

**UNIVERSITY OF GONDAR**  
**COLLEGE OF MEDICINE AND HEALTH SCIENCES**  
**SCHOOL OF BIOMEDICAL AND LABORATORY SCIENCES**  
**DEPARTMENT OF MEDICAL MICROBIOLOGY**



**Prevalence and associated factors of smear positive pulmonary tuberculosis and multi-drug resistance tuberculosis among homeless individuals in Dessie and Debre Birhan towns, Northeast Ethiopia**

**By: Tsedale Semunigus (B.Sc)**

**Advisors: Prof. Feleke Moges (PhD, Professor)**

**Dr. Belay Tessema (PhD, Assistant Professor)**

**A thesis submitted to School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar for partial fulfillment of the requirements for degree of Master of Science in Medical Microbiology**

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**By: Tsedale Semunigus (B.Sc)**

**Mobile: 0963724810**

**Email: [tsedalesemu@gmail.com](mailto:tsedalesemu@gmail.com)**

<b>Advisors</b>	<b>Sign</b>
1. Prof. Feleke Moges	_____
2. Dr. Belay Tessema	_____

<b>Examiners</b>	<b>Name</b>	<b>Sign</b>
1. External examiner	_____	_____
2. Internal examiner	_____	_____

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## List of abbreviations

AFB	Acid-Fast Bacilli
AIDS	Acquired Immune Deficiency Syndrome
BMI	Body Mass Index
CDC	Centers for Disease Control and prevention
DNA	Deoxyribo Nucleic Acid
DR	Drug-Resistant
DST	Drug Susceptibility Test
FM	Fluorescence Microscopy
HIV	Human Immunodeficiency Virus
INH	Isoniazid
LED	Light Emitting Diode
MDR	Multi - Drug Resistant
MTB	<i>Mycobacterium tuberculosis</i>
MTBC	<i>Mycobacterium tuberculosis</i> Complex
PCR	Polymerase Chain Reaction
PTB	Pulmonary Tuberculosis
RIF	Refampicin
rpoB	RNA Polymerase Beta subunit
TB	Tuberculosis
USA	United State of America
WHO	World Health Organization



## Abstract

**Background:** Tuberculosis (TB) remains one of the globe's deadliest communicable diseases. The homeless individuals are at high risk to acquire TB and multi-drug resistant TB (MDR-TB), because of their poor living conditions and risky behaviors. Tuberculosis and MDR-TB in the homeless individuals can pose a risk to entire communities. However, the magnitude of the problem in Ethiopia is not yet determined.

**Objectives:** The aim of this study was to determine the prevalence and associated factors of smear positive pulmonary TB (PTB) and MDR-TB among homeless individuals in Dessie and Debre Birhan towns, Northeast Ethiopia.

**Materials and Methods:** A community based cross-sectional study design was conducted from February to April 2015. Using an active screening with cough of  $\geq 2$  weeks, 351 TB suspects homeless individuals were participated in this study. Data were collected by using pre-tested and structured questionnaire. Spot-morning-spot sputum sample were collected and examined for acid-fast bacilli (AFB) using florescence microscopy by Auramine O staining technique. All AFB positive sputum was further analyzed by GeneXpert for detection of *Mycobacterium tuberculosis* complex and rifampicin resistant gene. Bivariate and multivariate logistic regressions were applied to identify factors associated with smear positive PTB and P-value  $<0.05$  were considered as statistically significant.

**Results:** The prevalence of smear positive PTB and MDR-TB were 2.6% [95% CI: 1.3 – 5] and 0% respectively among TB suspect homeless individuals. Extrapolation of this study finding implies that there were 505 smear positive PTB per 100,000 homeless individuals. Smoking cigarette regularly for greater than 5 years [AOR =10.08, 95% CI: 1.04, 97.66], body mass index lower than 18.5 [AOR = 6.94, 95% CI: 1.17, 41.07] and HIV infection [AOR = 6.75, 95% CI: 1.14, 40.07] were significantly associated with smear positive PTB.

**Conclusion:** This study indicates high prevalence of smear positive PTB, but MDR-TB was not found among TB suspect homeless individuals. The estimated point prevalence of smear positive PTB in homeless individuals was about 4.67 times higher than the general population. Smoking cigarette regularly for greater than 5 years, malnutrition and HIV infection were factors associated with smear positive PTB. Conducting active surveillance of TB and developing and implementing specific TB prevention and control strategies are highly recommended. Further research is needed to strengthen and explore the problem among homeless individuals in depth with large sample sizes and advanced diagnostic techniques.

