Pulmonary Mycobacterium Tuberculosis: *Drug Resistance Pattern and Associated Risk Factors* in Uniformed Service Hospitals of Addis Ababa, Ethiopia

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DEPARTMENT OF MEDICAL LABORATORY SCIENCES

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Abbreviations

Ag	Antigen
AFB	Acid Fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
AST	Antimicrobial Susceptibility Test
BA	Blood Agar
BHIA	Brain heart infusion agar
BMI	Body mass index
DR-TB	Drug-resistant tuberculosis
DST	Drug susceptibility testing
EMB	Ethambutol
EPHI	Ethiopian Public Health Institute
EPTB	Extra-pulmonary TB
FQ	Fluoroquinolones
HIV	Human Immunodeficiency Virus
INH	Isoniazid
LJ	Löwenstein-Jensen
LTBI	Latent TB infection
MDR-TB	Multidrug-resistant tuberculosis,
MGIT	Mycobacteria Growth Indicator Tube
NAAT	Nucleic acid amplification test
PTB	Pulmonary TB
PZA	Pyrazinamide
RIF	Rifampicine
STM	Streptomycin
TB	Tuberculosis
UCSD	University of California San Diego
WHO	World Health Organization
XDR-TB	Extensively Drug Resistant Tuberculosis
ZN	Ziehl Neelsen

Abstract

Background: Tuberculosis remains one of the formidable challenges to global health commitments and drug-resistant tuberculosis poses a major challenge to the control of TB. Members of army, prisoners, police and their families represent a segment of the national population that may be at increased risk for TB infection, disease, and transmission due to service in endemic areas and residence in congregate settings. Thus, there is a collective need determining the epidemiology, drug-resistant patter, and risk factors of tuberculosis among uniformed population for possible interventions.

Objective: The objective of this study was to determine the prevalence, drug-resistance pattern and associated risk factors of pulmonary mycobacterium tuberculosis in uniformed hospitals (Federal police, Defense, and prison administration hospital) in Addis Ababa, Ethiopia.

Methods: A cross sectional study design was performed on eligible tuberculosis suspected patients using a consecutive sampling technique from June to August 2016. Three sputum samples (spot-morning-spot) were collected from each participant and tested for tuberculosis using ziehl neelsen sputum smear microscopy, liquid culture (BACTEC Mycobacterium Growth Indicator Tube 960), and GenXpert MTB Rif diagnostic techniques. Structured questionnaires were used to collect socio-demographic data, Clinical signs and symptoms and other risk factors of the study participants. Data was sorted into working sheet, cleaned and analyzed using Microsoft Excel and the data was extracted by exporting to SPSS version 20.

Results: A total of 167 eligible and volunteer tuberculosis suspected patients were participated, where 109 (65.3%) were male. The mean age was 37 and the dominant age group was 35-44 (41.3%). Sixty-eight (40.7%) of the participants were from Federal police hospital, 39 (22.2%) from Prison hospital and 62 (37.1%) from Armed Force hospital, and 113 (67.7%) of participants live in camps. The prevalence of mycobacterium tuberculosis was 24 (14.4%) using Mycobacterium Growth Indicator Tube liquid culture, 11 (6.6%) in GenXpert and one was smear-negative tuberculosis. One case was identified as multi-drug resistant tuberculosis. The prevalence of tuberculosis was more among smokers, alcohol consumers and under weights (under 18.5 body mass index) with 72.7%, 63.6%, and 36.4%, respectively. History of contact

with known tuberculosis patient, cigarette smoking, malnutrition and alcohol abuse had significant association with mycobacterium tuberculosis at P = 0.00 and HIV at P = 0.014.

Conclusion: There is high prevalence of tuberculosis in uniformed hospitals, indicating possible transmission of TB especially within those living in the camp and prison. The study documented a number of risk factors which could facilitate exposures to tuberculosis infection. Thus, strong cooperation between uniformed authorities and the national tuberculosis control program is required to develop locally appropriate interventions potential for reducing tuberculosis transmission in members of army, prisoners, police and their families in Ethiopia.

1. Introduction

1.1. Background

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis*. It usually affects the lungs (pulmonary TB) but also other sites as well (extra-pulmonary TB). It transmits through the air when a person with PTB expels the bacteria. Most of the time, a relatively small proportion of people infected with M. tuberculosis will develop TB disease. But peoples with HIV develop TB disease much higher than others. TB is also more common among men mainly adults in their most economically productive age (1). It causes ill-health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide, after HIV. In 2012, 8.6 million new TB cases and 1.3 million deaths occurred (2). Health system weaknesses, lack of effective regimens and other treatment challenges are responsible for unacceptably low cure rates (1). The global burden of death and disease caused by TB is concentrated especially in low-income countries. Sub-Saharan Africa, including Ethiopia, is an area with high prevalence of TB infection. According to the World Health Organization 2013 report (2), Ethiopia ranks seventh among the world's 22 countries with a high tuberculosis burden.

Multi-drug resistant tuberculosis (MDR-TB) poses a major challenge to the control of TB. From a microbiological perspective, resistance is caused by a genetic mutation that makes a drug ineffective and this allows drug resistant mutants to become the dominant strain in a patient infected with TB (3, 4, 5). Programmatically, the treatment strategy need an adherence in which the patient follow a prescribed regimen by making themselves available or admitted and are less to develop MDR TB (6). Globally, the success rate of MDR-TB was 48%. Five of the 27 high MDR-TB burden countries achieved a treatment success rate of ≥70%: Ethiopia, Kazakhstan, Myanmar, Pakistan and Viet Nam. MDR-TB is human-made, which results from treatment with inadequate drugs or drug regimens, improper case management, and preventable transmission, and its presence generally reflects weak tuberculosis control (7). It requires a longer duration of therapy with close monitoring and specialized treatment facilities. Patients remain infectious for longer periods and the MDR-TB causes accelerated disease leading to substantial mortality. The average treatment duration is longer than that of drug susceptible TB. This makes adherence to

treatment more challenging to achieve (8). If patients who have TB are not promptly diagnosed, treated and tested for drug susceptibility, resistance may be detected too late to permit a cure. And also an inadequate treatment with second-line drugs may result in XDR-TB (9).

The proportion of new cases with MDR-TB was 3.5% in 2013 worldwide and has not changed compared with recent year and it is a growing threat to national TB programs world-wide, like to XDR-TB, which decreases chances of survival and success further (10). Over 48 countries have reported at least one case of XDR TB (11) and on average, an estimated 9% of patients with MDR-TB had XDR-TB (12). In 2013, 58% of the 4.9 million PTB patients notified globally were bacteriologically confirmed via a WHO recommended test, including rapid tests such as Xpert MTB/RIF (13). The uniformed service members deployment and living condition was reported to have greater risk of TB and MDR-TB than other people (14, 15).

1.2. Statement of the problem

TB remains one of the world's fatal communicable diseases. In 2015, an estimated 10.4 million people developed TB and 1.8 million died from the disease, 0.4 million of whom were HIV-positive (16). Despite the availability of effective chemotherapy and ability to prevent and treat, TB remains a major health problem in most of the countries of the world (17) and represents more than a quarter of the world's preventable death (18). TB and HIV co-infection and the exponential increase in drug resistance are greatly responsible for the resurgence of TB (19).

MDR-TB is a critical problem worldwide (20); with more than 400,000 cases of MDR-TB emerge every year globally (21). MDR-TB complicates management of patients due to increased pressure on public health systems and cost of the treatment. It further aggravates the emergence of XDR TB, defined as MDR-TB plus resistance to a fluoroquinolone and at least one of the three second-line injectable drugs (Amikacin, Kanamycin or Capreomycin) (22). The fact that XDR-TB requires longer, more expensive and more toxic treatment regimens, that at the same time are less likely to cure the disease (22), further worsens the situation. Inappropriate or inadequate TB treatment programs are assumed to be responsible for generating MDR-TB, and TB patients with a history of previous treatment are as much as 10 times more likely to have MDR disease than those without previous TB therapy (23).

Members of army, prisoners, police and their families represent a segment of the national population that may be at increased risk for TB infection, disease, and transmission due to service in endemic areas and residence in congregate settings, particularly in countries with a high incidence of TB. The extent of TB in prisons, which is much greater than in the community, is often underreported. The high number of imprisoned persons, extremely overcrowded conditions, poor ventilation, late case detection, and the lack of respiratory isolation and inadequate treatment of infectious cases, high turnover of prisoners through repeated transfers within the prison system, release and recidivism and poor general health of inmates facilitate the spread of tuberculosis (24). MDR-TB may also be more common in prison settings as factors that encourage transmission of regular TB will enhance the spread of MDR-TB (24). Prisons represent a high risk setting for development and transmission of MDR-TB. In many countries where the incidence of TB is very high, prisons have been found to play a significant role in the epidemiology of DR-TB (25).

In Africa, TB in prisons is not well documented. However, according to the 2005 WHO report, the average TB incidence and prevalence rates of TB in sub-Saharan Africa, where Ethiopia is also located, was 363/100,000 and 475/100,000 respectively (26). A study in Botswana showed higher prevalence of smear-positive PTB in prisons compared to the civilian population (27) that shows more has to be done on associated risk factors and drug resistance profiles for better MTB management and prevention.

Lack of effective diagnosis and treatment challenges are responsible for unacceptably low cure rates and spread of MDR-TB and XDR-TB. Lack of data in Ethiopian uniformed service members (police, military and prison administration) on MTB prevalence, drug resistance profile and associated risk factors is a great problem to scale up management of TB. Therefore, this study will try to fill the gap and can be used as a bench mark for further studies.

1.3. Significance of the study

- Evaluating and assessing the prevalence and associated risk factors which will have significant importance to ensure that individuals with TB signs and symptoms are correctly diagnosed and get better treatment and also it is a key to control and prevent drug resistance based on identified risks associated with the occurrence of drug resistant tuberculosis.
- To determine the yield of drug resistance TB using DST and geneXpert methods and it is advantageous for scaling up an efficient programmatic management and surveillance of drug-resistant TB.
- Magnify the use of prompt detection of TB among prisoners, police and military recruits which are a good preventive and controlling mechanism and it should be ensured through a combination of screening methods using well structured questionnaires and different type of diagnostic methods. This will make an excellent diagnostic and treatment success.
- To fill the gap of document in availability on MTB prevalence, drug resistance profile and associated risk factors and to put a bench mark for future studies.

2. Literature review

2.1.Prevalence of PTB

Ethiopian federal ministry of health 2009 report showed that the first patients were admitted for MDR TB treatment in Ethiopia in 2009 in rehabilitation isolation ward in St. Peter's hospital in Addis Ababa. In the same year 45 MDR TB patents were enrolled initially in the second phase at St. Peter's hospital. Therapy was initiated on 811 MTB patients in 2012, of this 9 of them were XDR-TB (29). A study done in North Gondar Prison, Ethiopia, show high prevalence of TB with possible active transmission within the prison and the point prevalence of smear positive TB was 1482.3 per 100,000 populations which was 9.1 times higher than the TB in the general population (30).

The prevalence of tuberculosis recorded in Zambian prisons in 2011 is 18 times the national prevalence estimate of 0.35% (28) and even the TB incidence rate in Rio de Janeiro State prisons, Brazil, was 30 times higher in 2004 than in the general population (31). Study done in the active component of United States military showed that the rate of TB disease is 0.6 per 100,000 population (32), which is very close to the Centers for Disease Control and Prevention's goal for TB elimination, defined as less than one per million (33). Study done in Turkish Armed Force showed that 629 conscripts with TB were detected. Of these, 574 were aged between 20-24 years and 392 were smear-positive. The incidences of TB and smear-positive cases in Turkish Armed Force conscripts were calculated at respectively 76 and 47 per 100,000 populations (34).

