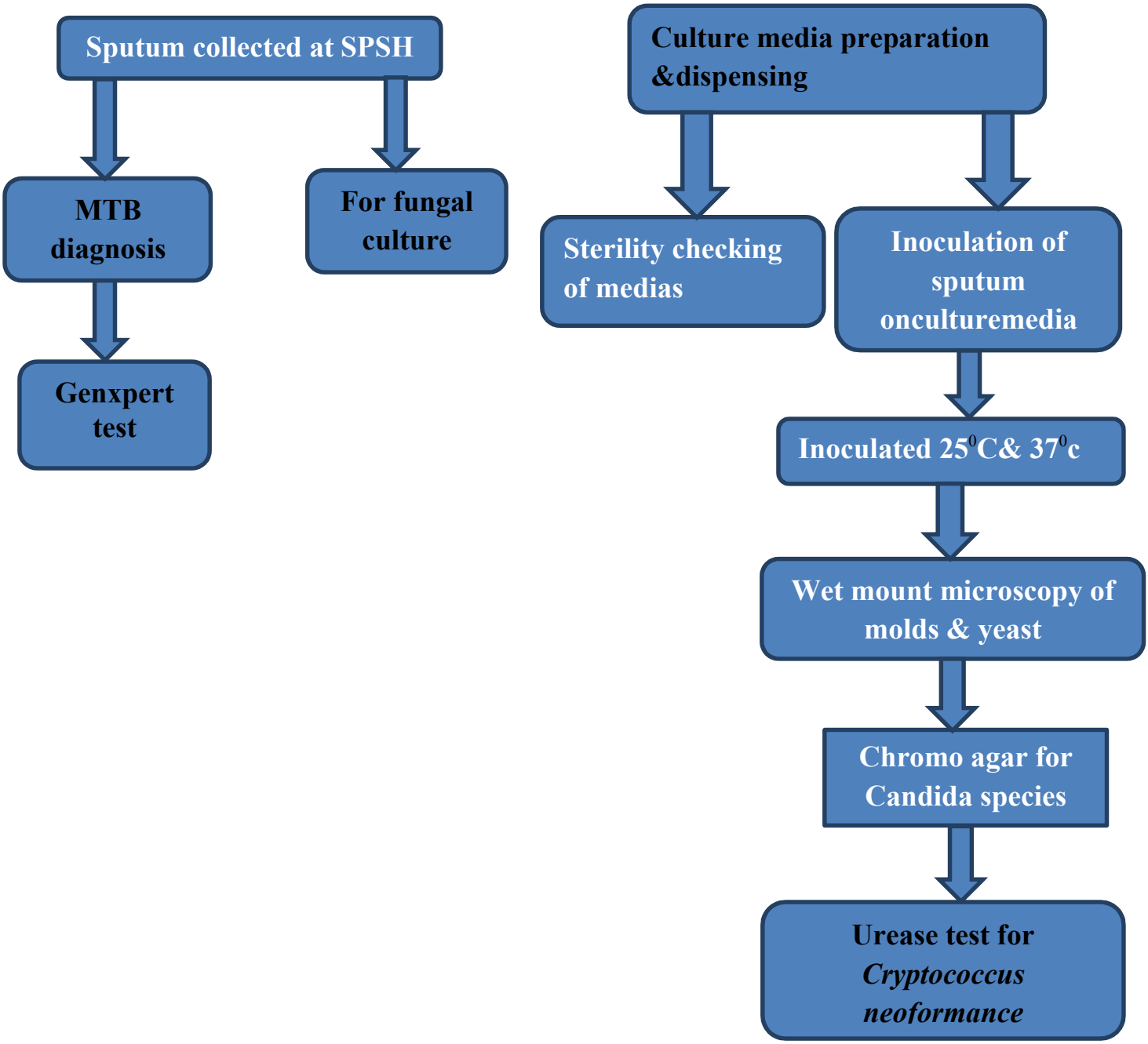

Figure1. Diagram of work flow

4.6.4.2Mycological Analysis

Sample collection

Duplicate sputum sample from tuberculosis suspected patients referred to St. Peter specialized hospital were collected with wide mouthed, clean, 50ml capacity, sterile, disposable containers, polypropylene centrifuge tubes, translucent, screw capped container after detail patient instruction. Sputum in one of the containers was used for the investigation of tuberculosis while sputum in the second container was used for mycological investigation.

Inoculation and incubation

Unprocessed sputum was inoculated directly onto duplicate Sabouraud Dextrose Agar (SDA) tubes supplemented with chloramphenicol (Oxoid, Basingstoke, UK) under safety cabinet level II. All inoculated tubes were then transported to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University. One of the tubes was incubated at 25^0C while the other one was incubated at 37^0C aerobically for up to four weeks. Culture plates were examined twice a week for any fungal growth.

Identification

Fungi were identified by studying their microscopic, macroscopic characteristics and by using an array of biochemical and assimilation tests according to Kern and Blevins [47]. Briefly, cultures of mycelia fungi (molds) were identified by examining macroscopic and microscopic characteristics of their colony. Texture, rate of growth, topographyand morphology of conidia (asexual spore), the nature of reproductive structures and pigmentation of the front and the reverse side of the culture were employed for macroscopic identification. Microscopic identification of mold isolates was performed by placing pieces of colony from SDA to clean microscopic slide and staining with lactophenol cotton blue. After placing a cover slip, the characteristics of conidia and mycelia of each isolate were studied microscopically. Yeasts were identified by employing an array of biochemical and assimilation test procedures [47,48] and using CHROMagar Candida culture medium (Becton Dickinson) as per the instruction of the manufacturer.

4.7 Quality assurance

4.7.1 Data Quality Assurance

The quality and volume of sputum samples and appropriate labeling of specimens was checked before analysis. Expiration date Xpert kit, SDA, Chromo agar and BHI media were inspected; daily preventive maintenance procedure for Xpert analyzer was inspected prior to testing. Prepared SDA, Chromo agar and BHI media were checked for sterility by incubating at 25^0-37^0C. Data was cleaned and checked for completion before analysis and pre-test was done before regular data collection start.