**Keywords:** Homeless individuals, Tuberculosis, Risk factors, Northeast Ethiopia

# 1. Introduction

## 1.1. Background

Tuberculosis (TB) is an airborne chronic infectious disease mainly caused by *Mycobacterium tuberculosis* (MTB). Occasionally the disease can also be caused by *M. bovis* and *M. africanum* (1). The tubercle bacilli are a rod-shaped called “acid-fast bacilli (AFB)” due to their lipid-rich cell wall ability to retain the primary stain even after decolorizing with acid-alcohol. In addition, the bacilli are non-motile, non-spore forming and slow-growing with a generation time of 12 to 18 hours(2). The bacilli are also obligate aerobes, grow most successfully in areas of the body with lots of blood and oxygen due to this it most commonly affects the lungs, pulmonary TB (PTB), but may also affect any organ or tissue outside of the lungs, extra pulmonary TB (EPTB)(3).

Tuberculosis is mostly transmitted by inhalation of infected droplet nuclei, which are discharged in the air when a person with untreated PTB coughs, sneezes, spits and sings(4). A single cough can generate about 3,000 droplet nuclei and in areas with poor ventilation, the bacilli could remain suspended in the air for several hours. If inhaled, a droplet nucleus is small enough in size (5 to 10 micron) to reach an alveolus in the lung. Once the bacilli reach the alveolar region, alveolar macrophages engulf and control multiplication of bacilli in 90% - 95% of the exposed individuals and no apparent disease is noted i.e. latent infection stage. Only about 5-10% of such infected persons (primary infection) develop active disease in their lifetime without underlying health problems. The most common symptom of PTB is a productive cough for 2 weeks and more duration, which may be accompanied by other symptoms (shortness of breath, chest pains, hemoptysis, loss of appetite, weight loss, fever, night sweats and fatigue)(5).

Globally PTB accounts for 85 % of all TB cases; among them smear positive PTB comprises 75% to 80%. Smear positive PTB are the most infectious and most likely to transmit their disease in to the surroundings; thus, they are the focus for infection control. Therefore, the identification of TB suspects (cough for 2 weeks and more duration) and screening them by examination of sputum allows discovering those who are transmitting the disease(6). The most common method used for diagnosing PTB in adult is sputum smear microscopy, in which the bacilli are observed in sputum samples examined under a microscope. However, culturing mycobacteria is the gold standard method but not feasible as smear microscopy. Following recent breakthroughs in TB diagnostics, the use of rapid molecular tests, like genexpert to diagnose TB and drug-resistant TB(DR- TB ) is increasing(7). Since TB is a curable disease, treatment is often success and involves a

combination of anti-TB drugs. However, if untreated it leads to deaths within 5 years in at least half of the patients and about 25 to 30% could remain chronically ill, as a result continuing to spread the disease in the community(5).

Multi-drug resistant TB (MDR-TB) is MTB resistant to at least isoniazid (INH) and rifampicin (RIF), the two most common powerful first-lines anti-TB drugs(8). The emergence of DR strains is a natural phenomenon, exclusively associated with random chromosomal mutations of MTB(9). It is largely being the consequence of human error as a result of poor patient management. With an appropriate and timely treatment with second line anti-TB drugs, MDR-TB is also curable(10).

Poverty, malnutrition, over-crowded or unsanitary living conditions, low socioeconomic status, drug abuse, cigarette smoking, alcoholism, close contact with active TB cases, human immunodeficiency virus /acquired immune deficiency syndrome(HIV/AIDS) and increasing numbers of homeless people are the greatest risk factors for the acquisition of active TB(3). The synergy between TB and HIV is strong, being infected with HIV increases susceptibility to infection with MTB, the risk of progression to active TB disease. It also increases the chance of re-infections and relapses of TB. The life time risk of HIV infected individuals to develop TB is 20-37 times higher than HIV uninfected individuals(6). In the developing world TB rates are highest among young adults, this leads to grave socio-economic consequences in a country(7).

