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Culture Isolation of Smear Negative Pulmonary Tuberculosis and Drug Susceptibility Pattern Among Presumptive Pulmonary Tuberculosis Patients at Felegehiwot Referral Hospital, Bahirdar, Ethiopia

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THESIS SUBMITTED TOBAHIR DAR UNIVERSITY COLLEGE OF MEDICINE AND HEALTH SCIENCES, DEPARTMENT OF MEDICAL MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE MASTERS OF SCIENCE DEGREE IN MEDICAL MICROBIOLOGY.

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Wisdom at the source of Blue Nile

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Abstract

Back ground: Tuberculosis is an infectious (TB) disease caused by *M. Tuberculosis*. TB generally affects the lung but can also affect other parts of the body. Patients with pulmonary tuberculosis (PTB) spread the disease through tiny airborne droplets into the surrounding air during coughing, sneezing, speaking and laughing from peoples who have active TB in their lungs .The aim of this study was to determine the prevalence of pulmonary tuberculosis using a standard culture method and its drug susceptibility pattern among smear negative presumptive pulmonary tuberculosis (SNPTB) patients at the Felege Hiwot Referral Hospital (FHRH), Bahir Dar, Ethiopia.

Methods: Institutional based cross sectional study design was conducted among smear negative presumptive pulmonary tuberculosis (SNPTB) suspected patients visiting FHRH from January 2017 to June 2017. Convenient sampling technique was employed to select the study subjects. Sputum sample was collected from patients who had sputum smear negative result by Auramine O Fluorescence staining method. All smear negative pooled sputum samples were further processed for culture using conventional Lowenstein-Jensen (LJ) solid medium and automated BACTEC MGIT 960 system liquid medium at Amhara public health institute, TB Laboratory. Moreover, molecular line probe assay (LPA) was done for culture positive MTB complex isolates. Descriptive statistics and multivariate logistic regression analysis were done using SPSS version 23.

Result: Out of the 242 florescence microscopy negative cases, 20/242 (8.3%) were found to be positive for acid fast bacilli (AFB) using overnight bleach concentration technique. The overall proportion of smear negative but culture positive PTB cases were 27/233 (11.6%). Out of this 2/27 (7.4%) were isolated as Non-Tuberculosis Mycobacterium (NTM). The remaining 9/242 (3.7%) specimens were contaminated and excluded from the analysis. Significant difference was not observed between solid and liquid culture systems in terms of mycobacterial recovery rate. LPA was done for twenty five *Mycobacterium tuberculosis* complex (MTBC) isolates. Of these, 4/25(16%) of isolates were identified as mono resistance to rifampicin on *rpoB* gene locus of MTBC. Variables (age 29-39years and ≥40 years, rural residence, being illiterate, retreatment case, contact history with PTB patients, abnormal chest x-ray finding, fever, hemopityasis and

purulent sputum were significantly associated with smear negative culture positive PTB (p<0.05)). Furthermore, mono resistance to rifampicin was significantly associated with retreatment cases (p<0.05).

Conclusions: In the studied site, high prevalence of culture positive miss classified PTB cases were documented from smear negative presumptive PTB patients. Fluorescence microscopy sputum examination skill can be one possible reason for this high discrepancy. Therefore, in-service training for laboratory professionals and regular quality assurance is likely to reduce false negative and increase the yield of smear positive results. Moreover, sputum culture should be used at the district level to decrease smear negative cases among presumptive SNPTB patients. Likewise, overnight bleach concentration sputum smear FM is recommended for routine diagnosis of SNPTB in resource limited areas; when culture is not available.

Keywords: Culture, Drug susceptibility pattern, Smears negative PTB, FHRH, Bahir Dar.

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Acronyms

AFB	Acid Fast Bacilli
Ag	Antigen
AIDS	Acquired Immune Deficiency Syndrome
BSL-2	Bio Safety Level two
CSA	Central Statistical Agency
DNA	Deoxy ribo Nucleic Acid
DST	Drug Sensitivity Testing
FHRH	Felege Hiwot Referral Hospital
FM	Fluorescence Microscopy
HIV	Human Immunodeficiency Virus
LPA	Line Probe Assay
LJ	Lowenstein Jensen
MDR	Multi Drug Resistance
MGIT	Mycobacterium Growth Incubator Tube
MTB	Mycobacterium Tuberculosis
NALC- NaOH	N-acetyl-L-cysteine-sodium hydroxide
NTM	Non-Tuberculosis mycobacterium
OR	Odds ratio
PTB	Pulmonary Tuberculosis
RMP	Rifampicin
SNPTB	Smear Negative Pulmonary Tuberculosis
SPPTB	Smear Positive Pulmonary Tuberculosis
SPSS	Statistical Package for Social Sciences
TB	Tuberculosis
WHO	World Health Organization
ZN	Ziehl-Neelsen

1. Introduction

1.2. Background

Tuberculosis is an infectious (TB) disease caused by *M. Tuberculosis*. TB generally affects the lung but can also affect other parts of the body (WHO, 2014a). Patients with pulmonary tuberculosis (PTB) spread the disease through tiny airborne droplets into the surrounding air during coughing, sneezing, speaking and laughing from peoples who have active TB in their lungs (WHO,2015). Transmission is directly associated to the number of bacilli being expelled in the air, time and intensity of exposure to the contaminated air and immune status of the exposed individual (Dheda *et al.,* 2010). TB always occurred excessively among disadvantaged populations such as the homeless, malnourished, immune compromised and overcrowded (Dye, 2010). TB remains a major public health problem, accounting for 10.4 million incident cases and 1.4 million deaths worldwide. Likewise drug resistance TB has exacerbated the burden with the estimated 580,000case and 250,000 deaths in the same year (WHO, 2016). Ethiopia has been listed as one of the 22 high-TB burden countries with mortality and incidence rate of 32 and 224 cases per 100,000 populations, respectively (WHO, 2014b).

Diagnosis of active TB is commonly based on clinical symptoms, chest X-ray, as well as microscopic examination and culture (Konstantinos, 2010). According to WHO 2014 TB report, out of 5.2 million PTB patients only 58% of the cases were confirmed by laboratory methods such as smear or culture (WHO, 2015). On the other hand, the remaining 42% of the patients were diagnosed using clinical criteria alone (symptoms, history and x-ray). Majority of populations affected by smear negative TB are found in economically poor countries, where sputum smear examination with a conventional light microscope is the primary method for diagnosing PTB (Karen, et *al*., 2006, Young *et al*., 2008), because of the lack of sensitive, rapid and accurate diagnostic tests applied in resource-limited settings.

Sputum microscopy for AFB is the cornerstone of TB diagnosis globally, while smear microscopy is specific, fast, low cost, and minimal requirement of equipment and technical skills and easily identifying the most contagious patients (WHO, 2012), though, methods like ZN lacks sensitivity, it fails to detect up to 50% TB cases (Steingart, *et al*., 2006). In contrast,

Fluorescence Auramine O staining and bleach concentration smear have better sensitivity and increases the positivity rate sputum sample over up to 10 % (Kumar *et al*., 2017). As approximately 5000-10,000 bacilli per ml of specimen are required for a positive smear, false negative results are obtained when the mycobacterial load in the clinical samples tested is insufficient for detection (Laserson *et al.,* 2005).

AFB culture, a more sensitive diagnostic tool and the gold standard for TB diagnosis, requires only 10-100 bacilli for a positive culture; however, results are obtained only after several weeks (Dinnes *et al.,* 2007). The role of more sensitive tests techniques like direct FM sputum smear and concentration technique needs to be reassessed, using culture as the gold standard for TB diagnosis to decrease the proportion of smear negative PTB and to increase early detection of smear negative TB (Swai1 *et al*., 2011, Steingart *et al*., 2006).

1.2. Statement of the problem

1.2.1. Global tuberculosis
Almost one-third of the world population is infected with MTB (WHO, 2012). The disease disproportionately affects people in resource-poor settings (Dye, 2010), more than 80 % of the TB cases and 78 % of deaths from the disease occurred in developing countries (WHO, 2014b).