4.7.2 Quality Control

4.7.2.1 Pre-analytical

A falcon tube was issued to patients. Patients were primarily 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 inspected 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.

4.7.2.2 Analytical

- Media preparation was performed according to media manufacturers' instruction on the media itself.
- Prepared SDA, Chromo agar and BHI media were checked for growth support by incubating at 25^0 - 37^oC.
- Sterility of each batch of prepared media was checked by incubating un inoculated media at 25^0 - 30^o C.

4.8 Data Analysis and Interpretation

Data entries done by excel and analysis were done using Statistical Package for Social Sciences (SPSS) software version 23. Descriptive statistics were used to describe the study participants in relation to relevant variables and statistical significant difference in the association between demographical characteristics (i.e. age, sex), tuberculosis result and fungal positivity using

binary logistic regression and odds ratio at 95% confidence interval with the aid of SPSS. Finally, the results were presented on words, graphs and tables.

4.9EthicalConsideration

This study was conducted with the ethical approval of ethical clearance were obtained from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University College of Health Sciences, Department of Laboratory Sciences and permission was obtained from Saint Peter's Specialized hospital research directorate. Personal information about the participants was treated confidentially. Each participant was informed briefly about the study, outcomes and confidentiality. Those showing interest to be included and willing to give consent were only included in the study. Information sheet, consent and assent forms were utilized for this agreement. Patients with positive pulmonary fungal infection were informed with their respective physicians and dealt accordingly.

4.10 Dissemination of Results

The final result of the study were submitted and presented to Addis Ababa University College of Health Sciences, Department of Laboratory Sciences. The result was reported to the host of the study, Saint Peter's specialized hospital research directorate. In addition, the research will be presented to other concerned bodies like professional associations and it will be submitted to peer reviewed journals for publication.

5. Results

5.1Demographic Analysis

A total of six hundred thirty six (n=636) study participants were enrolled in the present study, of which 327 (51.4%) were males and 309 (48.6%) were females as shown in table 1. Theages of the study subjects ranged from 1 year to 94 year with a mean age of 41 years. Approximately, about 40.6% of the participants were between the age's brackets of (25-44) as showed in table1.

Table 1: Socio-demographic, MTB result and pulmonary fungal result among MTB suspected cases, at Saint Peter's Specialized Hospital micro biology laboratory, Addis Ababa, Ethiopia, February to May, 2019.

Variables		Fungal pathogens		Positive percent (%)
		Pos(p)	Neg(n)	
Age group	<15	23	8	4.8
	15-24	64	21	13.3
	25-34	79	46	16.4
	35-44	109	24	22.6
	45-54	65	17	13.4
	55-64	69	24	14.3
	>64	74	13	15.3
Gender	Male	249	78	51. 5
	Female	234	75	48. 5
Genexperet	Positive	127	36	77.9
	Negative	356	117	73.7

5.2Detection and isolation rates of MTB and fungi in sputum

5.2.1 MTB detection

A total of 636 fresh early morning sputum samples were collected and examined for the presence of pulmonary fungal agents and *M. tuberculosis*, out of which 163(25.6%) patients were positive for MTB by genexpert MTB Rif assay. Male subjects presented more positive samples as compared to their female counterparts 87(53.3%) and 76(46.6%) respectively as indicated on table 1.

5.2.2Fungal pathogens isolated on culture

Among the 636 sputum samples analyzed 483/636(75.9%) were positive for fungal elements. In those483positive samples culture of 49subjects presented more than one fungal isolate shown at annex 10. Out of those 483 positive samples 684 fungal isolates were isolated and 562(82%) were yeasts, whereas 122(18%) were filamentous fungi. Out of 562 yeasts, Candida species were 494 (87%) among those candida species *C.albicans* were predominant 260 (52.6%) followed by *C. krusei* 121(24.4%) and *C.tropicalis* 113(22.8%).Other yeast Spp.was 62(9.7%)andCryptococcusspecies consists 6(1%) all isolated *Cryptococcus* species are *C.neoformance* table2.

Out of those 122(18%)filamentous fungi, the highest number were Aspergillusspecies 79 (62.9%), among them, 41 (31.4%) were *A.niger*followed by *A. fumigatus* 26 (20.4%), other aspergillus species 8(6.2%), *A.terreus* 3(2.3%),*A. flavus* 2(1.5%) and *A.glacus*(1.26%). Penicillium species 16(12.6%)the predominant Penicilliumspecies isolates were non *P.marneffei* species consists 11(68.7%) and the remaining isolated consisted by *P.marneffei*5(31.2%). Fuzarium specie consists 10(7.8%). Scopulariopsisspecies7(5.5%),Scedosporiumspecies 5(3.9%) the predominant Scedosporiumspecies isolates were*Scedosporium apiospermum* 3(60%) followed by *Scedosporium Prolificans* 1(20%)and OtherScedosporiumspecie1(20%).Rhizopus specie consists 4 (3.1%),Acremoniumspecies2 (1. 5%),Alternariaspecies were2 (1. 5%) and Paecilomycesspeciesconsists 1(0.8%) as indicated in table 2.

Table 2: Fungal isolates in sputum from pulmonary tuberculosis suspected patients, at Saint Peter's Specialized Hospital micro biology laboratory, Addis Ababa, Ethiopia, February to May,2019.