Study done on MDR- PTB among 148 young Korean soldier patients showed that MTB resistant to at least one drug was isolated from 18 patients (12.2%), Drug resistance rates were higher in previously treated cases (24.1%) compared to new cases (9.2%). MDR-TB was isolated from 12 patients (8.1%). The study also showed that previous treatment of tuberculosis was a significant independent risk factor for MDR TB. However, military rank, smoking, positive AFB smear and cavity on chest computerized tomography were not associated with development of MDR TB (35)

Research on PTB and DR in Dhaka Central Jail, the Largest Prison in Bangladesh showed that among 1,781 TB suspects 245 (13.8%) were positive for AFB on microscopy and/or culture. The

prevalence rate of sputum- positive PTB was 2,227/100,000. 53 cases (21.6% of 245 cases) were AFB negative on microscopy but were found positive on culture and drug resistance profile to isoniazid, rifampicin, streptomycin and ethambutol was 11.4%, 0.8%, 22.4% and 6.5% respectively (36)

A systematic literature review done on MDR-TB epidemiology and genetic diversity in East Africa from 1997-2012 shows a prevalence range from 0.4% in Tanzania to 4.4% in Uganda among new cases and from 3.9% in Tanzania to 17.7% in Uganda among recurrent cases (37).

A nested study done on Isoniazid prophylaxis for tuberculosis prevention among HIV infected police officers in Dar es Salaam showed that the prevalence of PTB among asymptomatic HIV-1 infected Police Officers was 21.6% (38).

2.2. Associated risk factors for MTB infection

A cross sectional study done in Amhara national regional state, Ethiopia, from 2012-2013 on 606 presumptive MDR-TB cases screened showed that the Age at range of 21-30 years old, being female and TB history of defaulters were significantly associated with having MDR-TB. the prevalence of MDR-TB was 15.3% , rifampicin and isoniazaide mono resistance were 2.8% and 2.5% respectively. (39).

A case control study done in Addis Ababa on patients who underwent first-line treatment showed the factors that were significantly associated with MDR-TB and these were drug side effects during first-line treatment; treatment not directly observed by a health worker; interruption of treatment of at least a day; duration of treatment between 2 and 7 months; and retreatment with the Category II regimen. But in their study, HIV infection was not significantly associated with the occurrence of MDR-TB (40).

A meta-analysis study done on Sub Saharan Africa shows the risk of developing DR-TB to at least one anti-TB drug was about 3 times higher in individuals who had a previous history of anti- TB treatment than new TB cases and the risk of having MDR-TB in previous anti-TB treated TB case was more than 5 fold higher than that of a new TB cases (41).

A nationwide MDR-TB survey conducted in Uzbekistan from 2010 - 2011 and 1037 patients were included. MDR-TB was detected in 165 treatment-naïve and 207 previously treated

patients. MDR-TB was significantly associated with age under 45 years, imprisonment, previous treatment and not owing a home (42).

2.3. Drug resistance profile

In 2008, there were an estimated 440,000 incident MDR TB cases globally; among those tested, 5.4% of MDR-TB patients were XDR-TB (43). A DR-TB surveillance done by WHO in 2013 shows that 35% of new cases and 75% of previously treated cases have MDR-TB in Eastern Europe and central Asian countries (44).

Study done in northern Karnataka region, India, showed that ZN staining for AFB was positive in 113 patients (75%), while AFB culture by LJ medium yielded growth in 66 cases (44%). Thus, a total of 66 AFB culture-positive samples by LJ medium were subjected for DST. DST was done for Isoniazid, Rifampicin, Pyrazinamide, Ethambutol and Streptomycin after isolation by using the resistance proportion method. From a total of 66 AFB culture-positive specimens, 20 (30.3%) cases were sensitive to all the five drugs while 46 (69.7%) cases showed resistance to one or more drugs. Among these, the resistance to RIF was highest (80.4%), while resistance to INH, PZA, EMB and STM were observed to be 60%, 58.7%, 52.1% and 63%, respectively (45).

A cross sectional study in South Africa in 2008 done on 1317 children, DST was undertaken in 148 of the 204 children who had culture confirmed TB. The prevalence of Isoniazide-resistance was 14.2% and the prevalence of MDR-TB was 8.8% (46).

Studies conducted in Ethiopia from 1984 to 2001 shows the initial resistance to isoniazid from 2% to 21% and streptomycin from 2 to 20%. MDR-TB was also reported in about 1.2% of new cases and 12% of re-treatment cases but in all studies which included ethambutol susceptibility test, ethambutol resistance is either nil or very low (below 0.5%) (47). A drug resistance study done in southwestern Ethiopia from 136 TB patients shows, Resistance to at least one drug was 18.4% (INH-13.2% and STM-8.1%). But there was no statistically significant difference by sex, age, HIV status and history of being imprisoned. Mono resistance against INH is 7.4% and MDR-TB was observed in two patients (1.5%) (48). A primary DR to anti-TB study done in Amhara region, Ethiopia, showed that from a total of 112 MTB isolates 93 yielded valid DST results and 28 (30.1%) were resistant to one or more of first line anti-TB drugs. One isolate

(1.0%) was MDR, five (5.4%) were classified as poly-resistant and 22 showed single drug resistance to either STM (n = 19) or INH (n = 3) (49).

3. Objective of the study

3.1. General objective

To determine prevalence, drug resistant pattern and associated risk factors of pulmonary mycobacterium tuberculosis among Federal police, defense and prisoners at uniformed service hospitals in Addis Ababa, Ethiopia.

3.2. Specific objectives

- To determine the prevalence of pulmonary tuberculosis in the study sites
- To determine drug resistance profile of MTB in the study sites
- To compare the yield of drug resistance TB using MGIT and gene Xpert methods
- To determine the associated factors for MTB infection in the study sites

Hypotheses

There is no difference in MTB and DR TB prevalence with this study and other studies

4. Materials and Methods

4.1. Study design

This research followed a cross sectional study design.

4.2. Study setting and period

The study has taken place in Federal police, defense and prison hospitals from June to August 2016.

Ethiopian federal police referral hospital was established in 1963 and one of the 2 police hospitals which is located in Addis Ababa to serve as a referral hospital for all police members and their family. It also serves as a referral from different police clinics located in addis ababa and the laboratory give service for about 10 TB patients per week.

Armed force general and teaching hospital is located in Addis Ababa to give service for military officers and their family with their different clinics. The laboratory gives diagnostic service for about 8 TB patients per week.

Federal prison administration general hospital is located in Addis Ababa and gives medical service to prisoners, prison police officers and their family. About 5 patients diagnosed for TB per week and also it serves as a referral hospital for patients from Zeway, Shewa Robit and Deredawa prison administrations eventhogh they have their own medical centers within their sites.

4.3. Source population

The source population of this study consisted of all out and in-patients coming to Police, defense and prison hospitals during the study period.

4.4. Study population

All TB suspected patients who fulfilled the inclusion criteria

4.5. Study variables

- Dependent – MTB prevalence, drug resistance profiles

- Independent – educational status, age, sex, living condition, contact history, HIV, diabetics, smoking, alcohol abuse

4.6. Sample size and sampling method

The sample size was calculated as follows

$$n = Z_{1-\alpha/2}^2 \frac{P(1-P)}{d^2}$$

Where: $Z_{1-\alpha/2}$ = is standard normal variant (at 5% type 1 error ($p<0.05$) is 1.96

P = 10.4% prevalence was used from a prier research done in North Gondar prison (31)

d = absolute error or precision (5% = 0.05)

Therefore the sample size with 15% contingency was, n= 167

A consecutive sampling technique was performed. Therefore, all TB suspected patients who were sent to TB clinic for sputum laboratory examination during the study period and fulfilling the inclusion criteria were included in the study.

4.7. Inclusion and exclusion criteria

4.7.1. Inclusion criteria

- All patients of above or equal to 15 years age came to laboratory for AFB test as many children do not cough and produce sputum effectively compared with adults and sputum smears from children are more likely to be negative (50).
- New and re-treatment patients suspected for MTB
- Those who volunteer to give adequate amount and triple sputum samples

4.7.2. Exclusion criteria

- Patients who are on TB treatment
- Those who are suspected for extra pulmonary TB

4.8. Data collection

4.8.1. Socio demographic and other clinical data

Well-structured questionnaires were used to collect socio-demographic data of the study participants. Clinical signs and symptoms of study participants and other risk factors were also collected by a physician and assigned technician attending each hospital (Annex 1 and 2).

4.8.2. Specimen collection

Three sputum samples (spot-morning-spot) were collected in containers that are sterile, clear, plastic, leak-proof and screw cap from each participant. Either two on-the-spot samples with one early morning specimen (from out-patients) or three early morning samples (from admitted patients)

Three receptionists and three runners were recruited for collection, handling and transportation of samples from clients of outpatient and inpatient from different wards. The technologist /technicians assigned for normal routine work got a familiarizing min-orientation for two consecutive afternoons (two half days) about the aim of study, how it is going to be done, techniques and materials to be used in all methods (ZN, Culture and GeneXpert MTB/RIF) to work together for every steps in accordance to the normal work procedure.

Voluntary patients were informed to take deep breaths through their mouth to get mucous from deep in their chest; Opening the cup and holding it close to their mouth; Spit the mucous into the cup without getting any on the outside of the cup and then Screw the lid on tightly to prevent leakage. Only intending participants with at least two sputum samples positive for AFB and negative after 3 consecutive sputum samples were enrolled into the study. This was conformed with the WHO's standard definition of a smear positive TB case which stipulates at least two initial sputum smear examinations (direct smear microscopy) positive for AFB (51). Therefore, smear microscopy positive and negative sputum specimens were transported in cold-box for further analysis.

4.8.3. Laboratory methods

The study included different laboratory testing procedures:

4.8.3.1. Zehel Nelseen AFB sputum smear microscopy

In Federal police, Defense and prison hospital laboratories, ZN smear microscopy for AFB was carried out. The air dried smear was treated with carbol fuschin, acid alcohol and methylene blue for 5, 3 and 1 minutes respectively, after complete slide wash with water in each step. Using with oil immersion, Acid-fast bacilli were seen as a bright red and rod shaped, slender rods, some time with one or more granules. The bacilli may occur singly, V-shaped forms, cords, or as clumps, and fragments of bacilli often seen during treatment (52, 53) (See Annex 5).

4.8.3.2. GenXpert MTB Rif

In Prison Administration General Hospital Laboratory and Federal Police Refferal Hospital Laboratory the sputum specimen was transferred into the MTB/RIF cartridge and entered into the GeneXpert instrument. By starting the test on the system software, the GeneXpert automates all following steps, including sample workup, nucleic acid amplification, detection of the target sequence and result interpretation. Xpert MTB/RIF result can indicate that MTB was not detected; MTB detected and rifampicin not resistant or MTB detected and resistant to rifampicin. (54) (See Annex 5).

4.8.3.3. MGIT culture and AST

EPHI is one of recognized reference centre for the research and isolation of Mycobacterium tuberculosis in Ethiopia. At EPHI, the samples were mixed with equal volume of 4% sodium hydroxide and sodium citrate for digestion and decontamination for liquid culture, MGIT, process. The BACTEC™ MGIT™ 960 System is designed for the rapid detection of mycobacteria in all types of clinical specimens except blood and urine. The system includes a liquid culture medium (BBL™ MGIT™ Mycobacteria Growth Indicator Tube), a growth supplement and an antibiotic mixture (BBL™ MGIT™ PANTA™). The BACTEC™ MGIT™ Growth Supplement provides substances essential for the growth of mycobacteria. BBL MGIT PANTA contains a mixture of antimicrobial agents used to suppress the growth of contaminating bacteria. A fluorescent compound is embedded in silicone on the bottom of each of the MGIT broth tubes. This compound is sensitive to the presence of oxygen dissolved in the broth. Initially, the large amount of dissolved oxygen quenches the emissions from the compound and little fluorescence can be detected. Later, actively respiring microorganisms consume the oxygen and allow the fluorescence to be detected. MGIT™ 960 System monitors the tubes for increasing

fluorescence. Analysis of the fluorescence is used to determine if the tube is instrument-positive; i.e., the test sample contains viable organisms. Culture tubes which remain negative for a minimum of 42 days (up to 56 days) and which show no visible signs of positivity are removed from the instrument as negatives (55) (See Annex 5).