The diagnosis and management of PTB in sputum smear-negative patients is also a challenge for today's medical practice, 30-60% of all pulmonary TB cases are reported as smear negative globally (WHO, 2007). The rates of SNPTB have been rising in countries with HIV epidemics especially in developing countries due to the use of smear microscopy technique which is low sensitive (Siddiqi *et al.* 2003).

SNPTB is associated with poor treatment outcomes and excessive mortality in settings used diagnostic of TB supported on; clinical and radiographic elements (Swail *et al*., 2011) and smear microscopy (Karen, et *al*., 2006). Untreated SNPTB cases are also responsible for approx. 13-20% of TB transmission (Tostmann *et al.*, 2008, WHO, 2012). Moreover, increase the risk of mortality 20% -25% (Shah *et al.,* 2012, Getahun *et al.,* 2007) among cases.

1.2.2. Tuberculosis in Ethiopia

TB is one of the most important infectious diseases responsible as 3rd cause of hospital admission and the second top causes of death in Ethiopia (WHO, 2012).

According to 2015 WHO report, Ethiopia is among 30 high-burden countries of TB with annual estimated incidence of 192 per 100,000 and death rate of 30 per 100, 000 (including, HIV positive people). Similarly, drug resistance TB continues to be a serious threat for the current national TB control effort in Ethiopia, 3,300 cases of MDR/RR-TB and 113 cases of second line drug resistance cases were reported on same year (WHO, 2016).

In Ethiopia, laboratory diagnosis of TB remains less sensitive technique, mainly in a stage of Ziehl Nelseen (ZN) smear (FMOH, 2013). Although, Aura mine O Fluorescence staining and bleach concentration smear increases the positivity rate up to 10 % (Kumar *et al*., 2017), still the proportion of microscopic undiagnosed TB cases are high in study setting. Recently, the number of registered smear-negative TB cases transcended the smear positive cases. According to the national TB prevalence survey in 2010/11 smear-negative cases accounted for 57% of culture positive cases (Kebede *et al.,* 2014).

Studies done in Ethiopia showed that, low detection of smear-positive PTB and over-diagnosis of SNPTB was also major problems in Ethiopia (Mengistie *et al*., 2005, Tadesse *et al.,* 2016). More than 80% of the cases of SNPTB were not confirmed by culture, pointing to a high rate of over-diagnosed cases (Desta *et al*., 2009). Similar study conducted in Cuba showed that, up to 60% of the cases diagnosed as SNPTB did not have the disease when re-evaluated by an expert panel (Sevy *et al.* 2008). This indicates that numerous patients are receiving unnecessary anti TB treatment that leads to unwanted drug effect, cost and improper management.

Although, SNPTB is a real challenge for TB diagnosis and management, little information is available in Ethiopia related to the prevalence of smear negative culture positive PTB and drug susceptibility pattern of MTB isolates. This study was carried out to determine the proportion of SNPTB using standard culture methods and drug susceptibility pattern of isolates among presumptive SNPTB patients in Ethiopia, Bahir Dar, Felege Hiwot Referal Hospital.

2. Literature review

2.1. Burden of smear negative Tuberculosis

Smear-negative pulmonary tuberculosis occurs less frequently than smear-positive pulmonary tuberculosis, but it is an infectious communicable form of TB (Desta *et al.*, 2009).Cohort study conducted in Holland revealed that, smear-negative culture-positive pulmonary TB is responsible for 13% of PTB (Tostmann *et al.*, 2008). A cross sectional study done on smear-negative PTB patients in the United States of America; among 159,121 cases of culture confirmed PTB 37% were sputum smear negative (Shah *et al*. 2012).

Another study done in Vietnam showed that, among 390 SNPTB patients, the prevalence of sputum culture result positive for MTBC and NMT were 22 (5.64%) and 1(0.35%), respectively (Nguyen *et al.,*2012). Similar study conducted in Cambodia confirmed that, among 776 SNPTB suspected patents, the overall prevalence of culture-confirmed smear negative PTB was at 62 (7.9%) (Tamhane *et al.,*2009).

A retrospective study was undertaken of the medical records of 122 active TB cases in Mexico from 2009 to 2012 and 97(80%) of the cases were reported negative sputum smears. All samples were confirmed as positive for *Mycobacterium tuberculosis* complex in culture; meaning that eight out of ten actual cases are being missed when sputum smear is the only diagnostic tool in asymptomatic patients with abnormal chest X-rays(Assael *et al.,*2013).

The study conducted in Tanzania indicates that, among 637 PTB suspected patients, 104 (81.9%) were FM smear negative at the reference laboratory. Of these, 13 (12.5%) were confirmed as culture positive PTB (Nyagosya *et al*., 2008). Similar study in Tanzania showed that, among 413 SNPTB suspected samples, 127(30.8 %) were found to be culture positive (Swai1*et al,.*2011).

A study conducted in Ethiopia, St. Peter Tuberculosis Specialized Hospital showed that, among 297, PTB suspected patients 247(83.2%) were smear negative. Of these, 43(17.4%) were found to be positive on culture (Desta *et al*., 2009). Similar study done in Northern Ethiopian prisons indicated that the overall prevalence of SNPTB cases was at 8%. Moreover, contact history to PTB patients, educational status, presence of cough and night sweating were found to be predictors of PTB positivity (Biadglegne *et al*., 2014).

Recent study also conducted among 1761 TB patients registered for treatment in Bahir Dar, Felege Hiwot Referral Hospital indicated that, the proportion of SNPTB was at 548 (31.1 %) (Zenebe *et al*., 2016). Another study done at Jimma University showed that, among185 smear-negative presumptive PTB cases, 19 (10.3%) were identified as culture proven TB (Tadesse *et al.,* 2016).

A systematic review of 83 studies showed that AFB concentrated sputum smear increase 17% of sensitivity over direct FM sputum examination (Steingart *et al.,* 2006). A study conducted in Zimbabwe among 300 PTB patients started TB treatment 139(46.3%) were smear-negative PTB patients. Of these 51(36.7%) were positive after concentration of specimens and an extra 30 (21.6%) samples were also identified as positive on culture (Apers *et al.,* 2004).

Comparative study done in Bangladesh on direct versus concentrated smear microscopy among 915 specimens showed that, 841(91.9%) specimens were found negative for AFB. Of these 87(9.5%) were become positive after concentration. About 17.1% culture positive cases were found to be negative on both direct and concentrated smear microscopy (Uddin *et al.,* 2013).

2.2. Factors associated with Sputum smear negative results

Although sputum smear microscopic technique is low in cost, specific, and rapid technique false-negative results for smears from patients with PTB are a problem (WHO, 2012). In many Sub-Saharan countries, the number of patients registered with SNPTB has increased, due to several factors such as co-incident of HIV infection, poor diagnostic practices, mistaking other respiratory infections or diseases for tuberculosis (Munyati *et al.,* 2005).

Multicenter study conducted in Africa and Asia showed that, factors like, dyspnea, localized interstitial radiological abnormalities and recurrent bacterial or parasitic infections were independently associated with smear negative TB (Chartier *et al*.,2011). A study conducted in Taiwan identified that, propensity for smear negative sputum smear may arise from inadequate smears, sputum type and improper reading of the slides and additional training can improve the accuracy (Chiang *et al.,* 2005*).* Similar study conducted in four African countries showed that, low grade positivity of sputum smear is more frequent among extremes of age (Rieder *et al*., 2009).

2.3. Anti- TB drug susceptibility Pattern

Resistance to drugs is due to particular genomic mutations in the specific genes of *M.tb*. Mutations that confer resistance to anti-TB drugs occur naturally and spontaneously (Zhao *et al*.2014). Rifampicin and isoniazid are the two most important drugs in TB treatment. Resistance to these two drugs necessitates the use of second line drugs that are toxic, poorly tolerated, expensive, require prolonged period of treatment, and have poor treatment outcomes (WHO, 2014a).