Fungal isolates	**Frequency**	**Percent (%)**
Filamentous fungi	122	18
Yeast	562	82
Filamentous fungi species		
Aspergillus Spp.	79	62.9
Penicillium Spp.	16	12.6
Fuzarium Spp.	10	7.8
ScopulariopsisSpp.	1	0.8
ScedosporiumSpp.	6	4.9
RhizopusSpp.	5	3.1
Acremonium Spp.	2	1. 6
Alternaria Spp.	2	1. 6
Paecilomyces Spp.	1	0.8
Yeast species		
Candida Spp.	494	67
Other yeast Spp.	62	9.7
Cryptococcus Spp.	6	1
Aspergillus Spp.		
A.niger	41	31.4
A. fumigatus	26	20.4
Other aspergillus Spp.	8	6.2
A.terreus	1	0.8
A. flavus	2	1. 5
A.glacus	1	0.8
PenicilliumSpp.		
non *P.marneffei*	11	68.7
P.marneffei	5	31.2
Scedosporium species		
S.apiospermum	4	60
S.prolificans	1	20
Other scedosporiumSpp.	1	20
Candida species		
C.albicans	260	52.6
C.krusei	121	24.4
C.tropicalis	113	22.8
***Cryptococcus* species**		
C.neoformance	6	100

NB:49 subjects presented more than one fungal isolate (mixed species), all species and mixed species that isolated from the sputum culture were stated as it was attached an annex 10.

5.2.3Distribution of fungal pathogens according to socio-demography

Pulmonary fungal isolates were distributed in all age groups. However, the age group of (35-44) years of age was the one containing the highest number 109(22.6%)of fungal isolates as

indicated and the least 23(4.8%) was recorded in age group < 15 as shown in table1.However, there was no association found between age group and pulmonary fungal infection. (P value was greater than 0.05 in all ager groups as shown on table 5).

Among 483 pulmonary fungal positive cases 249 (51.5%) were male and 234 (48.5%) were female. Male subjects presented more positive samples as compared to their female counterparts 249(51.5%) and 234(48.5%) respectively 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 AlternariaSpp.,Paecilomyces Spp.and *S.profilicans* isolated from only in males and *A.glacus* isolated in female.

5.2.5Fungal and Tuberculosis co-infection

In 163 MTB positive cases fungal and TB co – infection was observed in 127 (77.9%) patients of whom 71 (55 %) patients were males and 56 (44%). *C.albicans*was the predominant fungi in MTB positive group, 82(45.8%) then followed *by C.tropicalis*, *C.krusei*, *A.niger*, Other yeast spp., Scopulariopsis spp., *A.fumigatus*, Penicillium sppAspergillusspp.,Fusarium spp.,*P.marneffei*, *S.profilicans* and *S.apiospermum* respectively. There was no association between fungal isolates and MTB positive case in this study (P=0.147) as shown table 5.

5.2.5.1Fungal and Tuberculosis co-infection in relation to socio-demography

Among from 163 tuberculosis positive cases co –infection was observed in 127(77.9 %) patients of in which71(55.9%) were covered by males and 56 (44.1%) were contained by females patients. Male subjects presented more co-infection by pulmonary fungal infection and pulmonary tuberculosisas compared to their female counterparts 71(55.9 %) and 56 (44.1%) respectively as shown in table 3.

Table 3: Fungal and tuberculosis co-infection in relation to gender and age group, at Saint Peter's Specialized Hospital micro biology laboratory, Addis Ababa, Ethiopia, February to May, 2019.

Variables		Co-infection	
		No	(%)
Age group	<15	10	7.9
	15-24	24	18.9
	25-34	37	29
	35-44	33	26
	45-54	9	7
	55-64	10	8
	>64	4	3.2
Gender	Male	71	55.9
	Female	56	44.1

5.2.5.3 Fungal species from co-infected cases in relation to socio-demography

Yeast isolated from co-infected cases 148 (80%)and C.*albicans* was the highestyeast species in co-infected cases by 82 (55.8%). Mold consists 31(20%) and *A.niger* was highest mold isolated from co-infected cases 14 (41%). *C.albicans*isolated in male co-infected cases 43(52%) and in female 39(48%).*A.niger* isolated in male co-infected cases 6(43%) and in female 9(57%) as shown in table 4.*C.albicans* was the highst isolate in the age group (35 -44) and (25-34) by24 (29%)and 19(23%) in co-infected cases. *A.niger* was the predominant mold in the age groups (25-34) by 6(42%) age group as indicated on table 4.

Table 4: Fungal species fromco-infected cases in relation to socio-demographic data, at Saint Peter's Specialized Hospital micro biology laboratory, Addis Ababa, Ethiopia, February to May, 2019.

Variables		Fungal pathogens													
		Ca	Ct	Ck	Oy	Cn	An	Af	As	Fs	Ps	Pm	Sa	Sp	Sc
Age group	<15	8	3	2	1	0	2	0	0	0	1	0	0	0	0
	15-24	16	5	5	2	0	1	0	1	1	0	0	0	0	1
	25-34	19	5	7	3	1	6	2	1	1	1	1	0	0	2
	35-44	24	10	6	1	1	2	1	0	0	0	0	1	1	1
	45-54	5	2	2	2	0	2	0	0	0	0	0	0	0	0
	55-64	3	3	2	1	0	0	0	0	0	1	0	0	0	0
	>64	7	1	1	0	0	1	0	0	0	0	0	0	0	0
Gender	Male	43	13	16	4	1	6	1	0	0	1	1	1	1	4
	Female	39	16	9	6	1	8	2	2	2	2	0	0	0	0
Total		82	29	25	10	2	14	3	2	2	3	1	1	1	4

Key: Yeasts[Ca= *C.albicans*, Ct= *C.tropicalis*, Ck= *C.krusei*, Oy= other yeast Spp., Cn= *C.neoformance*,].**Mold** [An= *A.niger*, Af=*A.fumigatus*, As= other aspergillus Spp., Fs=Fusarium Spp., Ps=Penicillium Spp., Pm=*P.marneffei*, Sa=*S.apiospermum*, Sp=*S.profilicans* and Sc= other scopulariospsis Spp.]

6. Discussion

The main objective of the present study was to determine the distribution of fungal isolates among pulmonary tuberculosis presumptive patients in the sputum sample.