## **1.2. Statement of the problem**

Tuberculosis remains one of the globe's deadliest communicable diseases. About one third of the world's population is estimated to be infected with tubercle bacilli and hence at risk of developing active disease. Currently TB ranks as the second leading cause of death from an infectious disease worldwide, next to HIV/ADIS. According to World Health Organization (WHO) global TB report 2014, globally an estimated 9.0 million people developed TB, of whom 1.1 million (13%) were HIV positive in the year 2013. In addition 1.5 million died from the disease, of these 360,000 were HIV positive(11). The number of TB deaths is unacceptably large given that most are preventable if people can access for diagnosis and the right treatment of the disease. Short-course regimens of first line anti- TB drugs that can cure about 90% of cases have been available for decades(1).

Nowadays the emergence of MDR-TB has become a series threat to public health and an obstacle to effective TB control worldwide(7). There were an estimated 480,000 new cases of MDR-TB

and 210,000 deaths from MDR-TB worldwide. The African region had about one quarter of the globe's TB cases (280/100,000 incident cases, more than double the global average of 126/100,000) and the highest rates of mortality. The region also had an estimated 44,000 MDR-TB cases among notified PTB cases in the year 2013. The proportion of TB cases co-infected with HIV is highest; overall, the region accounted for 78% of TB cases among people living with HIV(11). Tuberculosis and MDR-TB are the leading causes of morbidity and mortality in the horn of Africa (12).

Ethiopia is among the 22 high TB burden countries and among the 27 high MDR-TB burden countries in the world. Compounded with HIV/AIDS, TB has been a formidable risk to the country(5). According to WHO global TB report 2014, Ethiopia had an estimated 211 prevalent TB case per 100,000 populations and a total of 30,000 TB related deaths. There were 20,000 HIV-positive incident TB cases. Among patients with notified PTB cases in the year 2013, an estimated 1,400 MDR-TB cases were found in the country(11).

*Mycobacterium tuberculosis* can infect anyone who lives and breathes in proximity to active cases but predominantly affects the poor, homeless and other socially marginalized groups who live in overcrowded conditions and/or lack access to healthcare(13). Globally 95% of new TB cases and 98% of deaths from TB are occurred in the developing countries(14).

Homelessness is a global problem; an estimated one hundred million to one billion people are homeless worldwide(15). Homelessness is becoming a common feature of cities and fast growing towns of the poor countries in Africa mainly due to a very high increasing rate of rural-urban migration and poverty(16). Ethiopian cities also have a realm of homeless people, where they thrive in rough streets, bus stations, around the church, abandoned buildings, market streets, and live in temporary shelter with relative inadequate facilities(17).

Because of poor living conditions and as they indulge in risky behaviors and lifestyle homeless people are exposed to many communicable disease(18). The death rate among this group of people is about 4 times higher than the general population(19). Homeless people are included in the high-risk classification for developing TB disease by Centers for Disease Control and prevention (CDC) as they suffer disproportionately from a variety of health problems and emergency shelters remain volatile TB transmission sites(20).

Several risk factors including over-crowded environments, poverty, malnutrition, low socio-economic status, close contact with TB patients, alcoholism, tobacco smoking, lack of stable

housing and HIV /AIDS combine to make TB more prevalent in the homeless individuals. In addition being homelessness are creating favorable conditions for development and transmission of MDR-TB because these groups are hard-to-reach groups, poor diagnostic and treatment services, more likely to incomplete and inadequate TB treatment and poor management of the disease including infection control(21, 22). Many homeless TB patients often will not regard their health as a high priority and may prioritize substance needs such as food, shelter and providing for any addiction(23). Tuberculosis in homeless individuals is especially problematic because it may be highly contagious and can presents as advanced disease with poor outcomes, including mortality(24).

These vulnerable populations in crowded congregate settings have potential for explosive transmission of TB and also resulted TB outbreaks(14). Even in low TB incidence countries such as Canada, TB disease remains concentrated in homeless with outbreaks and continue to challenge TB control programs(25). Generally 72% of domestic TB outbreaks investigated by CDC in the year 2002–2010 involved homelessness in developed countries(26). Therefore, appropriate health interventions should be done for homeless individuals to reduce the adverse outcomes of these communicable diseases(27). Currently, Ethiopia is working towards interrupting transmission of TB, and preventing emergence and spread of MDR-TB in the general population. In spite of these efforts, the problem remained a continuous challenge in the country (28).

Although, homelessness is one of the greatest risk factors for the acquisition of TB, and homelessness is a problem in Ethiopian cities, as to my knowledge the prevalence and associated factors of smear positive PTB and MDR-TB among homeless individuals in Ethiopia has not been reported. Therefore the aim of this study was to determine the prevalence and associated factors of smear positive PTB and MDR-TB among homeless individuals in Dessie and Debre Birhan towns, Northeast Ethiopia.