A study conducted in Nepal on drug susceptibility pattern of MTB isolates showed that, the highest rate of anti TB drug resistance was towards streptomycin (24.4 %) followed by isoniazid (23 %), rifampicin (17.8 %) and ethambutol (15.6 %). Highest percentage of resistance were observed on retreatment cases at (61.1 %) (Thapa *et al.*, 2016). Similar study conducted in china indicated that, resistance on Isoniazid and Rifampicin was 35.7% & 26.9% respectively (Zhao *et al*.2014).

Another study conducted in Ethiopia St.Peters Hospital showed that, among 37 smears negative PTB suspected patients 11/37(29.8%) of them were resistance to any of the first line anti-TB drugs tested. Smear negative PTB patients can harbor drug resistant strains like their smear positive counterparts (Desta *et al*., 2008).

A study conducted in Gondar, Ethiopia, showed that, among 250 PTB suspected patients 15(6%) were diagnosed as TB cases. Of these 6(2.4%) were smear negative but culture positive cases. Furthermore, all the isolates were sensitive to Rifampicin and Isoniazid (Alemayehu *et al.,* 2014). Similar study conducted in Northwest Ethiopia, Metema and west Armachiho, indicated that, the overall prevalence of MDR-TB was 5.7 % (2.3 % among new cases and 13.9 % among retreatment cases (Mekonnen *et al.,* 2015).

Another study conducted in East Gojjam, Ethiopia showed that, among 89 culture positive MTB strains, the prevalence of MDR-TB was at 3.37 % (Adane *et al*., 2015). Similar study conducted in Ethiopia, Amhara region among 606 presumptive MDR-TB cases showed that, the prevalence of rifampcin mono resistance (MDR TB/RMP) was 110(18.2%) (Nigus *et al.,* 2014*).

2.4. Risk factors for the development of drug resistance

MDR-TB has been described as a man-made problem resulting from the use of inadequate drug regimen that select drug resistant tubercle bacilli, poor supervision of treatment by TB control programs and non-adherence by the patient (WHO, 2014a). Prescribing of single drugs or inadequate drug regimen by the care providers and adding a single drug to an already failing regimen can contribute to an increase in drug resistance (Quy *et al*., 2003).

Most of studies conducted in several parts of the world consistently report history of previous treatment as a risk factor for development of drug resistance TB (Zhao *et al.,*2014, Thapa *et a*l., 2016, Mekonnen *et al.,* 2015). Study from British indicated that, young age and reactivated PTB were reported to be a risk factor for MDR-TB (Moniruzzaman *et al*., 2006).

Another study conducted in Ethiopia, Oromia region showed that, occupation of farming, known TB contact history, Alcohol use, HIV infection, previous known TB history, and previous history of treatment were significantly associated with drug resistance (Tadesse *et al.,* 2016) similar study conducted in East Gojjam, Ethiopia showed that, among 89 culture positive MTB strains, age group of 25-34 years and previous history of treatment was significantly associated with drug resistance TB (Adane *et al*., 2015). Moreover, study conducted in Amhara regional state among 606 presumptive MDR-TB cases showed that, age at a range of 21-30 years old; being female and TB history of defaulters identified as risk factors of drug resistance TB (Nigus *et al.,* 2014*).*

2.5. Significance of the study

Management algorithms based on several features (direct sputum smear microscopy, clinical symptoms, response to antibiotic trials, and chest radiography) have been developed to improve case detection of PTB. These algorithms must be validated in each local condition because their performance may vary depending on numerous local factors such as the regional prevalence of respiratory disease and other TB like disease. Globally, the rates of smear negative TB has increased, particularly in areas where HIV prevalence is highly rampant, this situation is same in Ethiopia. In the study site, a lot of work has been done on smear-positive PTB, to assess the full prevalence of TB. In contrast, currently there is no data related to this field. Therefore; the current study will provide information on the status of SNPTB and anti-TB drug susceptibility

pattern in the study setting. Moreover, this information will help to improve the diagnosis, treatment and prevention of pulmonary tuberculosis.

3. Objective of the study

3.1. General objective

To determine the prevalence of culture positive pulmonary tuberculosis and drug resistance profile among smear negative presumptive PTB patients at Felege Hiwot Referral Hospital, Bahir Dar, Ethiopia

3.2. Specific objective

- To determine the prevalence of culture positive pulmonary tuberculosis among smear negative presumptive PTB patients

- To determine the prevalence of bleach concentration positive pulmonary tuberculosis among smear negative presumptive PTB patients.

- To identify risk factors associated with culture positive TB among smear negative presumptive PTB patients.

- To determine drug susceptibility pattern of Mtb isolates among smear negative presumptive PTB suspected patients.

- To identify factors associated to anti-TB drug resistance among culture positive SNPTB patients.

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4. Methods and materials

4.1. Study design and period

Institutional based cross sectional study was conducted from January 2017 to June 2017 at Felege Hiwot referral Hospital, Bahir Dar.

4.2. Study area

Felege Hiwot Referal Hospital is found at Bahir Dar town, the capital city of Amhara National Regional State, which is located 565 km away from Addis Ababa North west Ethiopia. Based on the 2007 Census conducted by Central Statistical Agency (CSA) Bahir Dar special Zone has total population of 221,991.Of them 108,456are men and 113,535 women. Of the total population 180,174(81.16% are urban inhabitants, the rest of population are living at rural kebeles around Bahir Dar. The economic status of the population is established on agriculture and trade.   In addition to Felege Hiwot Referral Hospital (FHRH), there are two private Hospitals and more than 10 health institutions (Higher clinics, Medium clinics and Health center in the town (CSA, 2007).

FHRH is a tertiary referral hospital with around 450 beds and serving over 7 million people from the surrounding area. There are over 600 members of staff employed by the Hospital and a further 350 employed by Bahir Dar University. The hospital provides obstetrics, pediatrics, internal medicine, Ophthalmology, Gynecology, ENT and Orthopedics services.

4.3. Population

4.3.1.Source population

 All patients who were diagnosed as PTB based on clinical criteria at Felege Hiwot Referral Hospital, Bahir Dar, Ethiopia

4.3.2. Study population

  All direct FM   smear negative presumptive PTB patients at Felege Hiwot Referral Hospital, Bahir Ddar, Ethiopia.

4.4. Sample size determination

The required sample size of the study population was determined using single population proportion formula. According to the study done at St. Peter's Tuberculosis Specialized Hospital, prevalence of smear negative pulmonary tuberculosis was 17.4% (Desta *et al.,* 2009).

Therefore, the sample size was calculated based on the given proportion as:

$$n = \frac{(Z\,\alpha/2)^2 * (1-p) * (p)}{d^2}$$

Where;

n = minimum sample size

$Z\,\alpha/2$ = 1.96 at 95% Confidence Intervals (CI)

P = proportion of smear negative PTB

d= margin of error 0.05 at 95% CI

$n = (1.96)^2 * (1-0.174) * (0.174)$ divide by $(0.05)^2$

n = 220, with 10 % non-respondent, n =242

4.5. Sampling technique
A convenient sampling technique was used to select study subjects until the required sample size obtained.

4.6. Variables

4.6.1.Dependent variable
Tuberculosis status with culture

Drug susceptibility pattern of isolates

4.6.2.Independent variables
Socio-demographic characteristics such as age, residence, marital status, educational status, clinical data, chest X- ray finding, cough, night sweats, fever.

4.7. Eligibility criteria

4.7.1. Inclusion criteria

All adult patients, presenting with cough ≥ 2 weeks, who gave spot- spot sputum sample for FM diagnosis and no AFB detected.

4.7.2. Exclusion criteria

Patients of <18 years with known tuberculosis, on anti TB treatment, have extra pulmonary TB, on antibiotics treatment within a month were excluded from the study.

4.8. Data collection methods and specimen analysis

4.8.1. Administration of questionnaire

A pretested structured questionnaire was used to collect socio-demographic characteristics and other explanatory variables of the client's by trained laboratory technologists.