Susceptibility testing in the MGIT 960 system is based on the same principles as isolation from sputum (detection of growth). DST is performed using an AST (antibiotic susceptibilitytesting) set or TB exit, which consists of a Growth Control tube and one tube for each drug. A known concentration of drug is added to a MGIT tube, along with the specimen, and growth is compared with a drug-free control of the same specimen. If the drug is active against the mycobacterial isolate (isolate susceptible), growth will be inhibited and fluorescence will be suppressed in the drug-containing tube; meanwhile, the drug-free control will grow and show increasing fluorescence. If the isolate is resistant, growth and its corresponding increase in fluorescence will be evident in both the drug-containing and the drug-free tube. The MGIT 960 system monitors these growth patterns and can automatically interprets results as susceptible or resistant. An isolate is defined as resistant if 1% or more of the test population grows in the presence of the critical concentration of the drug (55, 56). (Annex 5)

4.8.3.4. TB Ag rapid test and Blood agar

TB Ag rapid test and BA culture were performed on MGIT positive samples for identification and differentiation between MTB and Other Mycobacterium (non-tuberculosis mycobacteria (NTM)) after the sputum culture but before DST was performed. The SD Bioline TB Ag MPT64 Rapid test is an immunechromatographic method which can detect MPT64 antigens produced by M. tuberculosis complex (57) and also on BA/BHI, hence any growth on the BA/BHI plate after a period of 24 hours is considered as a contamination. If there are growth, it is due to other fastidious microorganisms not necessarily Mycobacterium tuberculosis (Annex 5).

4.9. Data management and analysis

Data was sorted into working sheet, cleaned and analyzed using Microsoft Excel and the data was extracted by exporting to SPSS version 20 (Armonk, NY: IBM Corp.) Then calculated and analyzed.

4.10. Data quality assurance

4.10.1. Validity of the laboratory where tests were done

The study findings can be significant not only for prisoners, police and army members and their family but also for the public at large to control and prevent TB drug resistance. The study will also have a diverse population which is more likely to represent the national population. ZN used to differentiate AFB from sputum smear microscopy samples has sensitivity of about 50–60% when compared with culture and the new diagnostic test Xpert MTB/RIF sensitivity when used as an initial test in place of smear microscopy was found to be 89% with a specificity of 99%. For detecting rifampicin resistance the sensitivity was 95% and specificity of 99% and it is rapid and can give result with in about two hours (58). MGIT culture medium serves as a gold standard.

4.10.2. Reliability of the results

The reproducibility of a laboratory result was checked with senior laboratory technologists/technicians to arrive at similar judgment with in predefined bounds. Standardized data collection and transporting materials like sterile, clear, leak-proof and screw cap sputum collecting containers, clear and easily understandable questionnaires and cold-boxes was used with all safety precautions.

4.11. Result dissemination

The study report was submitted to the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, EPHI, uniformed service hospitals and Addis Ababa regional health bureau. Results of this finding will also be sent to peer reviewed journal for publication and will be presented in annual conferences.

4.12. Ethical consideration

The study was approved by departmental research and ethics review committee (DRERC) of the Addis Ababa University, Department of Medical Laboratory Science. Official letters were written to the participating hospitals. Consent forms were prepared in English and Amharic to be read and signed (when agreed) by the voluntarily participating patients. Information obtained about voluntarily participating patients from the questionnaires, data capturing formats and from

their sputum will be totally kept anonymous. Participants have the right not to participate or withdraw from the study any time whenever they want. But whenever the result was beneficiary to the patient, the information was communicated to the appropriate health care workers.

5. Results

5.1. Socio demographic characteristics of study participants

A total of 167 eligible and volunteer PTB suspected patients were participated. The age distribution of the participants show that the mean age was 37 and the dominant age group was 35-44 years (41.3%) and more than half of participants (65.3%) were male. Sixty-eight (40.7%) of the participants were from Federal police hospital, thirty-nine (22.2%) were from Prison hospital and sixty-two (37.1%) were from Armed force hospital. Majority of participants 113 (67.7%) live in the camp and from them 20 (17.7%) were MGIT positive, 10 (8.8%) GeneXpert positive and one of it was Rifampicine resistant. The majority of study participants were 99(59.3%) married; 125(74.9%) had college/university educational background,. 122(73.1%) were employed (See Table 5.1).

Table 5.1- Demographic characteristics of participants in Uniformed Service Hospitals, Addis Ababa, Ethiopia, from June-August, 2016

Demographic characteristics	Response category	Number	Percent
Age in year	15-24	14	8.4%
	25-34	49	29.3%
	35-44	69	41.3%
	45-54	28	16.8%
	55-64	7	4.2%
	Total	167	100%
Sex	Female	58	34.7%
	Male	109	65.3%
	Total	167	100%
Facility	Federal police hospital	68	40.7%
	Armed force hospital	62	37.1%
	Prison hospital	37	22.2%
	Total	167	100%
Marital status	Married	99	59.3%
	Single	65	38.9%
	Divorced	3	1.8%
	Total	167	100%
Residence/Living condition	Own/Rented House	54	32.3%
	Camp/Dormitory	113	67.7%
	Total	167	100%
Educational status	College/University	125	74.9%
	Primary/Secondary school	32	19.2%
	No formal education	10	6.0%
	Total	167	100%
Employment	Employed	122	73.1%
	Unemployed	45	26.9%
	Total	167	100%

5.2. Clinical characteristics of study subjects

All the study participants had cough duration for more than or equal to two weeks and from those 19 (11.4%) had blood in their sputum. 96 (57.5%), 26 (15.6%), 66 (39.5%), 29 (17.4%), 7 (4.2%) of all study participants had chest pain, weight loss, fever, sweating at night, history of contact with known TB patient respectively. There was no TB treatment history from all study participants (See Table 5.2).

Table 5.2- Clinical characteristics of study subjects and Associated Risk Factors in Uniformed Service Hospitals, Addis Ababa, Ethiopia, from June-August, 2016

Clinical characteristics	Response category	Number	Percent
Cough duration for more than or equal to 2 weeks	Yes	167	100%
	No	0	0%
	Total	167	100%
Cough up Blood	Yes	19	11.4%
	No	148	88.6%
	Total	167	100%
Chest pain	Yes	96	57.5%
	No	71	42.5%
	Total	167	100%
Weight loss	Yes	26	15.6%
	No	141	84.4%
	Total	167	100%
Fever	Yes	66	39.5%
	No	101	60.5%
	Total	167	100%
Sweat at night	Yes	29	17.4%
	No	138	82.6%
	Total	167	100%
Contact history with known TB patient	Yes	7	4.2%
	No	160	95.8%
	Total	167	100%
TB treatment history	Yes	0	0%
	No	167	100%
	Total	167	100%

5.3. Co-morbidity and Behavioral characteristics of study participants

Among 167 patients participated in the study 2(1.2%) has responded as they had HIV, Six (3.6 %) participants are diabetic mellitus patients. underweight status shows that four (2.4%) are underweight (under 18.5 BMI) but all participants had no chronic renal failure, organ transplantation history and had no history of using Chemotherapy and Immunosuppressant drugs. (See Table 5.3)

Table 5.3- Co-morbidity and Behavioral characteristics of study participants in Uniformed Service Hospitals, Addis Ababa, Ethiopia, from June-August, 2016

Type of co morbidity	Response category	Number	Percent
HIV	Yes	2	1.2%
	No	155	92.8%
	Unknown	10	6.0%
	Total	167	100%
Diabetic mellitus	Yes	6	3.6%
	No	156	93.4%
	Unknown	5	3.0%
	Total	167	100%
Underweight less than 18.5 BMI	Yes	4	2.4%
	No	163	97.6%
	Total	167	100%
Cigarette smoking	Yes	29	17.4%
	No	138	82.6%
	Total	167	100%
Alcohol abuse	Yes	19	11.4%
	No	148	88.6%
	Total	167	100%
Chronic renal failure	Yes	0	0%
	No	167	100%
	Total	167	100%
Immunosuppressant drug/Chemotherapy	Yes	0	0%
	No	167	100%
	Total	167	100%
Current Organ transplantation history	Yes	0	0%
	No	167	100%
	Total	167	100%

5.4. Risk factors for MTB

The majority of MTB positive participants were between the age group of 35-44 (45.8%) and 25-34 (29.2%). Seventeen (70.8%) of them were male, and also the prevalence distribution shows 54.2%, 33.3% and 12.5% for federal police, armed force and prison hospitals participants during the study period. Based on their living status, those who live in camp/dormitory account 20 (83.3%) of all positive study participants and 7 (29.2%) had prier close contact with known TB patient. 4(16.7%), 11 (45.8%) and 12 (50%) were under weight (less than 18.5 BMI), cigarette smokers and alcohol consumers respectively. The risk factor association analysis shows that

contact history, underweight, cigarette smoking and alcohol abuse has significant association (P=0.000) and HIV had also significant association with MTB prevalence (P=0.014) (See Table 5.4)

Table 5.4 - Risk factors of MTB disease in Uniformed Service Hospitals, Addis Ababa, Ethiopia, from June- August, 2016

Risk Factors	Response category	Number	Percent	P value
Age in year	15-24	0	0%	
	25-34	7	29.2%	0.419
	35-44	11	45.8%	
	45-54	5	20.8%	
	55-64	1	4.2%	
	Total	24	100%	
Sex	Female	7	29.2%	
	Male	17	70.8%	0.539
	Total	24	100%	
Facility	Federal police	13	54.2%	
	Armed force	8	33.3%	0.114
	Prison hospital	3	12.5%	
	Total	24	100%	
Marital status	Married	14	58.3%	
	Single	8	33.3%	0.457
	Divorced	2	8.3%	
	Total	24	100%	
Residence/Living condition	Own/Rented House	4	16.7%	
	Camp/Dormitory	20	83.3%	0.077
	Total	24	100%	
Educational status	College/University	18	75%	
	Primary/Secondary	5	20.8%	0.858
	No formal education	1	4.2%	
	Total	24	100%	
Employment	Employed	20	83.3%	
	Unemployed	4	16.7%	0.222
	Total	24	100%	
Contact history with known TB patient	Yes	7	29.2%	
	No	17	70.8%	0.000
	Total	24	100%	
TB treatment history	Yes	0	0%	
	No	24	100%	-
	Total	24	100%	
HIV	Yes	2	8.3%	
	No	16	66.7%	0.014
	Unknown	6	25%	
	Total	24	100%	
Diabetic mellitus	Yes	3	12.5%	

	No	18	75.0%	0.911
	Unknown	3	12.5%	
	Total	24	100%	
underweight less than 18.5 BMI	Yes	4	16.7%	
	No	20	83.3%	0.000
	Total	24	100%	
Cigarette smoking	Yes	11	45.8%	0.000
	No	13	54.2%	
	Total	24	100%	
Alcohol abuse	Yes	12	50%	0.000
	No	12	50%	
	Total	24	100%	
Chronic renal failure	Yes	0	0%	
	No	24	100%	-
	Total	24	100%	
Immunosuppressant drug	Yes	0	0%	
	No	24	100%	-
	Total	24	100%	
Current Organ transplantation history	Yes	0	0%	
	No	24	100%	-
	Total	24	100%	

5.5. MTB Prevalence

Prevalence of MTB among 167 study participants using conventional AFB smear microscopy was 10(6.0%) while it was 11(6.6%) using concentrated AFB smear microscopy from MGIT positive tubes. MGIT MTB prevalence shows 24 (14.3%) and GeneXpert show 11(6.6%) MTB prevalence Rifampicin resistance was 1(4.2%) (See table 5.5).