In the present study, fungi were isolated in 843 (75.9%) of sputum samples which agree witha study conducted in Benin by Aluyi H*et al.,* (71%) (24), Muhammad T *et al.,* In Nigeria, who reported68% [36] and Kalyani A*et al.,*62% [14]. The present study 75.9 % distribution of pulmonary fungal element, 67% yeast and 17.8% filamentous fungi higher than study done in Kenya by Mwaura E,*et al.,* reported that 37.8 %, 29.1% and 8.1% respectively [26]. The high prevalence rate recorded in the present study could reflect the sputum samples in the early morning were collected from each patient and processed immediately after collection, so that isolates might not be lost in the process of preservation and alsothis difference may be due to the organism load or population differences but needs further investigations [36]. The other reason may be the variations in percentages are mainly attributed to differences in local prevalence of different species due to different environmental conditions, as well as to the various detection methods employed [3].

Yeast isolate were 82% and mold were 18% in our study. This result closely related with Shesh R et *al.,*65.31% [22], Yadu*et al*., who reported 62.3% [37].Our study finding indicated that Candida Spp. constitute most common fungus (72% of positive fungal culture) a scausative agents of pulmonary mycosis. Our findings relating to occurrence of Candida Spp.was related with previous reports of Bhutia T *et al.* 70%[3]. *C. albicans* was isolated in 46% among yeasts which correlates with Babita et al., who reported 44.3%in India [13],Yadu *et al.*(41.2%) [37] and *C. albicans,C.krusei* and *C.tropicalis* species were isolated in the present study which correlates with Madhusudan A *et al*[41]. Our finding was higher in number of isolates C.albicans, C.krusei and C.tropicalis than findings of Mwaura E, *et al.* in Kenya [26] and Cermeno V *et al.* in Venezuela [42]. This variation in prevalence was may be differences in local prevalence of different species due to different environmental conditions, as well as to the various detection methods employed [3] and this variation in the prevalence can be attributed to the change in the geographical distribution, genetic makeup of the patients, sample size and type of culture media used [41].

The predominant filamentous fungi isolated in the present study was Aspergillus species 79(11.5%)the highest isolates were (*A.niger* and *A.fumigatus* was isolated in 33.6% and 21.1% cases which was in agreement with Babita *et al.,*in India 33.3%.*A.niger* and *A.fumigatus* was isolated in 16.5% cases [13]. However, findings by Bai-ling L et al. in china showed highest prevalence of aspergillus species (55.9 %). This may be their methodology used to diagnosis the fungal element by aid of radiographic characterization and pathological examination [9].

In our study pulmonary mycoses occur in all age groups as shown in the study; however, age group 34–45 years was found to have the highest prevalence (23%), whereas age group <15 years had the lowest (4.9%) our result harmony with Muhammad T et al., showed age group (31–35) years was found to have the highest prevalence.Possible reason for high prevalence could be those age groups mostly engage in outdoor activities where they could have been exposed to the airborne form of these fungi [36].

In our study *C.albicans*, *C.krusei*, *C.tropicalis*, *A.niger*, *A.fumigatus*those isolate were dominate the age group which was correlate with the findings of Muhammad T et al. the lowest isolated was shown in age group was below15 years could be because very few individuals participated in this study.

Findings from this study revealed that 77.9% prevalence of co-infection, which in line with those reported by Taura D*et al.* (35) and Yadu *et al.* [37]. Muhammad T *et al*. 90.2 % prevalence of opportunistic fungal infections in co infected patients. That finding was highest than our due to apart from the ideal opportunistic relationship between fungus and TB infections of the lung, HIV infection also provides ideal immune suppression that allows these fungi to thrive favorably [37].

Candida co-infection in the present study we detected 55%, 17.4% and 15.6% prevalence of *C. albicans, C. tropicalis,* and *C. krusei* respectively. This result is in keeping with other similar studied Kali A *et al* (14). But Jain et al. reported *C. tropicalis* (9.1%) and *C. krusei* 6.06% [43]. Baradkar A et al. detected *C. tropicalis* 3.25%,Latha et al. documented *C. krusei* (5.10%) [44]showed lowest in number when compared to our result. These variations in percentages are mainly attributed to differences in local prevalence of different species due to different environmental conditions, as well as to the various detection methods employed [45].

In our study filamentous fungi isolated in co-infected cases were 31 (20%) and *A.niger* was highest mold isolated from co-infected cases 14 (41%).Our result was similar with study conducted by Kalyani C *et al*.[46].

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 suspected patients at high TB burden suspected hospital.

7.2Limitations

Due financial constraints and man power, Immune status, risk factors for fungal infection and drug susceptibility test were not performed in this study. Cultural isolation for the presence of MTB couldn't be performed due to some resource limitation at study period.

8. Conclusion and Recommendations

8.1 Conclusion

Pulmonary mycoses can be easily misdiagnosed and mistreated as pulmonary tuberculosis. Our study indicates that fungal etiology should also be sought in 75.9 % the clinically suspected pulmonary tuberculosis patients; otherwise the case may be missed or misdiagnosed. *C. albicans, C.krusei, C.tropicalis,* other yeast Spp., *A.niger* and *A.fumigatus*species dominate both MTB positive and negative patients.

Co- infection of fungal agents was also seen in (79.9%) of MTB positive subjects. *C. albican* was the predominant fungi in MTB positive subjects. Aspergillus speciesandCandida species were the predominant species among the positive subjects and therefore found to preferentially cause or help in complicating bronchopulmonary disorders hence, in the absence of specific predictions with regards to pulmonary symptoms, the possibility of fungal colonization needs to be explored.

8.2 Recommendations

- Since the isolation of fungal agents in presumptive TB patients was higher than MTBit self, so we recommend fungal screening in presumptive TB patients so that patients get theappropriate diagnosis and treatment. So, we recommend fungal screening would also be important for good treatment outcome of these patients
- As there is a high prevalence of fungal infection in both TB positive and negative patients, routine screening for fungal infection is recommended for proper diagnosis and early treatment.
- As our study provides baseline information in terms of indicating the great possibility of fungal infection among presumptive TB patients, hence, we recommend further study to be done to strength more findings regarding pulmonary fungal infection.