4.8.2. Specimen collection and transportation

Patients were asked to give about 5-10 ml of spot-spot sputum sample using clean, sterile, leak proof, wide mouth container (falcon tube) clearly labeled with patient identification and pooled. Consequently, all samples were stained by Fluorescent-Auramine O technique and examined using simple FM. Finally, smear negative sputum samples were selected consecutively and transported to Amhara Regional public health institute, TB Laboratory for concentration, culture and dug susceptibility test. Ice box was used to maintain the cold chain during transportation and each cup containing sputum samples were covered by plastic separately inside the ice box.

4.4.3. Concentration technique

Concentration technique was done for all FM smear negative spot samples as per the standard procedures. Sputum samples were mixed with 5% sodium hypochlorite solution for overnight sedimentation .The supernatant was discarded, the sediment mixed with the remaining fluid and smeared onto a labeled slide. Auramine O was used as staining dye to demonstrate AFB since it binds to the nucleic acid in the Mycobacterium nucleus. After being stained with auramine the smear was decolorized with acid alcohol which removes the dye from the background and finally potassium permanganate was used as a counter stain to cover the

background. The bacilli fluoresce white-yellow against the dark background under 250x objective FM.

4.8.3. Sputum culture

Each spot-spot sputum samples were pooled together and processed (digested and decontaminated) for culture according to the standard procedures using N-acetyl-Lcysteine NaOH (NALC-NaOH) method. Processed samples were centrifuged at 3,000 rpm for 15 minutes and neutralized by phosphate buffer (pH=6.8). Finally, 100μl of the neutralized sample was cultured into two LJ tubes and 0.5 ml samples to MGIT culture containing 7H9 broth enriching supplement and an antibiotic mixture for primary isolation of the organisms. The inoculated LJ medium tubes were incubated at 35 to 37°C for 3 to 8 weeks and observed once a week for the growth of *Mycobacterium* and samples inoculated on MGIT were loaded to MGIT 960 automated system and tubes were classified as negative after 42 days. *Mycobacterium* growth was confirmed by visual detection of colonial morphology on LJ medium/MGIT and microscopic examination of the colonies for acid fast bacilli (AFB) from the growth was done. Finally, SD Bioline rapid test was performed to detect specific MPT64 antigen for *Mycobactrium tuberculosis* complex.

4.8.4. Molecular drugs susceptibility testing

Drug sensitivity test was done for all culture positive specimens according to the standard procedure using molecular Line Probe Assay (LPA) method. Susceptibility to anti-TB drugs was defined as hybridization (presence of a band) to all the wild-type probes and no hybridization (absence of a band) to the mutant probes. The absence of hybridization of any wild-type and/or hybridization of any mutant gene indicates resistance to the respective drugs. Hybridization of wild-type and mutant genes indicated hetero-resistance or a mixed infection.

4.9. Quality control

Questionnaire was pretested before the actual data collection period for its appropriateness. Sputum smear positive and negative control slides were used for Fluoresce-auramine O staining every week to insure the quality of the reagents. For the quality of culture, the performance characteristics of each lot of LJ media was confirmed by using well characterized laboratory strains of MTBC and NTM. Sterility check was done by placing a random sample of the batch at

37°C for 48 hours and evaluated each slant for growth. DNA extraction positive (H37Rv) and negative controls and master mix controls was used for controlling the quality of LPA. In addition, data was checked daily for consistency and accuracy.

4.10. Data management and analysis

After data collection and specimen examination completed all laboratory results were recorded on a logbook during the study period. Questionnaires were coded and data entered to SPSS v.23 statistical software for analysis. Logistic regression analysis was done to identify possible association between the dependent and different explanatory variables. Variables that had p-value<0.2 was analyzed by multivariate regression model to identify potential predictors for culture confirmed TB status and anti-TB drug resistance and to avoid confounding factors by calculating adjusted odds ratio(AOR) with 95% confidence intervals (CI). All associations in multivariate model were considered significant if the p-value < 0.05. Percentage, mean and standard deviations were also used to present the result of socio demographic data, drug susceptibility and prevalence of culture confirmed PTB. Final results were displayed in the form of narration, tables and graphs.

4.11. Operational definitions

In accordance with the (WHO, 2015) tuberculosis report, the following definitions of TB case classifications were used.

Pulmonary tuberculosis suspects: are patients presenting with persisting cough for two weeks or more; productive cough with or without blood stained sputum, shortness of breath and chest pain; and loss of weight, intermittent fever, night sweats, loss of appetite, fatigue and malaise.

Case detection: The act of identifying active tuberculosis cases through sputum examination mainly among suspects attending a health facility for any reason.

Smear-positive pulmonary TB (SPPTB): A patient with at least two initial sputum smear examinations positive for AFB by direct microscopy, or a patient with one initial smear examination positive for AFB by direct microscopy, and radiographic abnormalities consistent with active PTB as determined by a clinician.

Smear-negative pulmonary TB(SNPTB): A patient having symptoms suggestive of TB with at least two sputum smear examinations negative for AFB by direct microscopy; and radiographic

abnormalities consistent with active **PTB**; and no response to a course of broad-spectrum antibiotics, or a patient whose sputum diagnosis is culture positive for *M. Tuberculosis,* but two initial sputum smear examinations negative by direct microscopy and a decision by a clinician to treat with a full course of anti tuberculosis chemotherapy.

New case: A patient who has never had treatment for tuberculosis or who has taken anti tuberculosis drugs for less than one month.

Retreatment case: Patients those receiving their first retreatment regimen after relapse, failure, or default.

4.12. Ethical clearance

Ethical clearance was obtained from research and ethical clearance committee of Bahir Ddar University, College of Medicine and Health Sciences. Then formal letter of permission was written for FHRH and Amhara Regional public health institute, TB Laboratory. Written and oral informed consent was also obtained from all study participants after explaining the purpose and the procedures of the study. All collected information and records was kept confidential and coded. Finally study participants who had culture positive findings were communicated to their physician for possible intervention.

4.13. Dissemination of results

The findings of the study would be communicated to regional health bureau, patients, Felege Hiwot referral hospital and other responsible bodies for possible intervention. The paper will be presented to Bahir Dar University College of Medicine and Health Sciences department of Medical Microbiology, Immunology and Parasitology, in different seminars or work shop and published on reputable journal.

5. Results

5.1. Socio demographic characteristics

A total of 242 sputum smear negative presumptive PTB patients were participated in this study. Of those, 9/242(3.7%) patients did not have final culture result because of culture contamination. Of the remaining 233 sputum smear-negative patients, 153/233(65.7%) were males. The mean age of the study participants was 29.21 (SD. ±15.35) and 144(61.8%) respondents were rural resident. The male to female ratio of the study participants was approximately 1:2. Ninety eight (42.1%) of the study participants were able to read and write. Majority, 226 (97.8 %) of PTB suspected patients were new case of TB history (Table 1)

Table 1: Socio-demographic characteristics of smear negative presumptive Pulmonary Tuberculosis patients at Felege Hiwot Referral Hospital, Bahir Dar, 2017.

Variable	Frequency	Percent (%)
Sex		
Male	153	65.7
Female	80	34.3
Age in years		
18-28	88	37.8
29-39	69	29.6
≥40	76	32.6
Residence		
Urban	89	38.2
Rural	144	61.8
Religion		
Orthodox	140	60.1
Muslim	63	27.0
Protestant	30	12.9
Educational status		
Illiterate	52	22.3
Read and write	98	42.1
Others	83	35.6
Occupation		
Farmer	118	50.6
Merchant	55	23.6
Government worker	21	9.0
Others	39	16.8
TB history		
New case	227	97.4
Retreatment	6	2.60

5.2. Prevalence of culture positive pulmonary tuberculosis

From a total of 242 FM sputum SNPTB patients participated in this study, 27/233(11.6%) of them were identified as culture positive for Mtb. Of these samples 25/231(10.8%) were identified as *Mycobacterium tuberculosis* complex using a rapid SD Bioline MPT64Ag test. The remaining culture positive 2/27(7.4%) samples were identified as NTM (Table 2). Significant difference was not observed between culture and BACTEC MGIT 960 and LJ medium in terms of mycobacterial recovery rate.