Table 5.5 - MTB prevalence using three different TB detection methods (AFB, MGIT and GenXpert MTB Rif) in Uniformed Service Hospitals in Addis Ababa, Ethiopia from June – August, 2016.

Methods Employed	Result	Number	Percent
Ziehl Neelsen			
Conventional AFB smear microscopy	Positive	10	6.0%
	Negative	157	94.0%
	Total	167	100%
Concentrated AFB smear microscopy (from positive MGIT tubes)	Positive	11	6.6%
	Negative	156	93.4%
	Total	167	100%
MGIT	Positive	24	14.3%
	Negative	143	85.1%
	Total	167	100%
GeneXpert MTB Rif	Positive and Rifampicin resistant	1	0.6%
	Positive and Rifampicin not resistant	10	6.0%
	Total positive	11	6.6%
	Negative	156	93.4%
	Total	167	100%

5.6. Drug resistance pattern

5.6.1. MGIT drug resistance profile

Drug resistance pattern done by MGIT drug sensitivity test shows that one (4.2%) was MDR TB which was resistant to rifampicin, isoniazid and ethambutol and the other two (8.3%) were resistant to streptomicine and isoniazid (See table 5.6.1)

Table 5.6.1 - Drug resistant profiles of MTB isolates using MGIT DST in Uniformed Service Hospitals, Addis Ababa, Ethiopia from June-August, 2016

Drug Resistance	Rif 1.0g	INH 0.1g	STM 1.0g	EMB 5.0g	# MTB (+)	Prop. DR-TB from MTB (+)
Mono drug resistance						0%
Two drug resistance		2	2		2	8.3%
Three drug resistance	1	1		1	1	4.2%
Four drug resistance						0%
Total	1	3	2	1	3	12.5%

5.6.2. GenXpert drug resistance profile

GeneXpert result showed that one (4.2%) was MTB positive and rifampicine resistant, 10 (41.7%) MTB positive and rifampicine not resistant and 13 MGIT positive isolates were GeneXpert negative (see table 5.6.2)

Table 5.6.2 GenXpert drug resistance profile in Uniformed Service Hospitals, Addis Ababa, Ethiopia from June-August, 2016

GeneXpert Rifampicine drug resistance	# of isolates	Prop. GenXpert (+) from MGIT (+)
GeneXpert (+) & rifampicine resistant	1	4.2%
GeneXpert (+) & rifampicin susceptible	10	41.7%
Total	11	45.9%

6. Discussion

Currently, the emergence of MDR and XDR (extensively drug-resistant) tuberculosis in the world is becoming a major health problem (59). According to 2016 WHO global TB report, worldwide the rate of decline in TB incidence remained at only 1.5 % from 2014-2015 and In 2015, there were an estimated 10.4 million new (incident) TB cases worldwide, of which 5.9 million (56%) were among men, 3.5 million (34%) among women and 1.0 million (10%) among children. People living with HIV accounted for 1.2 million (11%) of all new TB cases. Six countries accounted for 60% of the new cases: India, Indonesia, China, Nigeria, Pakistan and South Africa.

This study investigated the prevalence, drug resistance patterns and risk factors of Mycobacterium tuberculosis in the uniformed service hospitals (federal police, armed force and prison hospitals) from June to august 2016. In our study the prevalence was 24(14.4%) using MGIT liquid culture used as a gold standard, 11(6.6%) using GenXpert MTB Rif and ZN AFB concentrated smear microscopy methods and 10 (6%) by ZN AFB smear microscopy from 167 MTB suspected participants and it was higher than the result from the study done in Dhaka Central Jail, the Largest Prison in Bangladesh with the prevalence of 13.8% from 1,781 TB suspects using culture (36) ; and a study done in eastern Ethiopian prison from 371 PTB suspects, 22(5.9%) were new TB positives (60).

In our study, we found that the prevalence of MTB was high among males 17(70.8%) than females 7 (29.2%) but there was no significant association (P=0.539) and it agrees with the study done in Gombe state, Nigeria, where males had the highest prevalence of TB infection with 687 (54.96%) than females with 563 (45.04%) but there was significant association between sex and TB prevalence (61). Another study done on suspected PTB patients undergoing smear microscopy also shows that AFB sputum smear positive cases were less among female patients with female to male ratio of less than one (0.84/1.0) (62). A population based national TB prevalence survey among adults (> 15 years) in Pakistan from 2010 – 2011 showed that TB prevalence was 1.8 times higher among men than women. (63). But a study done in Afghanistan disagree with our result where PTB are higher among females than among males (64). Women

who are co-infected with TB and HIV are significantly more likely to die of TB than co-infected men. (65)

The prevalence of MTB in relation to age group showed that 35-44 years (45.8%) had higher prevalence followed by 25-34 years (29.2%) and 45-54 years (20.8%). Low MTB prevalence was seen in the age group of 55-64 years (4.2%). But there was no significant association between age group and MTB prevalence (P=0.419). This agrees with a TB nationwide prevalence survey done in the Gambia, 2012. Which showed that TB prevalence was higher in the 35-54 years age group (355, 95%CI: 219-490) (66). While another TB prevalence study in Rwanda showed that most of the positive patients were within the age group of 15-44 years and the highest percentage of TB was seen in the age group of 15-24 years (67).

Based on marital status, TB prevalence was high among married study participants 14(58.3%) followed by singles 8(33.3%) and the divorced had 2(8.3%) MTB prevalence. According to our result there was no statistically significant association between marital status and MTB prevalence (P=0.457). This agrees with the study done in Nepal, where the prevalence of PTB was higher on married (68). Significant association was seen between marital status and MTB prevalence on a study done in Amhara region, Ethiopia (69). Another study done in Aminu kano teaching hospital, north Nigeria also showed than there was a significant association between marital stastus and MTB prevalence (70). And also another study in Ambo hospital, west Ethiopia showed that marital status had significant association with MTB prevalence (71).

The prevalence of MTB based on educational status showed that those who had tertiary (college, university) educational background had more prevalence 18(75%) than those who had primary and secondary school educational background 5(20.8%) while those who had no formal educational background were 1(4.2) and there was no statistically significant association between educational status and MTB prevalence in our study. But other studies showed that those individuals with higher educational background were comparatively better informed about TB infection and they were also less likely to experience delays in seeking treatment (72) and also a study done in a primary health center, Zaria, Nigeria, showed that educational status has statistically significant association with very likely chance of detecting AFB in sputum (73). But our result disagrees with a cross sectional study done in Gondar town, north west Ethiopia which showed that study subjects with low educational status were more prone to have PTB (74).

The prevalence of MTB based on residence/living condition showed that those who live in camp/dormitory had high MTB prevalence 20 (83.3%) while those who live in their own/rented house had less MTB prevalence 4 (16.7%). The study also showed that residence/living condition has no sig association with MTB prevalence and it agrees with the study done in Harare, Zimbabwe, which showed that patients infected with a shared spoligotype were more likely to live in overcrowded conditions (75). Another study done in salvadore, Bahia, Brazil, showed that the incidence of TB was higher in neighborhoods with poor living conditions but the association between living condition and TB was not observed (76)

In our study, MTB prevalence based on close contact history with known PTB showed that those who had prier close contact history with known PTB patient had 7(29.2%) MTB prevalence. While, those who didn't had prier close contact with known TB patient was 17(70.8%) and there was significant association between close contact history with known PTB patient and MTB prevalence (P=0.000). This agrees with a cross sectional study done on prisoners in Wolayta zone, southern Ethiopia which showed that PTB was significantly associated with having history of close contact with known PTB patients at home, sharing a cell with a known PTB patient and stay greater than 24 months in current prison (77) and another study indicated that early tracing of symptomatic contacts of MDR TB case could be a high yield strategy for early detection and treatment of MDR TB cases in the community (78).

According to our study, MTB prevalence on those who respond as they don't have HIV in their blood were higher 16(66.7%) than those who respond as they don't know their HIV status 6(25%) and those who had HIV positive status were 2(8.3%). HIV status has significant association with MTB prevalence and a study done in Mexican institute of social security from 2006-2014 showed that PTB and HIV increase the probability of dying during treatment compared to the PTB cases without HIV. But the study indicated that there was no significant association between PTB with HIV and treatment failure (79). Another study on association between HIV/AIDS and MDR TB demonstrated that there was no association between MDR TB and HIV. (105) a study done in Kazakhstan also indicated that HIV positivity was not associated with MDR TB (80).

MTB prevalence based on diabetic status showed that those who respond as they don't have diabetic are higher 18(75%) than unknown status 3(12.5%) and those who respond as they don't

have 3(12.5%). Our study also indicated that there was no significant association between diabetics and MTB prevalence but it disagrees with the study which showed that diabetes was associated with an increase risk of the combined outcomes of treatment failure and death during TB treatment as well as relapse (81). Some studies signify that high prevalence of diabetics among the TB patients compared to general population suggested that screening of diabetes among the diagnosis of TB (82).

According to our study, MTB prevalence based on underweight (BMI less than 18.5) status show that those who were not underweight were higher 20(83.3%) than MTB positive underweight study subjects 4(16.7%) and there was significant association between underweight and MTB prevalence. This agrees with the study which indicated that low BMI is the most influential and significant risk factor for PTB incidence with bivariate test of BMI (83). Another study done in Amritsar city showed that BMI below 18.5 has significant association between TB and concluded as poor nutritional status is associated with unfavorable outcome in TB. (84)

MTB prevalence according to cigarette smoking showed that those study participants who had cigarette smoking habit were 11(45.8%) while non-smokers were 13(54.2%) and a significant association was observed (P=0.000) and it agrees with a study done in India which showed a positive association between tobacco smoking and PTB (85) and another study also indicated that smoking has been found to be associated with both relapse of TB and TB mortality (86). While a study done in Kuwait showed that smoking did not influence sputum smear conversation in tuberculosis. However, as expatriate smokers and smokers with advanced disease showed a delay in smear conversion and smoking should be discouraged in patients with PTB (87).

The prevalence of MTB based on alcohol consumption and dependency showed that half of all MTB positive study subjects were alcohol consumers 12(50%) and the result also showed that alcohol consumption has significant association with MTB prevalence and our result agrees with the study done in Nepal which indicated that alcohol dependency as a risk factor for TB with significant association (88), and another study done in India also revealed that alcoholism is independently associated with PTB with other risk factors such as age, sex and smoking (89).

Drug resistance profile in our study showed that one (4.2%) isolate was MDR TB which was resistant to Rifampicin, isoniazide and ethambutol. Other two (8.3%) isolates were resistant to

streptomycine and isoniazide. A study done in Giza and Cairo on drug resistant pattern in cases of smear positive PTB shows 70.6% of drug resistance to at least one of the first line drugs and from that 31% were MDR TB cases (90) and it was higher than our study result. Another study done in Iran showed that 41 (16.3%) were resistant to at least one drug from out of 252 MTB isolates and the drug resistance profile showed that 30 isolates (12.0%) were resistant to streptomycine followed by isoniazide resistance, 20 isolates (8.0%), rifampicin, 15 isolates (6.0%) and ethambutol, 14 isolates (5.5%) and sixteen (6.3%) MTB isolates were MDR TB (91).

7. Strength and Limitation

7.1. Strength

There have been limited studies conducted in uniformed settings, thus this study fills information gaps in such settings which could be used as a reference for future studies.

7.2. Limitations

The study would have a better power if more specimens were included, but unable due to limited financing of the study.

8. Conclusion

The overall PTB prevalence in this study held in uniformed service hospitals which include federal police, armed force and prison hospitals was high at 14.4%, with possible horizontal transmission of TB, especially within those living in the camp and prison. The study documented a number of risk factors which could facilitate exposures to tuberculosis infection. Thus, strong cooperation between uniformed authorities and the national tuberculosis control program is required to develop locally appropriate interventions potential for reducing tuberculosis transmission in members of army, prisoners, police and their families in Ethiopia.