## **2. Literature review**

### **2.1. Tuberculosis in homeless individuals**

For the homeless individuals, TB has been a serious health threat since the early 20th century(22). Homelessness is associated with a variety of under-privileged social conditions, behavioral and environmental risks that expose people to TB(18). Because of these homelessness-related TB remains a widespread problem(29). Prevention and control of TB and MDR-TB among these vulnerable groups can be hampered by delayed diagnosis, and poor treatment adherence and loss to follow up(30). Although homeless population should be the strategic group in which untreated TB diagnosed, little is known especially in developing country.

### **2.2. Prevalence of tuberculosis in homeless individuals**

In many developed countries disproportionately high prevalence of active TB was reported among the homeless individuals, about 20 times higher than the general population(31). In Africa, where HIV/AIDS, poverty and chronic malnutrition are unacceptably rampant, the homeless individuals probably have a high burden of TB. However, published information about TB in African homeless individuals is very scarce.

A cross-sectional analysis conducted in United State of America (USA) showed that of 185,870 TB cases reported from the 50 States and the District of Columbia from 1994 through 2003, 11,369 (6.1%) were either culture confirmed or smear positive TB among homeless individuals. The annual proportion of TB cases associated with homelessness was (6.1%-6.7%)(32). Another retrospective study conducted in the same country from 2004 to 2009 showed that the prevalence of TB was 3.28 % among the homeless individuals. Tuberculosis was reported to be considerably greater among the homeless population compared to the state average(33).

A retrospective study conducted in USA from 1994 to 2010 showed that of 270,948 reported TB cases, 16,527 (6%) were found in homeless individuals. The TB incidence rate among homeless individuals ranged from 36 to 47 cases per 100,000 populations in the year 2006 to 2010. Compared to the general population, homeless individuals had an approximately 10-fold increase in TB incidence(34). Tuberculosis is more prevalent respiratory infection among homeless individuals, the estimated annual PTB rate for Canada and the USA is 2/100,000 but the homeless individuals comprise a disproportionate burden of TB (1.6% to 6.8%)(35).

Tuberculosis outbreak associated with a homeless shelter in Kane County, Illinois, between 2007 and 2011 showed that despite the overall decline in TB incidence in the USA, a total of 28 outbreak of TB occurred among homeless individuals(36). A prevalence survey of respiratory diseases conducted in Marseilles, in 2005 showed that among 221 homeless individuals, active PTB was found in 2 (1%) of individuals(37).

A prospective study conducted in Medellin city, Colombia between July 2006 and December 2007 among homeless individuals showed that the prevalence of TB was 7.9%. In this case, 426 homeless individuals enrolled in the study and 34 were diagnosed as culture positive for MTB(38). A systematic review and meta-analysis of TB, hepatitis C virus, and HIV prevalence in homeless individuals showed that, of these infectious diseases the prevalence of TB ranged from 0.2% to 7.7%(19).

A cross-sectional study conducted in Japan at the Osaka socio-medical center hospital between July 2007 and March 2008 showed that the prevalence of active TB was 1.5% among homeless individuals. According to annual TB patients' registry database, the prevalence of active TB among residents in the Airin district was 0.65% in 2007, and the homeless individuals accounted for 79.3% of TB patients in that district. These estimates of active TB prevalence in homeless individuals were noticeably higher than those of the total Japanese population and the Osaka city population in 2007(29).

A cross-sectional study conducted in Seoul, South Korea between October 2009 and April 2010 showed that the prevalence of active PTB diagnosed by chest radiography were 24.86%. This showed that the prevalence of active TB among homeless participants was much higher than the general population(24). Another cross-sectional study conducted in Rome from November 2006 to November 2007 showed that prevalence of active TB among homeless individuals was 3.86%(39).

An intervention study conducted in North-eastern Poland between September and January 2011 showed that among 121 homeless individuals, the prevalence of PTB was 4.13%. In this study participants were first screened by chest radiography, then sputum samples were examined by molecular testing and culture(40). A cohort study conducted in London on July 2003 with 1941 eligible patients showed that an extremely high prevalence of TB was seen in homeless individuals (780/100,000) than the overall prevalence of TB (27/100,000). Homelessness was significantly associated with infectious TB, comprise 17% of TB cases among eligible patients(41). A study on vulnerability of homeless people to HIV, TB and Viral Hepatitis

in Tehran, Iran, between June and August 2012 with a convenience sample of 593 homeless individuals showed that the prevalence of active PTB was 1.2% and HIV infection were (3.4%)(27).