Table 2: Culture results of smear negative pulmonary tuberculosis among presumptive PTB patients at Felege Hiwot Referral Hospital, Bahir Dar, 2017.

Laboratory Test	Frequency	Percent (%)
Culture (n=233)		
Positive	27	11.6
Negative	206	88.4
MPT64Ag test (n=27)		
MTBC	25	92.6
NTM	2	7.4

NTM=None-tuberculosis *mycobacterium, MTBC=Mycobacterium tuberculosis complex*

5.3. Prevalence of Pulmonary tuberculosis with bleach concentration technique

Out of the 242 FM sputum SNPTB suspected patients, 20/242 (8.3%) of them were positive for AFB using bleach concentration technique. Likewise all concentration positive samples were positive on culture but additional seven samples were become positive on LJ media which were negative on concentration technique (Table 3).

Table 3: Comparison of sputum concentration FM and culture results among smear negative Presumptive PTB patients at Felege Hiwot Referral Hospital, Bahir Dar, 2017.

		Concentration smear (n=242)		
		Positive	Negative	Total
	Positive	20(8.3%)	7(3%)	27(11.6%)
Culture (n =233)	**Negative**	0(0.0%)	206(88.4%)	206 (88.4%)
	Contaminated	0(0.0%)	9(3.7%)	9(3.7%)
	Total	20(8.3%)	222(91.7%)	242(100.0%)

5.4. Factors associated with smear negative pulmonary tuberculosis

For the potential risk factors of culture confirmed smear negative PTB, both bivarate and multivariate logistic regression analysis was employed. Multivariate logistic regression analysis was performed for all variables which had (P $\leq$ 0.2) from bivariate analysis to avoid the possible confounding factors. After adjustment, age, residence, educational status, previous history of TB, Fever, history of contact with TB patients, chest x-ray finding, sputum appearance were significantly associated with culture confirmed SNPTB (p<0.05).

PTB suspected patients in the age range of 29-39 years were 5.6 times (AOR=5.6, 95% CI: 1.6-19.6) and age groups greater than or equal to 40 years were 4 times (AOR= 4, 95% CI: 1.1-14.6) more likely to acquire culture positive SNPTB than compared to patients in the age range of 18-28years. PTB suspected patients who reside in rural were 11.9 times (AOR=11.9, 95% CI: 2.4-25.2) more likely to acquire culture positive SNPTB than urban. Moreover, illiterates had 6.5 fold higher risk (AOR=6.5, 95% CI: 1.3-32) to acquire culture positive SNPTB than able to read and write. Furthermore, retreatment cases were 48times (AOR=48, 5.3-43.5) more likely to aquire culture positive SNPTB than new cases. Likewise, clinical characteristics and chest x-ray findings like; PTB suspected patients who had fever (AOR=4.6, 95% CI: 1.13-18.6), contact history with PTB patients (AOR=15, 95% CI: 3.9-15.6) and abnormal chest x-ray were (AOR= 3.6, 95% CI: 1.01-12.9) found to be significantly associated with culture positive SNPTB. PTB patients who had bloody sputum (hemopityasis) had 5.5 fold higher risk (AOR=5.5, 95% CI: 1.20-25.0) and purulent sputum 4.5 fold higher risk (AOR=4.9, 95% CI: 1.43-16.6) to acquire culture positive SNPTB than watery sputum appearance respectively. In contrast to bivariate analysis abnormal chest x-ray finding shows significant association in multivariate analysis (p<0.05) (Table 4).

Table 4: Association of possible factors for culture positive PTB among smear negative presumptive PTB patients at Felege Hiwot referral Hospital, Bahir Dar, 2017.

Variables	Total n=233	Culture positive PTB	COR(CI),p value	AOR(CI),p value
Sex				
Male	153 (65.7%)	17(11.1%)	1.00	
Female	80(34.3%)	10(12.8%)	1.18(0.51-2.71),0.70	
Age in years				
18-28	88 (37.8%)	6(6.8%)	1.00	
29-39	69 (29.6%)	12(16.4%)	2.88(1.02-8.11),0.04	5.6(1.6-19.6),0.007*
≥40	76(32.6%)	9(12.2%)	1.89(0.64-5.59),0.2	4.0(1.1-14.6),0.04*
Residence				
Urban	89 (38.2%)	5(5.7%)	1.00	
Rural	144 (61.8%)	22(15.3%)	2.96(1.08-8.12),0.04	11.9(2.4-25.2),0.003*
Educational status				
Illiterate	52 (22.3%)	14(26.9%)	4.60(1.64-12.94),0.004	6.5(1.3-32),0.02*
Read & write	98 (42.1%)	7(7.1%)	0.96(0.31-2.98),0.9	0.8(0.20-4.0),0.8
Others	83 (35.6%)	6(7.4%)	1.00	1.00
Occupation				
Farmer	118 (50.6%)	15(12.7%)	1.75(0.48-6.39),0.4	
Merchant	55 (23.6%)	5(9.1%)	1.20(0.27-5.35),0.8	
Gov't worker	21(9.0%)	4(21%)	3.20(0.64-16.1),0.3	
Others	39 (16.8%)	3(7.7%)	1.00	
TB history				
New case	224 (97.4%)	22(9.8%)	1.00	
Retreatment	7(2.6%)	5(71.4%)	23(4.20-125),0.00	48(5.3-43.5),0.001*
Cough (>2weeks)				
Yes	221(94.8%)	26(11.8%)	1.2(0.15-9.90),0.9	
No	12(4.3%)	1(10%)		
Sputum production				
Yes	220(94.4%)	26(11.8%)	1.3(0.16-10.9),0.7	
No	13(5.6%)	1(9.1%)	1	
Night sweat				
Yes	182(78.1%)	24(13.2%)	2.3(0.67-8.08),0.2	1.0(0.20-4.56),0.9
No	51(21.9%)	3(6.1%)	1	
Fatigue				
Yes	190(81.5%)	25(13.2%)	3(0.68-13.0),0.2	1.9(0.35-10.3),0.5
No	43(18.5%)	2(4.9%)	1	
Shortness of breath				
Yes	145(62.2%)	22(15.2%)	2.9(1.06-8.00),0.04	1.14(0.27-4.50)0.9
No	88(37.8%)	5(5.8%)	1	

Excessive weight loss

Yes	121(51.9%)	22(11.2%)	4.7(1.70-12.80),0.03	3.0(0.95-9.64),0.1
No	112(48.1%)	5(4.5%)	1	
Fever				
Yes	150(64.4%)	23(15.3%)	3.5(1.16-10.50),0.03	4.6(1.13-18.61),0.03*
No	83(35.6%)	4(4.9%)	1	
Contact history				
Yes	109(46.8%)	24(22%)	11(3.30-38.40),0.01	14.8(3.90-15.6),0.01*
No	124(53.2%)	3(2.5%)	1	
Chest x-ray finding				
Normal	168(72.1%)	23(13.7%)	1	
Abnormal	65(27.9%)	4(6.3%)	0.43(0.42-1.29),0.1	3.6(1.01-12.90)0.04*
Sputum appearance				
Hemopityasis	27(11.6%)	6(22.2%)	6.6(1.85-23.71),0.004	5.49(1.20-25.0),0.03*
Purulent	83(35.6%)	16(19.3%)	5.5(1.94-15.80),0.001	4.9(1.43-16.65),0.01*
Watery(saliva)	123(52.8%)	5(4.1%)	1	

* Variables had significant association, COR= Crude Odds Ratio, AOR= Adjusted Odds Ratio