9. Recommendation

Awareness creation on getting immediate medical support on those who had close contract history with known TB patient, HIV positives and underweight should be done and also behavioral change on cigarette smokers and alcohol abusers should also be done. There should

be a separated room for TB positive members for those who live in camps. Strong cooperation between authorities and the national tuberculosis control programs required to develop locally appropriate interventions to reduce transmission. The impact of implementing TB control measures need to be further studied in order to improve TB control among inmates.

10. References

1. World Health Organization, Global Tuberculosis Report 2014, WHO Geneva, Switzerland, 2014.
2. World Health Organization, Global Tuberculosis Report 2013, WHO Geneva, Switzerland, 2013.
3. NTP. National Guidelines and Operational Manual for Tuberculosis Control. 4th Edition. Dhaka: National Tuberculosis Control Programme, DGHS, MOH&FW. 2009. https://www.scribd.com/doc/57371572/Bangladesh-National-Guidelines-and-Operational-Manual-for-Tuberculosis-Control
4. Iinternational union against tuberculosis and ling disease, clinical trial to test first all-oral MDR-TB treatment regimen, http://www.theunion.org/what-we-do/research/clinical-trials , [accessed May 28, 2015]
5. Törün T, Güngör G, Ozmen I, Bölükbaşi Y, Maden E, Biçakçi B, et al. Side effects associated with the treatment of multidrug-resistant tuberculosis. http://www.ncbi.nlm.nih.gov/pubmed/16468160 [accessed on May 29, 2015]
6. Niyi Awofeso, Anti-tuberculosis medication side-effects constitute major factor for poor adherence to tuberculosis treatment, *Bull World Health Organ*. 2008 Mar; 86(3): doi: 10.2471/BLT.07.043802
7. Cobelens FGJ, Heldal E, Kimerling ME, Mitnick CD, Podewils LJ, Ramachandran R, et al. Scaling Up Programmatic Management of Drug-Resistant Tuberculosis: A Prioritized Research Agenda. PLoS Med. 2008; 5(7)
8. Out A. Umoh V. Habib A. Ameh S. Lawson L. and Ansa V. Drug Resistance among Pulmonary Tuberculosis Patients in Calabar, Nigeria., *Pulmonary Medicine*, 2013;Volume 2013, Article ID 235190,
9. Mario C. Raviglione, Ian M. Smith. XDR Tuberculosis-Implications for Global Public *Health*, *N Engl J Med*. 2007; 356:656-659, DOI: 10.1056/NEJMp068273

10. Balabanova Y, Nikolayevskyy V, Ignatyeva O, Kontsevaya I, Rutterford CM, Shakhmistova A, et al. Survival of Civilian and Prisoner Drug-Sensitive, Multi- and Extensive Drug- Resistant Tuberculosis Cohorts Prospectively Followed in Russia. *PLoS ONE*.2011; 6(6): e20531. doi:10.1371/journal.pone.0020531
11. Shah NS, Wright A, Bai GH, Barrera L, Boulahbal F, Martin-Casabona N, et al. (2007) Worldwide emergence of extensively drug-resistant tuberculosis. *Emerg Infect Dis*. 2007; 13: 380–387.
12. World Health Organization, multidrug-resistant tuberculosis (MDR-TB) 2014 update, http://www.who.int/tb/challenges/mdr/mdr_tb_factsheet.pdf [accessed on February 10, 2015]
13. WHO, Global Health Observatory (GHO), what services are there to diagnose TB? Situation in 2013, http://www.who.int/gho/tb/epidemic/diagnosis/en/ [accessed on March 14, 2015]
14. Maboud Azad Aminjan, Seyyed Reza Moaddab, Mohammad Hosseini Ravandi, and Behzad Kazemi Haki Prevalence of Tuberculosis among Veterans, Military Personnel and their Families in East Azerbaijan Province Violators of the last 15 Years *Acta Medica Iranica*, Vol. 53, No. 10 (2015)
15. Hamid Hussain, Saeed Akhtar and Debra Nanan, Prevalence of and risk factors associated with *Mycobacterium tuberculosis* infection in prisoners, North West Frontier Province, Pakistan, *International Journal of Epidemiology* 2003;32:794–799 DOI: 10.1093/ije/dyg247
16. World Health Organization, Global Tuberculosis Report 2016, WHO Geneva, Switzerland, 2016.
17. World Health Organization, *Tuberculosis Control: WHO Report 2010*, WHO, Geneva, Switzerland, 2010
18. World Health Organization, WHO Report on Global Tuberculosis Control. Surveillance, Planning, Financing. World Health Organization Switzerland, 2007.
19. Becerra M.C, Pachao-Torreblanca I.F, Bayona J. Celi R. Shin S.S, Kim J.Y, et al., "Expanding tuberculosis case detection by screening household contacts," *Public Health Reports*, 2005;. 120, 3, . 271–277.

20. World Health Organization. Drug and multi drug-resistant tuberculosis (MDR-TB). Frequently asked questions. http://www.who.int/tb/challenges/mdr/faqs [accessed on January 02, 2015].
21. World Health Organization. Stop TB Partnership. The global MDR-TB and XDR-TB response plan 2007-2008. http://www.who.int [accessed on April 05, 2015]
22. World Health Organization (WHO), extensively drug-resistant tuberculosis (XDR.TB): recommendations for prevention and control. *Weekly Epidemiol Record* 2006; 81: 430 432.
23. Faustini A, Hall AJ, Perucci CA. Risk factors for multidrug resistant tuberculosis in Europe: a systematic review. *Thorax*. 2006; 61: 158–163.
24. World Health Organization, Tuberculosis control in prison, a manual for programme managers, WHO/CDS/TB/2000.281. 2000.
25. Coninx R, Mathieu C, Debacker M, Mirzoev F, Ismaelov A, de Haller R, et al. First line tuberculosis therapy and drug-resistant Mycobacterium tuberculosis in prisons. *Lance.1999;t* 353: 969–973.
26. World Health Organization. Global tuberculosis control Epidemiology, strategy, Financing report, WHO. 2009.
27. Center for Disease Control and Prevention, Rapid assessment of Tuberculosis in Large prison of Botswana. *MMWR Morb Mortal Wkly Rep.2003;*52(12):250–252. http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5212a3.htm
28. Katie R Maggard, Sisa Hatwiinda, Jennifer B Harris, Screening for tuberculosis and testing for human immunodeficiency virus in Zambian prisons, *Bull World Health Organ*. 2015;93:93–101.
29. Fantahun Biadglegne, Ulrich Sack and Arne C Rodloff, Multidrug-resistant tuberculosis in Ethiopia: efforts to expand diagnostic services, treatment and care. *Antimicrobial Resistance and Infection Control*, 2014; 3:31.
30. Moges B, Amare B, Asfaw F, Tesfaye W, Tiruneh M, Belyhun Y, et al. Prevalence of smear positive pulmonary tuberculosis among prisoners in North Gondar Zone Prison, northwest Ethiopia.*BMC Infectious Diseases* 2012;12:352.

31. Sanchez AR, Massari V, Gerhardt G, Barreto AW, Cesconi V, Pires J, et al. Tuberculosis in Rio de Janeiro prisons, Brazil: an urgent public health problem. *Cad Saude Publica*. 2007; 23: 545–552.
32. Aaron, C. L. Mancuso, J. D. Using the tuberculosis cohort review to evaluate and improve the U.S. Army's tuberculosis control program, j*journal of MSMR*. 2013; . 20 . 5 plesae fllow vancover style !!!
33. Geiter L, editor. Ending Neglect: The Elimination of Tuberculosis in the United States. Washington, D.C.: National Academy Press, 2000
34. Ciftci F, Tozkoparan E, Deniz O, Bozkanat E, Kibaroglu E, Demirci N., The incidence of tuberculosis in the armed forces: a good reflection of the whole population. *Int J Tuberc Lung Dis*. 2004;8(8):965-8.
35. Lee SW, Jeon K, Kim KH, Min KH. Multidrug-resistant Pulmonary Tuberculosis Among Young Korean Soldiers in a Communal Setting. *Journal of Korean Medical Science*.2009; 24(4):592-595. doi:10.3346/jkms.2009.24.4.592.
36. Banu S, Hossain A, Uddin MKM, Uddin MR, Ahmed T, Khatun R, et al. Pulmonary Tuberculosis and Drug Resistance in Dhaka Central Jail, the Largest Prison in Bangladesh. *PLoS ONE*. 2010; 5(5): e10759. doi:10.1371/journal.pone.0010759
37. Benson R. Kidenya,, Lauren E. Webster, Sehan Behan, Rodrick Kabangila, Robert N.Peck, Stephen E. Mshana. Epidemiology and genetic diversity of multidrug-resistant tuberculosis in East Africa, *Tuberculosis (Edinb).* 2014; 94(1): doi:10.1016/j.tube.2013.08.009.
38. Bakari M, Moshi A, Aris EA, Chale S, Josiah R, Magao P et al. Isoniazid prophylaxis for tuberculosis prevention among HIV infected police officers in Dar es Salaam. *East afr med j*. 2000: 77(9):494-7.
39. Daniel M. Nigus, Wondemagegn M. Lingerew, Bayeh A. Beyene1, Aschalew A. Tamiru, Martha T. Lemma and Mulat Y. Melaku, Prevalence of Multi Drug Resistant Tuberculosis among Presumptive Multi Drug Resistant Tuberculosis Cases in Amhara National Regional State, Ethiopia. *J Mycobac Dis.* 2014; 4 (3)
40. Asres Berhan, Yifru Berhan, Desalegn Yizengaw, a meta-analysis of drug resistant tuberculosis in Sub-Saharan Africa: how strongly associated with previous treatment and HIV co-infection, Ethiop J Health Sci. 2013; 23:3

41. Hirpa S, Medhin G, Girma B, Melese M, Mekonen A, Suarez P, et al. Determinants of multidrug-resistant tuberculosis in patients who underwent first-line treatment in Addis Ababa: a case control study. *BMC Public Health*. 2013; 13:782. doi:10.1186/1471-2458-13-782
42. Ulmasova DJ, Uzakova G, Tillyashayhov MN, Turaev L, van Gemert W, Hoffmann H,et al. Multidrug-resistant tuberculosis in Uzbekistan: results of a nationwide survey, 2010 to 2011. *Euro Surveill*. 2013; 18(42)
43. WHO, Multidrug extensively drug-resistant TB (M/XDR-TB), Global report on surveillance and response. World Health Organization. WHO/HTM/ TB/2010.3. 2010.
44. World Health Organization, drug resistant TB surveillance and report, WHO, global tuberculosis report of 2014, 2015.
45. Gaude GS, Hattiholli J, Kumar P. Risk factors and drug-resistance patterns among pulmonary tuberculosis patients in northern Karnataka region, India. *Niger Med J* 2014;55:327-32, http://www.nigeriamedj.com/text.asp?2014/55/4/327/137194
46. Lee Fairlie, Natalie C Beylis, Gary Reubenson, David P Moore, Shabir A Madhi, High prevalence of childhood multidrug resistant tuberculosis in Johannesburg, South Africa: a cross sectional study. *BMC Infectious Diseases*, 2011; 11:28.
47. Abate G. Drug-resistant tuberculosis in Ethiopia: problem scenarios and recommendation, *Ethiop Med J*. 2002 ;40(1):79-86
48. Abebe G, Abdissa K, Abdissa A, Apers L, Agonafir M, de-Jong BC, et al. Relatively low primary drug resistant tuberculosis in southwestern Ethiopia. *BMC Research Notes* 2012;5:225. doi:10.1186/1756-0500-5-225.
49. Yimer SA, Agonafir M, Derese Y, Sani Y, Bjune GA, Holm-Hansen C. Primary drug resistance to anti-tuberculosis drugs in major towns of Amhara Region, Ethiopia. *APMIS*. 2012; 120: 503–9.
50. Tuberculosis Coalition for Technical Assistance. International Standards for Tuberculosis Care (ISTC). The Hague: Tuberculosis Coalition for Technical Assistance, 2006.
51. World Health Organization, *Framework for Effective Tuberculosis Control. World Health Organization Document 1994*, WHO/TB/94.179:1-7.
52. Kenneth Todar, *Mycobacterium tuberculosis* and Tuberculosis http://textbookofbacteriology.net/tuberculosis.html