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10. Annexes

Annex I: Participant Information sheet, Consent/Assent

My name is **Solomon Bati**. I am a laboratory technologist postgraduate student at Addis Ababa University. Now I am conducting a study entitled **Prevalence anddistribution of fungal species in sputum from pulmonary TB suspected 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 **Prevalence and distribution of fungal species in sputum from pulmonary TB suspected patients at Saint Peter's specialized hospital**, Addis Ababa, Ethiopia. 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: Solomon Bati (BSc, Msc student), DMLS; AAU: +251947484151

Email- seleado@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 I: 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

researcher to give my specimen for the mentioned study.

Participant name _____Signature/fingerprint: _____Date _____

Witness's name_____ Signature: _____Date _____

Investigator's name_____ Signature: _____Date_____

Annex II: 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: _____ date_____

Witness's name_____Signature:_____date _____

Investigator's name_____ Signature: _____date _____

Annex III: Parental/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: _____ Date _____

Investigator's name_____Signature: _____Date _____

Annex IV: Amharic Versions of Patient Information Sheet

እኔሰለሞን ባቲ በአዲስአበባዩኒቨርሲቲ፣ጤናሳይንስኮሌጅ፣

የህክምናላቦራቶሪሳይንስትምህርትክፍልየሁለተኛዲግሪተማሪስሆንየምርምርስራየንበመስራትላይእገኛለሁ።

እርስዎምበዚህጥናትላይእንዲሳተፉተጋብዘዋል።

በጥናቱለመሳተፍፈቃደኛሆነዉከተስማሙመስማማትዎንየሚያሳይዶክመንትላይእንዲፈርሙእጠይቃሁ።

መግቢያ

የጥናቱርዕስ**Prevalence anddistribution of fungal species in sputum among pulmonary TB presumptive patients at Saint Peter's specialized hospital, Addis Ababa Ethiopia.**ማለትምበመተንፈሻአካላትላይበሽታንየሚያመጡፈንገሱህችስርጭትበሚልርዕስእያጠናሁእገኛለሁ።

ይህጥናትምበተሳታፊሙለፈቃዮኝነትላይየተመሠረተነዉ።

ከጥናቱተሳታፊየሚጠበቁ

በዚህጥናትለመሳተፍየሚስማሙከሆነየአክታናሙናእንዲወሰድመስማማትይጠበቅቦታል።

የጤናባልሙያከእርሰዎናሙናዉንይሰበስባል።

ከተወሰደዉምናሙናላይየሚገኙመረጃዎችየእርሶንማንነትየማይገልጹማስረጃዎችንማለትምስም፣

አድራሻናየመሳሰለትመረጃዎችሳይጨምርናለዚህጥናትአገልግሎትብቻየሚዉልመለያቁጥርበመጠቀምከዚህሆስፒታልዉጭለሚ

ገኙለሥራዉአግባብነትላላቸዉሰዎችቢነገርየማይቃወሙመሆኑንመስማማትይጠበቅቦታል።

ናሙናሰጡማለትበሽታውይገኝብዎታልማለትአይደለም።

በእርሰዎናሙናውስጥየበሽታአምጭተህዋስቢገኝከጤናባለሙያዉአስፈሊጊውንህክምናያገኛሉ።

የመረጃዉሚስጥራዊነት

ማንኛዉምየሰጡትመረጃእናከተወሰደዉናሙናላይየተገኘዉየላቦራቶሪዉጤትየሚዉለዉለጥናቱአላማብቻነዉ።

ይህንማህደርሊያገኙየሚችሉየተወሰኑየጥናቱተባባሪሠራተኞችብቻናቸዉ::

ከዚህምበላይስለእርስዎያለዉንማንኛዉንምመረጃየይለፍ-ቃልባለዉየኮምፒዉተርየመረጃማህደርዉስጥእንዲቀመጥይደረጋል::

ተሳታፊዉየሚያጠፋዉጊዜ

የተዘጋጀዉንየስምምነትቅጽለመፈረምናናሙናለመስጠት3-7ደቂቃያስፈልጋል::

በጥናቱበመሳተፍየሚስከትላቸዉችግሮች

ናሙናበሚሰበሰብበትወቅትምነምአይነትችግርአያስከትልቦትም::

በጥናቱበመሳተፍየሚያስከትላቸዉጥቅሞች

ይህጥናትየማስተርስዲግርመመረቂያእንደመሆኑመጠንበመሳተፍዎየሚያገኙትየገንዘብጥቅማጥቅምየለም::

ሆኖምበቀኑበሚደረገዉየላቦራቶሪምረመራለህኪምዎበመስጠትአስፈላጊዉንህክምናእንዲያገኙይደረጋልእንደአስፈላጊነቱምየካልቸርዉጤቶችንምጠብቆአስፈላጊዉንምርመራይደረግሎታል::

ለወደፊትበተመሳሳይሁኔታዉስጥላሉበሽተኞችበመረጃላይየተመሰረተህክምናለመስጠትያግዛልከፈለጉየላቦራቶሪዉጤቶችንበነፃ ያገኛሉእንዲሁምስለአስፈላጊዉህክምናከህኪምዎጋርይነጋገራሉ::

የጥናቱተሳታፊዎችመብት

ትብብርዎሙሉበሙሉበፍቃደኝነትላይየተመሠረተናተሳትፎዎንመተውናበማንኛውምሰዓትጥናቱንማቆምይችላሉ::

በጥናቱዉስጥያሎትንተሳትፎበማንኛዉምጊዜየማቋረጥሙሉመብትዎየተጠበቀከመሆኑምበላይራሶንከጥናቱበማግለልዎምክንያትየሚቀርብዎትምንምዓይነትየሆስፒታልአገልግሎትአይኖርም::

ከዚህምበተጨማሪጥናቱንበተመለከተማንኛዉንምዓይነትጥያቄየመጠቅናገለፃየማግኘትመብትአለዎት::

የላቦራቶሪምርመራዉጤቱንምበነፃማግኘትይቻላል::

ግንኙነትናጥያቄ

ይህንጥናትበተመለከተወይምከዚህጋራበተዛመደመልኩስለሚያጋጥዉድንገተኛችግርወይምጥያቄካሎትበሚከተለዉአድራሻይ ጠቀሙ::

ሰለሞን ባቲ

(ቢ.ኤስ.ሲ) ሞባይል+251947484151, ኢ-ሜይል፤seleado@@gmail.com

አዲስአበባዩኒቨርሲቲየጤናሳይንስኮሌጅየሕክምናላብራቶሪሳይንስትምህርትክፍል!