5.5. Drug susceptibility pattern of culture positive pulmonary tuberculosis

Line probe assay was performed for 25 rapid MPT64 antigen positive MTB complex isolates to determine the magnitude of gene mutations conferring resistance to anti-TB drugs among smear negative culture positive PTB patients. Of these 16.0% of the isolates showed gene mutation resistance to rifampicin on *rpoB* gene locus of MTB complex. However, resistance to Isonazide, was not observed in this study. Considering rifampicin mono resistance as surrogate marker for MDRTB (WHO, 2016), the prevalence of MDRTB with mono resistance was 16% (Fig.1).
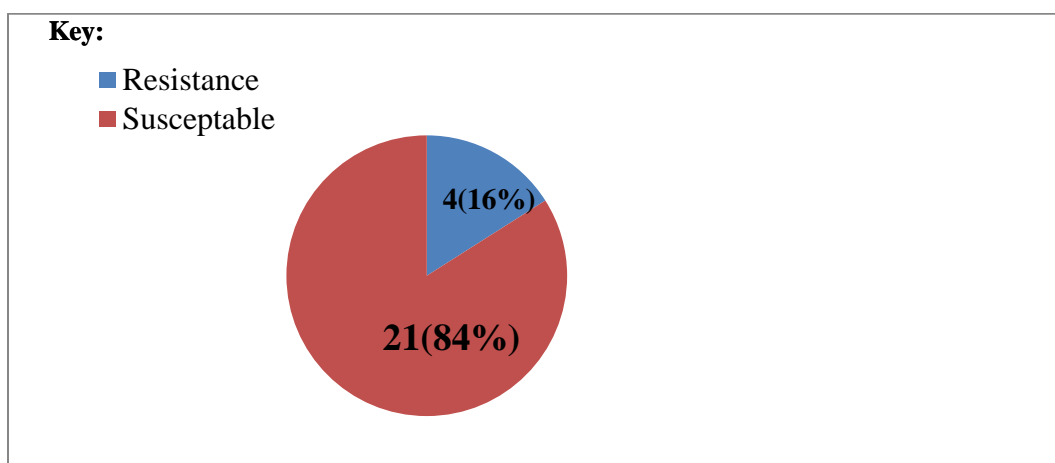

Fig.1: Drug susceptibility pattern of MTB isolates among culture confirmed smear negative presumptive PTB patients, at Felege Hiwot Referral Hospital, Bahir Dar, 2017.

5.6. Factors associated with anti-TB drug resistance

Logistic regression analysis was done to identify the possible associated socio demographic factors for anti- TB drug resistance. In the bivariate logistic regression analysis, previous history anti-TB treatment and religion being protestant was possibly considered as statistically associated with anti-TB drug resistance (p < 0.2). In multivariate logistic regression analysis, individuals with previous history of anti-TB treatment were twenty times (AOR=20, 95% CI: 2.53-11.3) more likely to develop resistance to rifampcin (RMP) compared to those no history of previous anti-TB treatment. However, religion was excluded from the model (Table 5).

Table 5: Association of possible factors with rifampcin resistance among smear negative presumptive PTB patients at Felege Hiwot Referral Hospital, Bahir Dar, 2017

Variables	RMP result (n=25)		COR (CI),p value	AOR (CI),p value
	Resistance	**Susceptible**		
Sex				
Male	2(13.3%)	13(86.7%)	0.44(0.04-5.01), 0.5	
Female	1(10%)	9(90%)	1	
Age in years				
18-28	2(33.3%)	4(66.7%)	0.33(0.02-5.02),0.4	
29-39	1(8.3%)	11(91.7%)	1.83(0.09-34.8),0.7	
≥40	1(25%)	6(28.6%)	1	
Residence				
Urban	1(20%)	4(80%)	1	
Rural	3(15%)	17(85%)	1.42(0.12-17.5),0.8	
Religion				
Orthodox	2(11.1%)	16(88.9%)	1	
Muslim	1(25%)	3(75%)	0.38(0.03-5.6),0.5	0.56(0.12-25.6),0.8
Protestant	1(33.3%)	2(66.7%)	0.25(0.12-4.2),0.2	0.44(0.07-26.6),0.7
TB history				
New case	1(4.8%)	20(95.2%)	1	
Retreatment	2(50%)	2(50%)	6(2.9-12.7),0.01	20.0(2.53-11.3), 0.01*

* Variables had significant association**,** COR= Crude Odds Ratio, AOR= Adjusted Odds Ratio

6. Discussion

Smear-negative pulmonary TB has become an increasing important clinical and public health problem in Ethiopia due to the low availability of laboratory service like culture at the district level (Tadesse *et al*., 2016). The incidence and prevalence of smear negative culture positive PTB is poorly documented in the country.

This study reveals that among smear negative presumptive PTB patients, the prevalence of smear negative culture positive PTB was at 11.6%. This finding strongly suggests the need for rapid and accurate diagnostic tool for detecting PTB cases in patients having negative sputum smears. Furthermore, the study finding strongly suggests the need of quality assurance in general. Our finding was higher than with the previous studies done on the prevalence of smear negative culture positive pulmonary tuberculosis Ethiopia 10.3% (Tadesse *et al*., 2016). In contrast, our finding was found to be lower compared to the study conducted in Mexico 80% (Assael *et al.,*2013), USA 37% (Shah *et al* ., 2012), Bangladesh 17.1% (Uddin *et al.,* 2013) ,Tanzania 30.8% (Swai1 *et al*.,2011), Tanzania 12.5% (Nyagosya *et al.,*2008), Zimbabwe 21.6% (Apers *et al.,* 2004) and Ethiopia, St. peters Referral Hospital 17.4% (Desta *et al*., 2009). This variation might be due to the difference in the study population, patient status and the sample size.

The present study identified 2 (7.4%) NTM. This finding was higher than the study conducted in Vietnam, 1(0.3 %) (Nguyen *et al.,* 2012). In contrast, our study was comparable to the previous study done in Ethiopia, 3(7%) NTM (Desta *et al.,* 2009).

The utility of sputum concentration techniques have been consistently demonstrated for the diagnosis of TB (Steingart*et al.,* 2006). Our study showed that, the use of overnight bleach sedimentation sputum smear FM increased sensitivity in identifying positive TB cases, compared to the direct FM method, while using positive culture as the gold standard. In this study, the overall prevalence of SNPTB using sputum concentration technique was 8.3%. The likely reason of this high prevalence is due to the high sensitivity of concentration method or the poor detection ability of AFB by laboratory professionals using direct FM. This finding was lower than the study conducted in Bangladesh, 9.5% (Uddin *et al.,* 2013), Zimbabwe, 36.7% (Apers *et al.*, 2004). The difference might be due to observer variation in different study settings. In this study, fortunately, specimens contaminated on culture result were not observed among

concentrated smear positive samples and all of them were included in the analysis. Of twenty seven MTB isolates 20 (74%) of smear negative specimens were found to be positive in both concentrated smear FM and culture method. In contrast, 7(26%) of specimens, that were negative with the concentration method, were found to be positive by culture. The difference might be due to the high sensitivity of the culture or unexpected technical errors of concentration method.

Like other developing countries, the diagnosis of PTB in Ethiopia relies mainly on clinical screening, chest x-ray which is non specific for PTB diagnosis. Our study identifies combination of clinical symptoms which could be a sensitive predictor for smear-negative PTB like; fever, abnormal chest x-ray finding and Hemopityasis. This finding was consistent with the study conducted in Iran (Alavi-Naini *et al*., 2012) but inconsistent with previous study which failed to identify neither single nor combination of symptoms as predictors for smear-negative PTB (Nguyen *et al*., 2012). This inconsistency may reflect the disparity in the studied population and observer variation.

The study conducted in Northern Ethiopian prisons showed that, contact history to TB patients, educational level, cough and night sweating were found to be predictors of TB positivity among smear-negative PTB cases (Biadglegne *et al*., 2014). Likewise in this study, contact history of contact with PTB patient and being illiterate were significantly associated with culture confirmed smear negative PTB. Moreover, age group of 29-39 years & age group greater than or equal to 40 years and being rural residences were also found to be factors significantly associated with culture confirmed smear negative PTB. This finding indicated that PTB affects the productive age groups of the populations in the study area.