53. Ethiopian public health institute, national tuberculosis reference laboratory, standard operating procedure for Zehel Neelson staining technique.
54. WHO, Xpert MTB/RIF implementation manual: technical and operational 'how-to'; practical considerations. ISBN: 978 92 4 150670 0, 2014. http://apps.who.int
55. Salman H. Siddiqi, Sabine Rüsch-Gerdes , *Mycobacteria Growth Indicator Tube (MGIT) Culture and Drug Susceptibility Demonstration Projects, MGIT procedure manual. https://www.finddx.org*
56. Ethiopian public health institute, national tuberculosis reference laboratory, standard operating procedure for MGIT drug susceptibility testing
57. Kumar, MPT 64 Antigen detection for Rapid confirmation of M.tuberculosis isolates. BMC Research Notes 2011 4:79
58. TB CARE I. International Standards for Tuberculosis Care, Edition 3. The Hague, TB CARE I, 2014.
59. Burki T. Tuberculosis-resistance, funding, and drugs. Lancet Infect Dis 2010; 10: 297-8
60. Abebe D, G. Bjune, G. Ameni, D. Biffa, F. Abebe Prevalence of pulmonary tuberculosis and associated risk factors in Eastern Ethiopian prisons, INT J TUBERC LUNG DIS 15(5):668–673
61. Ibrahim S, Abubakar UB, Danbirni S; Usman A, Kudi AC, Loveth L et al. Causes and Prevalence of Tuberculosis from Sputum of Patients based on Sd Bioline® and Genotype Mbtc® Assay in Gombe State, Nigeria. J Microbiol Biotechnol, 2017, 2(1).
62. Tehseen Iqbal, Muhammad Abdul Raziq, Zafar Hussain, Naveed Anjum, Salman Atiq, Gender Differences among Suspected Pulmonary Tuberculosis Patients undergoing Sputum Smear Microscopy, Ann. Pak. Inst. Med. Sci. 2011; 7(1): 14-17
63. Qadeer E, Fatima R, Yaqoob A, Tahseen S, Ul Haq M, Ghafoor A, et al. Population Based National Tuberculosis Prevalence Survey among Adults (>15 Years) in Pakistan, 2010–2011. PLoS ONE ,2016, 11(2): e0148293. doi:10.1371/journal. pone.0148293
64. Sabawoon W, Sato H, Sex Difference in Tuberculosis in Afghanistan: A National Cohort Study. Mycobac Dis, 2012, 2:115. doi:10.4172/2161- 1068.1000115
65. United nations development program, gender and tuberculosis, 2015

66. Ifedayo MO Adetifa, Lindsay Kendall, Adedapo Bashorun, Christopher Linda, Semeeh Omoleke, David Jeffries et al. A tuberculosis nationwide prevalence survey in the Gambia, Bulletin of the World Health Organization; BLT.14.151670
67. Claude Mambo Muvunyi, Florence Masaisa, Claude Bayingana, Andre Musemakweri, Leon Mutesa and Teresa Carbonell Hernandez, Prevalence and diagnostic aspects of sputum smear positive tuberculosis cases at a tertiary care institution in Rwanda, African Journal of Microbiology Research ,2010,Vol. 4(1) pp. 088-091 Available online http://www.academicjournals.org/ajmr
68. Bishnu R Tiwari, Surendra Karkb, Prakash Ghimire, Bimala Sharma, Sarala Malla, Factors associated with high prevalence of pulmonary tuberculosis in HIV-infected people visiting for assessment of eligibility for highly active antiretroviral therapy in Kathmandu, Nepal, *WHO South-East Asia Journal of Public Health* 2012;1(4):404-411
69. Mitku AA, Dessie ZG, Muluneh EK, Workie DL. Prevalence and associated factors of TB/HIV co-infection among HIV Infected patients in Amhara region, Ethiopia. Afri Health Sci 2016;16(2): 588-595. http://dx.doi.org/10.4314/ahs.v16i2.29
70. Zubairu Iliyasu and Musa Babashani, Prevalence and Predictors of Tuberculosis Coinfection among HIV-Seropositive Patients Attending the Aminu Kano Teaching Hospital, Northern Nigeria, Epidemiol 2009;19(2):81-87 doi:10.2188/jea.JE20080026
71. Ephrem T, Mengiste B, Mesfin F, Godana W. Determinants of active pulmonary tuberculosis in Ambo Hospital, West Ethiopia. Afr J Prm Health Care Fam Med. 2015;7(1), Art. #608, 8 pages. http://dx.doi. org/10.4102/phcfm.v7i1.608
72. Nazrul Islam Mondal, Nazrul Hoque, Rocky Khan Chowdhury, Jeffrey Howard, Socio Demographic Factors Affecting Knowledge Level of Tuberculosis Patients in Rajshahi City, Bangladesh, HCPP White Paper Series, 2016, No. 3
73. Ogboi S. J, Idris S. H., Olayinka A. T. and Ilyas Junaid, Socio demographic characteristics of patients presenting pulmonary tuberculosis in a primary health centre, Zaria, Nigeria, Journal of Medical Laboratory and Diagnosis, 2010,Vol. 1(2) pp. 11 14, Available online http://www.academicjournals.org/JMLD
74. Martha Alemayehu, Abiy Tigabu, Solomon Yunkura, Fana Hagos, and Birehanemeskel Tegene, "Prevalence of Smear Positive Pulmonary Tuberculosis and Associated Risk Factors among Pulmonary Tuberculosis Suspected Patients at Private Health Institutions

in Gondar Town, Northwest Ethiopia: A Cross-sectional Study." *American Journal of Infectious Diseases and Microbiology*, 2017: vol. 5, no. 1: 60-65. doi: 10.12691/ajidm-5-1-3.

75. R S Heyderman,M Goyal, P Roberts, S Ushewokunze, S Zizhou, B G Marshall, et al. Pulmonary tuberculosis in Harare, Zimbabwe: analysis by spoligotyping, *Thorax* 1998;53:346–350
76. Erazo C, Pereira SM, Costa MCN, Evangelista-Filho D, Braga JU, Barreto ML.Tuberculosis and living conditions in Salvador, Brazil: a spatial analysis. Rev Panam Salud Publica. 2014:36(1):24–30.
77. Bayu Begashaw, Abera Beyamo Mekiso, and Tegene Legesse, "Prevalence of Pulmonary Tuberculosis and Associated Factors among Prisoners in Wolaita Zone, Southern Ethiopia: Cross-sectional Study." *American Journal of Public Health Research*, 2016: vol. 4, no. 4: 142-148. doi: 10.12691/ajphr-4-4-4.
78. Addisalem Titiyos, Degu Jerene and Fikre Enquselasie, The yield of screening symptomatic contacts of multidrug-resistant tuberculosis cases at a tertiary hospital in Addis Ababa, Ethiopia, *BMC Res Notes (2015) 8:501* DOI 10.1186/s13104-015-1442-z
79. Cabrera-GaytaÂn DA, Niebla-Fuentes MdR, Padilla-VelaÂzquez R, Valle-Alvarado G, Arriaga- Nieto L, Rojas-Mendoza T, et al. Association of Pulmonary Tuberculosis and HIV in the Mexican Institute of Social Security from 2006-2014. PLoS ONE 11(12): e0168559. doi:10.1371/journal. pone.0168559
80. S. van den Hof, A. Tursynbayeva, T. Abildaev, M. Adenov, S. Pak, G. Bekembayeva. et al. Converging risk factors but no association between HIV infection and multidrug-resistant tuberculosis in Kazakhstan, Int J Tuberc Lung Dis, 2013: 17(4):526–531: http://dx.doi.org/10.5588/ijtld.12.0703
81. -Elorriaga G, Del Rey-Pineda G. Type 2 Diabetes Mellitus as a Risk Factor for Tuberculosis. J Mycobac Dis, 2014, 4: 144. doi:10.4172/2161- 1068.1000144
82. Mansuri S, Chaudhari A, Singh S, Malek R, Viradiya R. Prevalence of Diabetes among Tuberculosis Patients at Urban Health Centre, Ahmedabad. Int J Sci Stud 2015;3(4):115 118.
83. Galuh Chandra Irawan, Ani Margawati, and Ali Rosidi, Underweight increases the risk of pulmonary tuberculosis in adult, Universa M e d i c i n a , 2017:*Vol.36 - No.1*

84. Nagpal M, Devgun P, Chawla N. A study on nutritional status and change in body mass index with treatment outcome in smear positive pulmonary TB patients on DOTS in Amritsar city. Int J Med Sci Public Health 2015;4:454-457
85. C Kolappan and P G Gopi, Tobacco smoking and pulmonary tuberculosis, *Thorax* 2002: 57: 964-966, doi: 10.1136/thorax.57.11.964
86. C-Y. Chiang, K. Slama, D. A. Enarson, Associations between tobacco and tuberculosis, int j tuberc lung DIS. 2017: 11(3):258–262
87. A.T. Abal, B. Jayakrishnan, S. Parwerb, A. El Shamy, E. Abahussain, P.N. Sharma, Effect of cigarette smoking on sputum smear conversion in adults with active pulmonary tuberculosis, Respiratory Medicine (2005) 99, 415–420
88. *N Gyawali, R Gurung, N Poudyal, R Amatya, R Shrestha, LK Khana,* Tobacco and alcohol:The relation to pulmonary tuberculosis in household contacts, *Nepal Med Coll J 2012; 15(2): 125-128*
89. C. Kolappan, P. G. Gopi, R. Subramani, P. R. Narayanan, Selected biological and behavioural risk factors associated with pulmonary tuberculosis, Int J Tuberc Lung dis,2017: 11(9):999–1003
90. K. Aid Sobhy, S. Elawady, S. Abdel Latef, A. Abou Zeid, and M. Said, Patterns of drug resistance in cases of smear positive pulmonary tuberculosis in Giza and Cairo governorates, Egyptian Journal of Chest Diseases and Tuberculosis (2012) 61, 343–348
91. Mohammad Javad Nasiri, Faranak Rezaei, Samin Zamani, Davod Darban Sarokhalil, Abbas Ali Imani Fooladi, Hasan Shojaei et al., Drug resistance pattern of Mycobacterium tuberculosis isolates from patients of five provinces of Iran, Asian Pacific Journal of Tropical Medicine (2014)193-196, doi: 10.1016/S1995-7645(14)60019-5
92. Microbiology laboratory manual, April, 2014. http://www.who.int/tb/laboratory/mycobacteriology-laboratory-manual.pdf
93. Bodmer, T., Ströhle, A. Diagnosing Pulmonary Tuberculosis with the Xpert MTB/RIF Test. *J. Vis. Exp.* 2012; (62), e3547, doi:10.3791/3547.
94. Ethiopian public health institute, National Tuberculosis laboratory, standard operating procedure for MGIT culture workup, 2016.

11. Annex

11.1. Annex 1: English and Amharic subject information to respondents

Dear Sir/Madam;

My name is Nigusu Fentahun. I am currently a student of Addis Ababa University, College of Health Sciences, school of allied health sciences, and Department of Medical Laboratory Sciences. The objective of the study is to *Evaluate Drug Resistance Pattern and associated risk factors of mycobacterium tuberculosis*. Your cooperation and willingness for the study is very helpful. If you are voluntary, your leftover (surplus) samples will be tested for TB using sputum test free of charge as a benefit but no any other harm to you.