አማካሪ፣አዳነቢተዉ(ፒ.ኤች. ዲ) ሞባይል+251911039162, ኢ-ሜይል፤bitewadane@gmail.com

አዲስአበባዩኒቨርሲቲየጤናሳይንስኮሌጅየሕክምናላብራቶሪሳይንስትምህርትክፍል!

ለተጨማሪመረጃአዲስአበባዩኒቨርሲቲ፤የሕክምናላብራቶሪሳይንስትምህርትክፍልይጠይቁ;

ስልክ-+251112755170

ከዚህበታችየሚገኘውፊርማዎለእርስዎየተሰጠውንመረጃማንበብዎን፣መስማትዎንእናመገንዘብዎንየሚያሳይነው::

ከመፈረምዎበፊትእባክዎትንየጥናቱንዓላማ፣የተሳትፎጉዳትናጥቅሙ፣የመተው፣የማቋረጥ፣መብትናነፃነትእንዳለዎትይረዱ::

ተስማምተዋል? የጥናቱንመግለጫአንብበያለሁ/ ስምቻለሁእናምተረድቻለሁ::

መመሪያውምንእንደሆነናበእኔምንሊከሰትእንደሚችልተረድቻለሁ::በጥናቱላይለመሳተፍ፤

እስማማለሁ_____አልስማማም_____

Annex V: Amharic Versions Of Consent Form For Participants Older Than 18 Years of Age

የተሳታፊስምምነትቅጽ

ይህገጽ"**distribution of fungal isolates among pulmonary TB presumptive patients at Saint peter's specialized hospital, Addis Ababa Ethiopia"**ማለትምበመተንፈሻአካላትላይበሽታንየሚያመጡፈንገሱችስርጭትበቅዱስ ኢትዮጵያ"በሚልርዕስየተሳታፊስምምነትቅጽነዉ::በመሆኑምእባክዎንከዚህበታችየተዘረዘሩትንነጥቦችይረዱና፤ ለመሳተፍፈቃደኛሆነዉከተስማሙመስማማትዎንየሚያሳይፊርማዎንከታችበተሰጠውቦታሊይእንዱፈርሙእጠይቃለሁ::

1. እኔበመተንፈሻአካላትላይበሽታንየሚያመጡፈንገሱችስጭትat Saint Peter's specialized hospital, Addis Ababa Ethiopia".

የሚለዉጥናትአላማበደንብተገንዝቤአለሁ::

2.ከእኔየሚወሰደዉናሙናለጥናቱአላማብቻእንደሚዉልተረድቻለሁ::

3.ሁለምመረጃዎችእናየናሙናዉጤቱምስጢራዊመሆኑንተገንዝቤአለሁ::

4.በጥናቱላይበመሳተፌምንምየገንዘብክፍያእንደማላገኝተረድቻለሁ::

5.በጥናቱያለመሳተፍእንዱሁምበማንኛዉምጊዜየማቃረጥመብትእንዳለኝአዉቄአለሁ::

6.ሁለምመረጃዎችበአስተባባሪዉ/ዎችተገልጾልኝበደንብተረድቻለሁ::

የተሳታፊፊርማ:----------

የተሳታፊአድራሻ:----------

ቀን:----------

በስምምነቱወቅትየነበሩምስክሮች

1._____

2._____

ይህንንጥናትበተመለከተጥያቄቢኖርዎትወይምከዚህጋራበተዛመደመልኩስለሚያጋጥመዎት ድንገተኛችግርበሚከተለዉ አድራሻይጠቀሙ::

ሰለሞን ባቲ

ሞባይል:+251947484151

የሕክምናላብራቶሪሳይንስትምህርትክፍል

የጤናሳይንስኮሌጅ፤

አዲስአበባዩኒቨርሲቲ

ኢ-ሜይሌ፤seleado@gmail.com

ለተጨማሪመረጃ፡አዲስአበባዩኒቨርሲቲ፤የሕክምናላብራቶሪሳይንስት/ክፍልይጠይቁ፡፡

ስልክ፡+251112755170

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

ይህገጽማለትም distribution of fungal isolates among pulmonary TB presumptive patients at Saint peter's specialized hospital, Addis Ababa Ethiopia"ማለትምበመተንፈሻአካላትላይበሽታንየሚያመጡፈንገሱችስጭትat Saint peter's specialized hospital, Addis Ababa Ethiopia "በሚልርዕስእድሜያቸውከ18 ኣመትበታችለሆናቸውተሳታፊዎችስምምነትየመጠየቂያቅጽነው፡፡

ናሙናየምንወስድበትመሳሪያንጽህናውሙሉበሙሉአስተማማኝናከዚህበፊትጥቅምሊይያልዋለነው፡፡

ናሙናበምንወስድበትጊዜየሚኖርህመምየሚፈጥርስሜትምሆነአደጋየሚያስከትልሂደትየለውም፡፡

ለጥናቱየሚወሰደውናሙናለጥናቱአላማብቻይውላል፡፡

የናሙናውውጤትምስጥራዊነትየተጠበቀሲሆንበናሙናውውስጥየበሽታአምጭተህዋስቢገኝከጤናባለሙያዉአስፈላጊውንህክምናያገኛሉ፡፡በጥናቱላይበመሳተፍዎምንምየገንዘብክፍያአያገኙም፡፡

በጥናቱለመሳተፍየመፍቀድምሆነያለመፍቀድእንዱሁምበማንኛዉምጊዜየማቋረጥመብትአለዎት፡፡

(ስም) በጥናቱእንዲሳተፍ/ እንድትሳተፍይፈቅዳሉ?