The current study identifies the drug susceptibility pattern of twenty five MTB complex isolates. Of these 4(16.0%) of the isolates were found to be only mono resistance to rifampicin (which is surrogate marker for MDRTB/RMP (WHO, 2016). Although, the small sample size might limit us to generalize, our findings suggest that the precursors of MDR-TB are increasing in the study areas as RMP resistance. The possible reason of the highest proportion of resistance might be attributed to the fact that, RMP is largely used in Ethiopia for the treatment of other bacterial infections (Adane *et al.,* 2005). Moreover, this finding strongly suggests the need for rapid and

accurate diagnostic tools for detecting resistant TB cases in patients having negative sputum smears.

Our finding was relatively greater than the previous studies conducted in Ethiopia, East Gojjam, which was 3.37% (Adane *et al*., 2015) and Northwest Ethiopia, Metema and west Armachiho 5.7% (Mekonnen *et al.,* 2015). The possible explanation for this difference could be due to the fact that, this study was conducted at Felege Hiwot Referal Hospital in Bahir Dar , which is a referral site for complicated TB cases from most of the Amhara region. However, this finding was almost lower than the previous study conducted on drug resistance TB among smear negative culture positive PTB patients in Ethiopia, St. peters Referral Hospital, 29.8 % (Desta *et al*., 2008), RMP resistance in Amhara region 18.2% (Nigus *et al.,* 2014) and other country, prevalence of drug resistance in Nepal, 17.8% (Thapa *et al.*, 2016) and China 26.9% (Zhao *et al.,* 2014).

Several studies documented the association between a previous history of TB treatment and anti-TB drug resistance (Zhao *et al.,* 2014, Thapa *et al*., 2016, Mekonnen *et al*., 2015, Moniruzzaman *et al*., 2006). Similarly our finding showed that, previous history of anti-TB treatment as the only risk factor associated with anti-TB drug resistance.

In our study, most of the variables were not significantly associated with the drug resistance TB. This might be due to the small sample size included in the study; hence, further study with large sample size is recommended to identify additional risk factors.

7. Limitation of the study

Our study has some drawbacks these include; lack of data on HIV status of the patients whose sputum has processed for culture. We use small sample size due to shortage of resources; as multi-center studies including large number of samples could have generated more significant data. Finally the study has limited to clearly identify MTB complex as its species level.

8. Conclusion and recommendation

In the studied site, high prevalence of culture positive miss classified PTB cases were documented from smear negative presumptive PTB patients. Fluorescence microscopy sputum examination skill can be one possible reason for this high discrepancy. Therefore, in-service training for laboratory professionals and regular quality assurance is likely to reduce false negative and increase the yield of smear positive results. Moreover, sputum culture should be used at the district level to decrease smear negative cases among presumptive SNPTB patients. Likewise, overnight bleach concentration sputum smear FM is recommended for routine diagnosis of SNPTB in resource limited areas; when culture is not available.

Although, the presence of fever, abnormal chest x-ray, hemoptyasis and purulent sputum were identified as predictors for SNPTB; this study identified clinical symptom screening alone is not recommended for identification of SNPTB cases. Moreover, socio demographic factors like, being age group between 29-39 years, and age group greater than or equal 40 years, rural residence, illiterate, history of previous treatment and had contact history with TB patients were identified as predictors of culture confirmed SNPTB. Therefore, health education on the prevention, control and treatment of TB is needed in the study area, to decrease the health and economical impact of the country.

The current study also identified a high MDR-TB in the study area (considering rifampicin mono resistance as surrogate marker for MDR-TB). As anti -TB drug resistance is linked with pervious history of TB treatment, there is a need to strengthen Direct Observed Treatment Serves (DOTS) programme and expand MDR-TB diagnostic facilities so as to detect the cases in time and start appropriate treatment to prevent the spread of MDR-TB among SNPTB patient in the country.

Finally, further study should be done on large sample size with different setting and laboratory methods to identify additional predictor variables of smear negative culture positive PTB and to differentiate MTB complex at the species level.

9. Principal investigator

The undersigned agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the College of Medicine and Health Sciences in effect at the time of grant is forwarded as the result of this application.

Name of the student: **Asrat Ayalew**

Date._____   Signature _____

APPROVAL OF THE ADVISOR (s)

Name	Signature	Date
1. _____	_____	_____
2._____	_____	_____

EXTERNAL EXAMINER

Name   Signature   Date

_____   _____   _____

INTERNAL EXAMINOR

Name   Signature   Date

_____   _____   _____

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Annex-I: Questionnaire

Questionnaire for investigation of prevalence of pulmonary tuberculosis using standard culture methods and drug susceptibility pattern among PTB suspected smear negative patients at FelegeHiwot Referral Hospital, Bahir Dar, Ethiopia

Code No_____

Card No. _____

Tel-------------------------------

No	Question	Response	skip
Part-I: Patient Identification and demographic questions			
101.	Age	-----------------	
102.	Sex	1.Male 2. Female	
103.	Occupation:	1. Farmer 2. Merchant 3.Gov't worker 4. Other___ (specify).	
104.	Residence:	1.Urban 2.Rural	
105.	Religion	1. Christian 2. Muslim 3. Other _____ (specify)	
105.	Educational level:	1. Illiterate 2. Elementary 3. High school 4. College /university	
106.	TB history	1.New case 2.Retreatment cases	
Part-II: Clinical data (Symptom of TB)			
201.	Chronic cough (>2weeks):	1. Yes 2. No	
202.	Production of sputum/cough:	1. Yes 2. No	
203.	Haemoptysis (coughing up blood):	1. Yes 2. No	
204.	Night sweats:	1. Yes 2. No	
205.	Fatigue/tiredness:	1. Yes 2. No	
206.	Shortness of breath	1. Yes 2. No	
207.	Unexplained weight loss:	1. Yes 2. No	
208.	Fever:	1. Yes 2. No	
209.	Contact History with suspected TB Patients:	1. Yes 2. No	
210.	If yesQ209 with whom	1. Family 2. Friends 3.Neihbour 4.other(specify)__	
211.	Chest X-ray finding:	1. Normal 2. Abnormal 3. Not done	

Part –III .Laboratory request form (Laboratory data)

Name of Hospital _____Date ___/____/_____

Patient's register number_____

Laboratory serial number _____ Date specimen received _____

1. Gross appearance of sputum

               1. Haemoptysis   2. Purulent   3. Watery/saliva

2. Direct microscopic examination of FM smear result

               1. Not done   2. No AFB   2. AFB seen

3. Concentration FM examination result

               1. Not done   2. No AFB   3. AFB seen

4. Culture results using LJ/MGIT

               1. No growth   2. Contaminated   3. Have growth

MTB identified as: 1.*Mycobacterium tuberculosis*

                2. Non *Mycobacterium Tuberculosis* (NMT)

                3. Contaminated

Date _____ Signature _____

Annex - II: Information sheet to the respondents

Principal investigator: my name is_____ MSC student in Medical Microbiology track at Bahir Dar University, college of Medicine and health science, Department of Microbiology, Immunology and Parasitology. The purpose of the study is to determine the Burden of smear negative pulmonary tuberculosis and drug susceptibility test among pulmonary tuberculosis suspected patients. You are invited to participate in the study. If you give your consent to participate in the study, you will be asked to give answer to structured questionnaire by data collector, give two consecutive sputum samples and also to share your medical records only for the purpose of study. There is no direct financial benefit from study, but you will be communicated if there is a need for intervention .There is no risk to participate in the study. There will be no compensation for using your sputum sample. Confidentiality of your information will be used only for the purpose of the study and kept confidential; your identity will not be presented and will be kept secret .We will respect your withdrawer if you are not interested in the study at any time.

If you have any question or comment regarding the study, you can use the following address.