The study will be conducted through analysis of sputum sample by Ziehl Nelseen AFB, LJ culture and Gene Xpert methods. The information you provide will be used to improve TB diagnosis, treatment and to design appropriate public health interventions for future. Your name will not be written on the Log book of routine procedure rather code will be given. Your participation is voluntary and you may choose to stop at any time. Your participation does not have any influence for your service that you want to use. In addition, your participation in the study does not have any invasive procedure except for which your request to give three consecutive sputum samples as recommended by the health personnel. At the end of the study the results will be distributed to the concerned body. For the successes of our study, we will ask you to give us correct answer and support for our respective questions.

I would like to indicate that your name will not be written in the form and I assure you all the information you give will be kept strictly confidential. Your participation is voluntary and you are not obliged to give replicate sample, for the study purpose only, that you did not requested by your physician. If you are not comfortable with the study, please feel free to stop in any time you like.

Thank you for your assistance.

ለጥናቱ ተሳታፊዎች በንባብ የሚገለፅ መረጃ

እኔ ንጉሱ ፈንታሁን በአሁኑ ሰዓት የአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ የድህረ-ምረቃ መርሃ ግብር ተመራቂ ተማሪ ስሆን የዚህ ጥናት አላማ ቲቢ የተላመዳቸዉን የመዳህኒት አይነቶችን ና ተጓዳኝ ችግሮችን ለመለየት የሚረዳ ጥናታዊ ፅሑፍ ለማዘጋጀት ይሆናል፡፡

በዚህም ጥናት የእርሶ ትብብርና ፈቃደኝነት ለጥናቱ መሳካት በጣም ጠቃሚ ነው፡፡ ስለሆነም እርሶ ፈቃደኛ ከሆኑ ለቲቢ ምርመራ ወደ ላቦራቶሪ መጥተዉ ከሚሰጡት የአክታ ናሙና ላይ ተሰርቶ የሚተርፈውን ያለምንም ተጨማሪ ዋጋ እና ያለምንም ጉዳት እንደ ተጨማሪ ጥቅም ልንሰራልዎት እንዲፈቅዱልን እንጠይቃለን፡፡

ከዚህ የጥናት ሂደት የሚገኘው የተቢ ምርመራ ውጤት በመነሳት ተገቢውን አቅጣጫ ለማስቀመጥ ይረዳል፡፡ የእርስዎ ስም የማይገለጽ ሲሆን በምትኩ ኮድ ይዘጋጂልወታል እንዲሁም የርስዎ ዉጤት በምንም አጋጣሚ ለማይመለከተው አካል ተላልፎ አይሰጥም፡፡ የእርሶን ሚስጥር በተለየ መረጃ መዝገብ በተለየ ሚስጥር ቁጥር ስለሚቀመጥ ስጋት እንዳይገባዎ እያልን የእርሶ ተሳታፊነት ሙሉ ፈቃደኝነትዎን እና በምርጫዎ እንዲሆን እንፈልጋለን፡፡

ይህ ካልሆነ በማንኛውም ጊዜ የማቆም መብትዎ የተጠበቀ ነው፡፡ ከማንኛውም ተጽኖ ነፃ መሆንዎን ልንገልጽልዎ እንወዳለን፡፡ በስተመጨረሻ የጥናቱ ሂደት እንደ አስፈላጊነቱ ለቀጣይ የቲቢ ምርመራ ሂደት ይረዳ ዘንድ የሚመለከታቸው የባለድርሻ አካላት እንዲደርሳቸው እናደርጋለን፡፡

ስለትብብርዎ እናመሰግናለን!!!

11.2. Annex 2: English and Amharic informed consent form

I_____ here gives my consent to answer all questioners truly and give three consecutive sputum samples as instructed by health personnel and as requested by my physician for microscopy diagnosis of TB. I understand that there is no serious and no replicate sampling procedure at the beginning as well as at the end of the study. I understand this study is useful not only for me but also for other who go through TB diagnosis research. I know that it is for public benefit in future diagnosis of TB. I believe that at the end of study the result will also be explained for concerned body only for the purpose of the study like lab personnel, clinician working in TB clinic and so on.

Signature _____ Date_____

Thank you for helping and participating in this important study!

ለጥናቱ ተሳታፊዎች የተዘጋጀ የፍቃደኝነት መግለጫ ቅፅ

እኔ.....ሦስት ተከታታይ አከታ በጤና ባለሙያ ትዕዛዝ መሰረት ለቲቢ ምርመራ ለመስጠት ና ቃለመጠይቁን የሚያካሂደው ባለሙያ የሚጠይቀኝን ቃለመጠይቅ በትክክል ለመመለስ ፍቃደኝነቴን እገልፃለሁ፡፡ በጥናቱ ወቅትም ከመጀመሪያ እስከ መጨረሻ ምንም አይነት የሚጎዳ ሁኔታ ና ሀኪሙ ካዘዘልኝ አከታ ተጨማሪ የሆነ እንደማያስፈልግ ተረድቻለሁ፡፡ ጥናቱም ለእኔብቻሳይሆን ለሌሎች በቲቢ በሽታ ለተያዙ ህሙማን ሁሉ እንደሚጠቅም ፡፡ ጥናቱም በመጨረሻ ጊዜ ጥናቱ ሚመለከታቸው አካላት ብቻ ይፋ እንደሚሆን ተረድቻለሁ፡፡

ፊርማ፡_____ ቀን: ----/ ----/----

በዚህ ጠቃሚ ጥናት ስለተባበራችሁኝ ና ስለተሳተፉ አመሰግናለሁ፡:

11.3. Annex 3: English and Amharic study questionnaire

Questioner

Code: ..... Sex: M/F..... Age: ……

- Marital status Married ◯ Single ◯ Divorced ◯
- Level of Education University/College ◯ Primary/Secondary school ◯
  Have no formal education (Illiterate) ◯
- Living condition Own/Rented house ◯ Dormitory/camp ◯ Homeless ◯
- Employment status Employed ◯ Unemployed ◯
- Cough that lasts 3 weeks or longer Yes ◯ No ◯
- Coughing up blood or sputum (from deep inside) Yes ◯ No ◯
- Chest pain Yes ◯ No ◯
- Weight loss Yes ◯ No ◯
- Fever Yes ◯ No ◯
- Sweating at night Yes ◯ No ◯
- History of contact with a person known to have TB case Yes ◯ No ◯
- Tuberculosis treatment history NO ◯ Previously treated case ◯
  - If previously treated TB case, have you got the drug immediately after diagnosis
    Yes ◯ No ◯
  - If previously treated TB case, have you completed full course of treatment
    Yes ◯ No ◯

- **Do you have a history of:-**
  - HIV/AIDS Yes ◯ No ◯ Unknown ◯
  - Diabetes Yes ◯ No ◯ Unknown ◯
  - Sever Kidney disease Yes ◯ No ◯ Unknown ◯
  - Certain cancers Yes ◯ No ◯ Unknown ◯
  - Cancer treatment, such as chemotherapy Yes ◯ No ◯
  - History of smoking Yes ◯ No ◯
  - Alcohol consumption Yes ◯ No ◯
  - History of organ transplantation Yes ◯ No ◯
  - Under Weight (BMI less than 18.5) Yes ◯ No ◯

Amharic form

ኮድ: ..... ዖታ: ..... እድሜ: ……

- የጋብቻ ሁኔታ ያገባ ◯ ያላገባ ◯ የተፋታ ◯
- የትምህርት ሁኔታ ዩኒቨርስቲ/ኮሌጅ ◯ 1ኛ/2ኛ ደረጃ ◯ መደበኛ ት/ት ያልወሰደ ◯
- የኑሮ ሁኔታ የግል/የኪራይ ቤት ◯ ዶርም/ካምፕ ◯ ቤት አልባ ◯
- የስራ ሁኔታ ስራ ያለዉ ◯ ስራ የሌለዉ ◯
- 3 ሳምንትና ከዛ በላይ የቆየ ሳል አለ ◯ የለም ◯
- ከዉስጥ የሚወጣ አክታ/ደም አለ ◯ የለም ◯
- የደረት ህመም አለ ◯ የለም ◯
- ክብደት መቀነስ አለ ◯ የለም ◯
- ተኩሳት አለ ◯ የለም ◯
- የሌሊት ላብ አለ ◯ የለም ◯
- ቲቢ ከነበረበት ሰዉ ጋር ቆይተዉ/ኑረዉ ያዉቃሉ አዎ ◯ የለም ◯
- ከዚህ በፊት ለቲቢ መዳህኒት ወስደዋል አልወሰድኩም ◯ ወስጀ አዉቃለሁ ◯
  - ወስደዉ የሚያዉቁ ከሆን፣ መዳህኒቱን ቶሎ ነበር ያገኙት
    አዎ ◯ የለም ◯
  - ወስደዉ የሚያዉቁ ከሆን፣ መዳህኒቱን ጨርሰዋል
    አዎ ◯ አልጨረስኩም ◯

- **ከነዚህ ዉስጥ የትኛዉ ተከስቶብወት የዉቃል :-**
  - ኤች አይ ቪ/ኤድስ ላለብኝ ◯ የለብኝም ◯ አላዉቀዉም ◯
  - ስኳር ላለብኝ ◯ የለብኝም ◯ አላዉቀዉም ◯
  - ከባድ የኩላሊት በሽታ ላለብኝ ◯ የለብኝም ◯ አላዉቀዉም ◯
  - ካነሰር ላለብኝ ◯ የለብኝም ◯ አላዉቀዉም ◯
  - የካነስር መዳህኒት እወስዳለሁ ◯ አልወስድም ◯
  - ሲጋራ የማጨስ ልምድ አለብኝ ◯ የለብኝም ◯
  - አልኮል የመጠጣት ልምድ አለብኝ ◯ የለብኝም ◯
  - የሰዉነትዎን አካል አስቀይረዉ ያዉቃሉ አዎ ◯ አላዉቅም ◯
  - ከክንደት በታች (BMI<18.5) አዎ ◯ አልችልም ◯

11.4. Annex 4: Signs and symptoms of TB

Most of the time, symptoms of TB disease depend on where in the body the TB bacteria are growing. But generally, TB disease symptoms may include:

- A bad cough that lasts 3 weeks or longer.
- Pain in the chest.
- Coughing up blood or sputum (phlegm from deep inside the lungs)
- Weakness or fatigue.
- Weight loss.
- No appetite.
- Chills
- Fever
- Sweating at night

11.5. Annex 5: Laboratory procedures and result interpretations

11.5.1. I - ZN for AFB

1. Procedures

    1. Prepare smear and allow to air dry.
    2. Heat fix smear, (either on an electrics slide warmer at 65 to 75 ^{o}C for at least 2 hours), or pass slide through Bunsen burner flame. Don't over heat.
    3. Flood the stain with carbool fuchsine.
    4. Heat the slides to steaming with Bunsen burner, for 5 minutes. If the smear dries, add more stain but don't re heat.
    5. Wash slides with water (use tap water or water from reservoir bottles)
    6. Flood smear with acid alcohol, allow to distain for 3 minutes
    7. Wash smear again with water and drain
    8. Floods slides with methylene blue as a counter stain for 1 to 2 minutes
    9. Rinse with water, drain and air dry .do not blot
    10. Examine smears under oil immersion objectives lens of the microscope

2. Reporting of smear results

The smear should be searched in an orderly manner

- None…….. No AFB seen
- 1-9/100 fields……. ……Exact number
- 1-9 /10 fields……1+
- 1-9 /fields…….....2+
- >9 /fields……..…….....3+ (92)

11.5.2. GeneXpert MTB/RIF

When Expectorated Sputum Samples

1. Label each Xpert MTB/RIF cartridge with the corresponding specimen ID.
2. Transfer 1.0 ml expectorated sputum to a conical, screw-capped tube using a sterile transfer pipette. Alternatively, the entire specimen may be processed in the original leak-proof sputum collection container.
3. Add 2.0 ml Xpert MTB/RIF Sample Reagent (2:1; v/v) to the expectorated sputum using a sterile transfer pipette.
4. Replace the lid, and shake the tube vigorously 10-20 times. Alternatively, the mixture may be vortexed (30 sec).
5. Allow the tube to stand upright for 5 min at room temperature.
6. Shake the tube vigorously 10-20 times. Alternatively, use a vortex (30 sec).
7. Allow the tube to stand upright for another 10 min at room temperature.
8. Inspect the specimens: samples should be liquefied with no visible clumps of sputum.