ፈቃዮኛከሆኑ፤

የተሳታፊፊርማ፣---------- የፈቀደውግለሰብፊርማ፣----------

አድራሻ፣---------- ቀን፣----------

በስምምነቱወቅትየነበሩምስክሮች

1._____

2._____

ይህንጥናትበተመለከተጥያቄቢኖርዎትወይምከዚህጋራበተዛመደመልኩስለሚያጋጥመዎት

ድንገተኛችግርበሚከተለዉ

አድራሻይጠቀሙ፡፡

ሰለሞን ባቲ
ሞባይል፡+251947484151

የሕክምናላብራቶሪሳይንስትምህርትክፍል

የጤናሳይንስኮሌጅ፤

አዲስአበባዩኒቨርሲቲ
ኢ-ሜይሌ፤seleado@gmail.com
ለተጨማሪመረጃ፡አዲስአበባዩኒቨርሲቲ፤የሕክምናላብራቶሪሳይንስት/ክፍልይጠይቁ፡፡
ስልክ፡+25111275517

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

a. Sabouraud Dextrose Agar with Chloramphenicol

Approximate formula per liter of purified water

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

Ingredients per liter of distilled water:

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.

Principle

The medium contains proteose 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(121^0 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:

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

Final pH 6.7 +/- 0.2 at 25^0 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 121^0 C and 15 psi for 15 minutes.
4. Cool the agar to 50 to 55^0 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 wasready

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 25-37^{0}C for weeks sterility of each batch of prepared media was checked by incubating un inoculated media at 25-37^{0}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.

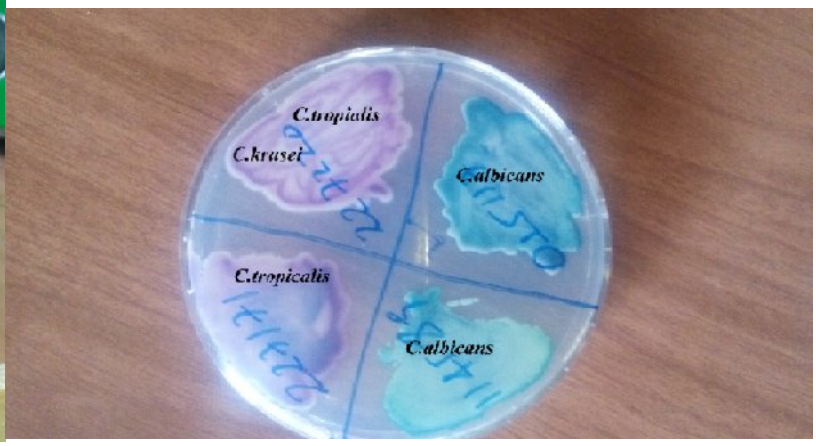
Post analytical

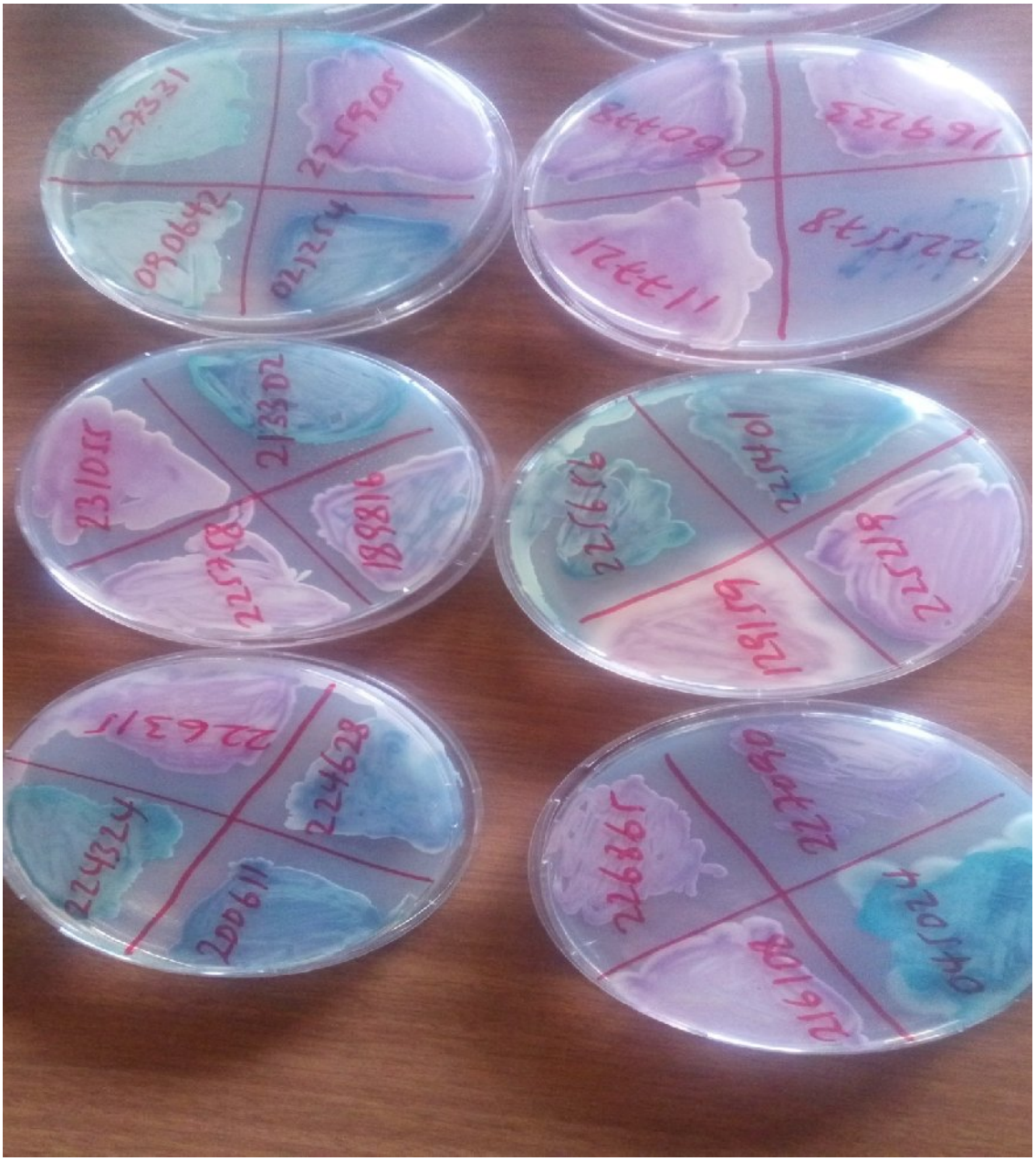
• Results generated was appropriately documented electronically