Principal investigator: Asrat Ayalew

Bahir Dar University, college of Medicine and health science, Department of Microbiology, Immunology and Parasitology

Phone number: 0911630599

Email: asratayalew@yahoo.com

Annex-III: Consent form

Your signature below indicates that you have read or listened to and understand the information provided to you about the study. Before you sign, please confirm that you understand the overall activity of the study. By signing, you are making a decision to participate in this study (to give sputum sample as recommended, to answer the question for data collector and share your clinical data). All the information which will be gain from you will be kept confidential and used only for study purpose. If you decide that you wish to withdraw or discontinue your participation in the study, you may do so at any time. Do you agree to participate?

I have read and/or listened to the description of the study and I understand what the procedures are and what will happen to me in the study. I agree to participate in it. I know that I can quit the study at any time.

Name/ID no……………………………….Signature ………………………………Date

Annex -IV: Amharic questionnaire and consent form

ባህርዳር ዩኒቨርስቲ የህክምና እና ጤና ሳይንስ ኮሌጅ ሜዲካል ባዮሎጅ ፤ ኢሚኖሎጅ እና ፓራሳይቶሎጅ ትምህርት ክፍል

የቲቢ በሽታ ላይ የ ሟጸረግ ጥናት

ማላያ ቁጥር ----------------------------

የ ካርድ ቁጥር ----------------------------

ስ/ቁጥር --------------------------------------

	ክፍል አንድ፡ ማህበራዊ ና ስነ -ህዝባዊ ጥያቄዎች		
ተ.ቁ	**ጥያቄ**	**ማጐስ**	**እላፍ**
101.	እድሜ	---------------	
102.	ጾታ	1. ወንድ 2. ሴት	
103.	የ መዳደሪያስራ	1. ገበሬ 2.ነ ጋዴ 3. የ መንግስት ሰራተኛ 4. ሌላ ከሆነ ይግለጹ ……….	
104.	የ ማሪያ አድራሻ	1. ገ ጠር 2. ከተማ	
105.	ሀይማኖት	1.ክርስቲያን 2.ሟሊም 3. ሊላካለ ጥቀስ-------	
106.	የ ትምህርት ደረጃ	1.ያልተማሪ 2. 1ኛ ደረጃ 3. 2ኛ ደረጃ 4. ከፍተኛ የ ት/ት ተቋም……	
107.	የ ቲቢ ህክምና ከአሁን በፊት ወስደዋል	1.አልወሰድኩም(ለ መጀመሪያ ጊዜ) 2.ወስጃለሁ (ድጋሟ	
ክፍል ሁለት፡ የ ቲቢ በሽታ ምልክት ጥያቄ			
108.	ከሁለት ሳምንት በላይ የሆነ ሳል አለዎት ወይ ?	1. አዎ 2 .የለኝም	
109.	አክታ አለዎት ወይ ?	1. አዎ 2 .የለኝም	የለኝካሆነ ወደጥቁ. 110
110.	አክታ ካለዎትደም አለው ወይ ?	1. አዎ 2 .የለኝም	
111.	ማታማታ ያልብዎታል ወይ ?	1. አዎ 2 .የለኝም	
112.	የ መድከምስ ሟጐ አለ ወይ ?	1. አዎ 2 .የለኝም	
113.	የ ትንፋሽ ማጠር ስሟጐ አለ ወይ ?	1. አዎ 2 .የለኝም	
114.	የ ክብደት መቀነስ አለ ወይ ?	1. አዎ 2 .የለኝም	
115.	ትኩሳት አለዎትወይ ?	1. አዎ 2 .የለኝም	
116.	የ ራስ ምታት ህመማአለ ወይ	1. አዎ 2 .የለኝም	
117.	በቲቢ በሽታ ከ ሟበረ ጠር ሰው ጋር ተገናኝተውያ ወቃሉ?	1. አዎ 2.የለም	
118.	ጥቁ. 109 ማጐሉ አ ወ ከሆነ	1.ከ ቤተ ሰብ 2.ከ ገ ጓ ደኛ 3.	

	ከማን?	ከጎረቤት 4.ሌላካለይግለጹ----------	
119.	የ ራጅ ውጤት ካለ (ባለሙያ የ ሞይሞላ ው)	1. ችግር የ ሌለበት 2. ችግር ያለበት 3. አልተሰራም	

የ ሞኂ ጃ ቅጽ

የጥናቱ ባለ ሞያ---------------------------------------እባላለሁ በባህርዳር ዩኒቨርስቲ የህክምና እና ጤና ሳይንስ ኮሌጅ ማይክሮባዮሎጅ ፤ ኢሞኖሎጅ እና ፓራሳይቶሎጅ ትምህርት ክፍል የህክምና ማይክሮ ባዮሎጅ የማስተርስ ተማሪ ነኝ፡፡ ስለሆነም የሞሞቂያ ጽሁፌን በቲቪ በሽታ ላይ ለማራት ስለፈለኩኝ እርሶም የምርምሩ አካል እንዲሆኑና ለሞደግሎት ቃለ ሞጠይቅ አስፈለጊውን ምላሽ እንዲሰጡኝ ና የአክታናሞዋትን ለሞሞረገው ጥናት ሞጠቀም እንድቸል እንዲፈቀድልኝ በትህትና እጠይቃለሁኝ፡፡ ስለዚህ ለጥናቱ አላማየ ሞሆን የአክታ ናሞ ሁለት ጊዜ እንዲሰውይደረጋል ፡ ፡ በጥናቱ ላይ በሞተፍ የሞገ ኙት የገንዘብ ጥቅም አይኖርም ነገርግን በጥናቱ በተደረገው ምርሞራ የቲቪ በሽታ ሞሩ ከተረጋገጠ ሞድሃኒቱን የሞገ ኙበት ሁኔታ ይመቻቻል፡፡ በጥናቱ ላይ በሞተፍ የሞርስበት ጉዳት አይኖርም ለጥናቱ ብለውለሞስጡ የአክታ ናሞ ማከካሻ የሚጠነገር አይኖርም ፡ ለጥናቱ ብለው የሞስጡን ማንኛውም ሞኂ ለጥናቱ አላማብቻ ነውየ ምንጠቀመ፤ የተሳታፊውን ማንነት በሞልጽ ሁኒታ በጥናቱ ወስጥ አይገባም፤ እነዲሁም የተሳታፊው ሞኂም ሞስጥርነቱ የተጠበቀ ይሆናል፡፡ በጥናቱ ላይ ሞስተፍ ካልፈለጉ እንዲገ ደዱ አይደረግም፤ እንዲሁም ሞስተፍ ጀምረው ሞጨረጥ ቢፈልጉ ሞጨረጥ ይቸላሉ፡፡ በጥናቱ ላይ ማንኛውም ጥያቄ ወይም አስተያት ካለ በሞስተለው አድራሻ ማግኘትይቸላሉ፡፡ **(ሞባይል ቁጥር ፡ 0911630599)**

ሞተማሞኛ ፎርም

ከዚህ በታች ያለውፎርማስለ ጥናቱ ግንዛቤ እንዳገኙ የማልጽ ስለሆነ ከሞረሞጣ በፊት ስለጥናቱ በቂ ሞኂ ማንኛሞን ያረጋገጡ፡፡በጥናቱ ላይ ለሞተፍ ማእትም ለምጠየቀው ሞጠይቅ ምላሽ እንዲሰጡ፤ የአክታ ናሞ ለሞስጡት ና አስፈለጊ ከሆነ የህክምና ካርዶትን ለጥናቱ አላማ ብቻ እንድጠቀም እንዲፈቀዱልኝ እየጠቁኝ የርሶም ሞኂም ስጥራዊነቱ የተጠበቀና ለጥናቱ አላማብቻየ ሞዋል ነው ፡

ስለሞደረገው ጥናት በቂ ግንዛ ቤእግኝቻለሁኝ፡፡ በጥናቱላ ይለሞስተፍ ማሞማጨን እገልጻለሁኝ፡፡ እነ ዲሁምበማንኛውምጊዜ ከጥናቱ ሞጨረጥእንደምቸል አረጋግጣለሁኝ፡፡

ካርድ ቁጥር---------------------------------------ፊርማ---------------------------ቀን--------------
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