When Concentrated Sediments

1. Label each Xpert MTB/RIF cartridge with the corresponding specimen ID.
2. Transfer 1.5 ml Xpert MTB/RIF Sample Reagent to a conical, screw-capped tube using a sterile transfer pipette.
3. Add at least 0.5 ml concentrated sediment to the Sample Reagent containing tube using a sterile transfer pipette.
4. Replace the lid, and shake the tube vigorously 10-20 times. Alternatively, use a vortex (30 sec).
5. Allow the tube to stand upright for 5 min at room temperature.
6. Shake the tube vigorously 10-20 times. Alternatively, use a vortex (30 sec).
7. Allow the tube to stand upright for another 10 min at room temperature.
8. Inspect the specimens: samples should be liquefied with no visible clumps of sputum.

Loading the Xpert MTB/RIF Cartridge

Note: Start the test within 30 minutes of adding the sample to the cartridge!

1. Check the labels on the Xpert MTB/RIF cartridge and the specimen ID.
2. Open the cartridge lid.
3. Using the sterile transfer pipette provided, aspirate the liquefied specimen into the transfer pipette until the meniscus is above the minimum
4. Mark and transfer the sample into the open port of the Xpert MTB/RIF cartridge. Note: Dispense slowly to minimize the risk of aerosol formation!

5. Close the cartridge lid.

Starting the Test

Note: Before starting the test, ensure that the GeneXpert Dx System is equipped with the GX2.1 software, and the Xpert MTB/RIF assay is imported into the software!

1. Turn on the computer, and then turn on the GeneXpert Dx instrument.
2. On the Windows desktop, double-click the GeneXpert Dx shortcut icon.
3. Log on to the GeneXpert Dx System software using your user name and password.
4. In the GeneXpert Dx System window, click Create Test. The Scan Cartridge Barcode dialog box appears.
5. In the Sample ID box, scan or type the sample ID.
6. Scan the barcode on the Xpert MTB/RIF cartridge. The Create Test window appears.
7. Click Start Test, and enter your password in the dialog box that appears.
8. Open the instrument module door with the blinking green light, and load the cartridge.
9. Close the door. The test starts and the light stops blinking and remains constantly green. When the test is finished, the light turns off.
10. Wait until the system releases the door lock at the end of the run, then open the module door and remove the cartridge.
11. Dispose of used cartridges in the appropriate waste containers according to your institution's standard practices.

Result Interpretation

Xpert MTB/RIF result can indicate that *M. tuberculosis* (MTB) was not detected, MTB was detected and was not resistant to rifampicin (that is, it is rifampicin susceptible), or that MTB was detected and it was resistant to rifampicin. A small proportion of tests may result in an error or invalid result; these tests need to be repeated (93).

11.5.3. MGIT liquid Culture Media

Procedure

Materials Provided: BBL MGIT Mycobacteria Growth Indicator Tubes and BACTEC MGIT 960 Supplement Kit, containing BACTEC MGIT Growth Supplement and BBL MGIT PANTA Antibiotic Mixture.

Inoculation of MGIT tubes:

BBL MGIT 7 mL Tubes must be used with a BACTEC MGIT 960 instrument.

1. Reconstitute a lyophilized vial of BBL MGIT PANTA Antibiotic Mixture with 15 mL of BACTEC MGIT Growth Supplement.
2. Label the MGIT tube with the specimen number.
3. Unscrew the cap and aseptically add 0.8 mL of Growth Supplement/MGIT PANTA Antibiotic Mixture. For best results, the addition of Growth Supplement/MGIT PANTA Antibiotic Mixture should be made just prior to specimen inoculation.
4. Add 0.5 mL of the concentrated specimen suspension prepared above. Also add a drop (0.1 mL) of specimen to a 7H10 agar plate or other mycobacterial solid agar or egg-based medium.
5. Tightly recap the tube and mix well.
6. Tubes entered into the instrument will be automatically tested for the duration of the recommended 42 day testing protocol.
7. Positive tubes, identified by the BACTEC MGIT 960 instrument should be subcultured and an acid-fast smear prepared (55).

11.5.4. MGIT drug susceptibility test

Inoculum from the MGIT tube: It is important that the growth is within the following recommended timeframe.

- The day a MGIT tube is positive by the instrument is considered Day 0.
- The tube should be kept incubated for at least one more day (Day 1) before being used for the susceptibility testing (may be incubated in a separate incubator at 37ºC + 1ºC).
- A positive tube may be used for drug susceptibility testing up to and including the fifth day (Day 5) after it becomes instrument positive. A tube that has been positive for more than 5 days should be subcultured in a fresh MGIT tube supplemented with MGIT 960 Growth Supplement and should be tested in a MGIT 960 instrument until it is positive. Use this tube from one to five days of instrument positivity as described above.
- If growth in a tube is on Day 1 or Day 2, mix well (vortex) to break up clumps. Leave the tube undisturbed for about 5-10 minutes to let big clumps settle on the bottom. Use the supernatant undiluted for inoculation of the drug set.
- If growth is on Day 3, 4, or 5, mix well to break up the clumps. Let the large clumps settle for 5-10 minutes and then dilute 1.0 ml of the positive broth with 4.0 ml of sterile saline. This will be a 1:5 dilution. Use this well mixed diluted culture for inoculation.

Procedure

1. Label 5 MGIT tubes for each test culture. Label one for GC (growth control, without drug) one for STR, one for INH, one for RIF, and one for EMB.
2. Aseptically add 0.8 ml of BACTEC 960 SIRE Supplement to each of the MGIT tubes. Use only MGIT SIRE Supplement and not MGIT Growth Supplement.
3. Aseptically add 0.1 ml (100 microliter) or properly reconstituted STR drug in the STR labeled tube. Similarly, add other drugs in the other labeled tubes. It is important to add the correct amount of drug to each tube. If possible, use a well calibrated micropipette for each addition. Use a separate pipette or micropipette tip for each drug. Do not add any drug to the GC tube.

4. Aseptically add 0.5 ml of the well-mixed culture suspension (inoculum) into each of the drug containing tubes using a pipette. Do not add to the control.
5. For the control, first dilute the test culture suspension 1:100 by adding 0.1 ml of the test culture suspension to 10.0 ml of sterile saline. Mix well by inverting the tube 5-6 times. Use this diluted suspension to add 0.5 ml into the growth control tube.
6. Tighten the caps and mix the inoculated broth well by gently inverting the tube several times.
7. Susceptibility test "Set Carriers" are provided in different numbers of drug combinations. For a routine SIRE test with critical concentration, a Set Carrier of five tubes is used). Place labeled tubes in the correct sequence in the set carrier (GC, STR, INH, RIF, EMB).
8. Enter the susceptibility set carrier into the BACTEC MGIT 960 instrument using the susceptibility test set entry feature. Ensure that the order of the tubes in the AST Set Carrier conforms to Set Carrier definitions. For example, GC, STR, INH, RIF, EMB for the SIRE standard testing.

Results and their interpretations

- The instrument monitors the entered susceptibility test set. Once the test is complete (within 4 to 21 days), the instrument will indicate that the results are ready. Scan the susceptibility Set Carrier and print the report. The instrument printout indicates susceptibility results for each drug. Results are qualitative: Susceptible (**S**), Resistant (**R**) or indeterminate (**X**).
- The instrument interprets results at the time when the growth unit (GU) in growth control reaches 400 (within 4-13 days). At this point, the GU values of the drug vial are evaluated.
- **S** = Susceptible – the GU of the drug tube is less than 100.
- **R** = Resistant – the GU of the drug tube is 100 or more.
- **X** = Error – indeterminate results when certain conditions occur which may affect the test, such as GU of the control reaches >400 in less than 4 days. In such situations, the test should be repeated with pure, actively growing culture which is confirmed to be *M. tuberculosis* complex. Certain drug resistant strains grow very slowly in the medium and the results may not be achieved within 13 days with the standard inoculum. In such a

case, the inoculum should be increased by decreasing the dilution of the culture suspension in order to get reportable results.

Quality control (QC)

It is extremely important to perform a quality control of drug susceptibility testing periodically. The minimum requirement is to test each new batch of reagents, such as SIRE drugs or MGIT medium. If the batch QC fails, all the results obtained within that batch, as well as the new batch of a reagent should be thoroughly reviewed and the testing should be repeated. Use *M. tuberculosis* H37Rv as a QC strain which is susceptible to all anti-tuberculosis drugs (92).

11.5.5. TB Ag MPT64 Rapid test

Test procedures

1. Remove the test cartridge from the foil pouch and place it on the BSC work area
2. Avoid touching the specimen placing area on the cartridge with hands
3. Label each cartridge with the sample identification number
4. Place 100µL of the prepared bacterial culture on the specimen placing area of the test cartridge. Pipette tips should be changed between samples
5. Examine the reading area of the test plate after 15 minutes

Result interpretation

- A color band will appear at the left section of the result window to show that the test is working properly. This band is the Control Line
- The right section of the result window indicates the test results. If another colour band appears at the right section of the result window, this band is the Test Line
- The formation of a purple to red line on the reading areas labelled [T] and [C] of the cartridge indicates a POSITIVE result
- The formation of a purple to red line on the reading area labelled [C] of the cartridge but not [T] indicates a NEGATIVE result
- If no line is observed on the reading area [C], technical errors or product damage has occurred. In this case, the test should be considered invalid and repeated using a new cartridge

11.5.6. Blood agar inoculation and reading

Procedure

1. Take the positive tube from the incubator
2. Inoculate in BA plate
3. Incubate blood agar at 37ºC for 48 hours, checking for growth of contaminants at 18-24 and 48 hours.
4. Record growth status in culture reading worksheet (94).

11.5.7. ZN AFB Smear of positive MGIT cultures

Procedures

1. Mix the MGIT tube
2. Using a sterile pipette remove a small aliquot
3. Place 1 drops of fixative and 1 drop of broth on the slide and spread it on a small area (2 x 1 cm).
4. Let the smear air-dry.
5. Heat-fix the smear by passing it over a flame a 2-5 times
6. Stain the smear with Ziehl-Neelsen method
7. Place a drop of oil on the stained and completely dried smear
8. Screen under a low power objective to locate stained bacteria
9. Switch to oil immersion objective lens for detail observation
10. Record results in culture reading worksheet
11. If the smear is negative for AFB and the broth is clear and no growth on BA, re-incubate the tube for further monitoring
12. After 3 days, visually inspect MGIT tube for growth and repeat AFB smear as above
13. If AFB smear remains negative, contamination is not found on the blood agar plate, the culture is considered to be negative (94).

11.6. Annex 6: English and Amharic study questionnaire

S.No	Sample ID	Laboratory test											
		AFB					Culture						Xpert MTB/ RIF
		1st	2nd	3rd	Result	AFB conc.ed	MGIT Culture	DST					
								RIF	INH	EMB	STM	Result	
1													
2													
3													
4													
5													
6													

11.7. Annex 7: Declaration

DECLARATION:

I the undersigned, declare that this thesis is my original work and has never been presented for the degree in any other university and that all the source materials used for this proposal have duly acknowledged.

I certify that all the information given here above are true.

Principal Investigator	**Signature**	**Date**
Nigusu Fentahun Asress	_____	_____

This research proposal has been submitted with our approval as academic advisors

Principal Advisors	**Signature**	**Date**
Mr. Kassu Desta (MSc, PhD Fellow, Ass. Prof)	_____	_____
Dr. Tsegahun Manyazewal (PhD)	Tsegahun Manyazewal	June 23, 2017
Mr. Zelalem Yaregal (MSc)	_____	_____