• 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 VIII: Some Candida species isolated on chromoagar media and *C.neoformance* on urease media





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 suspected 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. Genxpert MTB Rif assay

Positive	**Negative**

V. Culture result of mycological agent_____

Sample ID no	MRN	Age	Gender	XPert MTB		Inoculation date	**MYCOLOGY CULTURE (SDA and BHI)**				
							Check 1	Check 2	Check 3	Check 4	Check 5
				P	N						

VI. Macroscopic and microscopic result registered format

Sample ID	MRN	Macroscopic result	Microscopic result

Annex X: Fungal isolates in pulmonary tuberculosis suspected patients.

Fungal species	Frequency	Percent
A.flavus	1	0.2
A.fumigatus	22	3.5
A.fumigatus + *Fusarium Spp.*	1	.2
A.niger	24	3.8
A.niger + *A.terreus*	1	0.2
A.niger + *Fusarium Spp.*	1	0.2
A.niger + *Penicillium marneffei*	1	0.2
A.niger + *Penicillium Spp.*	3	0.5
A.niger + *Rhizopus Spp.*	1	0.2
A.terreus	1	0.2
Acremonium Spp.	1	0.2
Acremonium Spp. + *A.niger*	1	0.2
Alternaria Spp.	1	0.2
Aspergillus glacus group	1	0.2
Aspergillus Spp.	3	0.5
Aspergillus Spp. + *Fusarium Spp.*	1	0.2
Aspergillus Spp. + *Paecilomyces Spp.*	1	0.2
Aspergillus Spp. + *Scedosporium apiospermum*	1	0.2
C.albicans	164	25.8
C.albicans + *A.flavus*	1	0.2
C.albicans + *A.fumigatus*	1	0.2
C.albicans + *A.niger*	4	0.6
C.albicans + *Aspergillus Spp.* + *Fusarium Spp.*	1	0.2
C.albicans + *C.krusei*	3	0.5
C.albicans + *C.krusei* + *A.fumigatus*	1	0.2
C.albicans + *C.krusei* + *C.tropicalis*	2	0.3
C.albicans + *C.krusei* + Other yeast Spp.	1	0.2
C.albicans + *C.krusie* + *C.tropicalis*	1	0.2
C.albicans + *C.tropical* + Other yeast Spp.	1	0.2
C.albicans + *C.tropicalis*	60	9.4
C.albicans + *C.tropicalis* + *A.niger* + *Penicillium Spp.*	1	0.2
C.albicans + *C.tropicalis* + *C.krusei*	2	0.3
C.albicans + *C.tropicalis* + Other yeast Spp.	6	0.9
C.albicans + *Cryptococcus neoformans*	1	0.2
C.albicans + *Fusarium Spp.*	1	0.2
C.albicans + Other yeast Spp.	4	0.6
C.albicans + *Penicillium Spp.*	1	0.2
C.albicans + *Rhizopus Spp.*	2	0.3
C.albicans + *C.krusei* + Other yeast Spp.	1	0.2
C.albicans + Other yeast Spp.	1	0.2

C.krusei	43	6.8
C.krusei + *A.fumigatus*	1	0.2
C.krusei + *A.niger* + *Penicillium Spp.*	1	0.2
C.krusei + *C.tropicalis*	22	3.5
C.krusei + *C.tropicalis* + *A.niger*	1	0.2
C.krusei + *C.tropicalis* + Other yeast Spp.	3	0.5
C.krusei + *C.tropicalis* + *Scopulariopsis Spp.*	1	0.2
C.krusei + *Cryptococcus neoformans* + *A.niger*	1	0.2
C.krusei + *Cryptococcus neoformans* + *Aspergillus Spp.*	1	0.2
C.krusei + Other yeast Spp.	31	4.9
C.krusei + Other yeast Spp. + *Alternaria Spp.*	1	0.2
C.krusei + Other yeast Spp. + *Penicillium marneffei*	1	0.2
C.krusei + Others yeast Spp.	1	0.2
C.krusei + *Scopulariopsis Spp.*	1	0.2
C.krusuei + *Fusarium Spp.*	1	0.2
C.tropicalis	10	1.6
C.tropicalis + Other yeast Spp.	3	0.5
Cryptococcus neoformans	2	0.3
Cryptococcus neofrmans + *A.niger*	1	0.2
Fusarium Spp.	3	0.5
Fusarrium Spp. + *Scedosporium apiospermum*	1	0.2
Negative	153	24.1
Other yeast Spp.	9	1.4
Penicillium marneffei	3	0.5
Penicillium Spp.	5	0.8
Rhizopus Spp.	2	0.3
Scedosporium apiospermum	2	0.3
Scedosporium Prolificans + *Scopupariopis Spp.*	1	0.2
Scedosporium Spp.	1	0.2
Scopulariopsis Spp.	4	0.6
Total	636	100.0

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: Solomon Bati (B.Sc.).

Signature:_____

Date of submission:_____

This thesis has been submitted with our approval as advisors.

Advisor:

ADANE BITEW (MSc, PhD).

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.