The first Ethiopian National population based TB prevalence survey conducted between October 2010 and June 2011 showed that, a total of 46,548 participants were first screened by chest radiography and TB suggestive symptoms. Among them a total of 6,080 (13%) TB suspect participants sputum was examined and smear positive PTB were found in 47(0.77%) participants. The point prevalence of smear positive PTB among people aged 15 and above was 108/100,000 population(42).

### **2.3. Prevalence of multi-drug resistant tuberculosis in homeless individuals**

A cross-sectional study conducted in USA between 1994 and 2003 showed that drug susceptibility test (DST) was performed for 661 TB patients homeless individuals among them, MDR-TB was found in 18 (2.7%) homeless individuals(32). Another retrospective study conducted in the same country between 1994 and 2010 showed that for 2101 homeless TB patients genotype test was performed among them, MDR-TB was found in 24(1.1%) homeless individuals(34).

A cross-sectional analysis conducted in Busan Medical Center, Korea between January 2001 and December 2010 showed that DST was performed for 96 smear positive PTB homeless patients. The result indicated that 24 (25%) cases were resistant to one or more anti-TB drugs and 11 (11.5%) cases were MDR-TB(21). A cohort study conducted in London on July 2003 showed that of 77 culture confirmed TB cases, 30 (39%) cases were resistance to one or more anti-TB drugs and 5 (6.5%) cases were MDR-TB among homeless individuals(41).

### **2.4. Factor associated with tuberculosis in homeless individuals**

A cross-sectional study conducted in USA between 1994 and 2003 revealed that alcohol abuse, HIV infections and drug use were factors associated with TB among homeless individuals(32). Another retrospective study conducted in the same country between 2004 and 2009 showed that drug use, HIV/AIDS and environments such as congested living arrangements inside homeless place them at greater risk for contracting TB(33). A retrospective cohort study conducted in Montreal, Canada from 1996 to 2007 showed that HIV infection, smoking, alcohol and drug use were factors associated with TB among homeless people(43).



A cross-sectional study conducted in Seoul, South Korea showed that risk factors for active PTB were a history of previous TB and BMI less than 18.5(24). Similar study conducted in Rome from November 2006 to November 2007 shows that under nutrition, smoking and alcohol misuse are risk factors for active TB development and all these conditions are associated with homelessness(39).

### **3. Justification of the study**

Tuberculosis and MDR-TB among homeless individuals is a public health concern. The homeless individuals are more vulnerable than the general population to acquire TB and MDR-TB, as they indulge in risky behaviors and lifestyle. Prevention and control of TB among these risk groups is hindered by delayed diagnosis, onward transmission and poor treatment adherence leading to the development of MDR-TB. These populations also have worse treatment outcomes including mortality. So these groups require vigorous scrutiny and innovative treatment strategies. As TB prevalence is one indicator of millennium development goals, this study will be significant, especially in countries where the incidence and prevalence of TB is high.

In Ethiopia, even if there are a number of studies conducted on prevalence and associated factors of smear positive PTB and MDR-TB in different segment of population, the magnitude of the problem among homeless individuals is not yet determined. Better understanding of the characteristics of homeless individuals with smear positive TB and MDR-TB is critical for creating strategies to decrease disease incidence and for reducing associated factor in this vulnerable population. Thus, this community based cross-sectional study was conducted, to determine the prevalence and associated risk factors of smear positive PTB and MDR-TB among homeless individuals in Dessie and Debre Birhan towns, Northeast Ethiopia. This study may serve as a baseline for future studies in our country particularly in this target population.

Therefore, based on the findings of this study, recommendations might be provided to facilitate decision making to reduce potential sources of transmission and provide appropriate prevention and control measures for these high risk populations. Moreover, the result will have potentials for developing and implementing TB and MDR-TB prevention and control strategies in homeless individuals in the study area. Furthermore, as a disease is transmitted by aerosol it will persuade policy makers, program managers, health providers, community groups, nongovernmental organization and scientific communities to take action and conduct intervention for the well being of homeless people and general population at large.

## **4. Objectives**

### **4.1. General objective**

- To assess the prevalence and associated factors of smear positive pulmonary tuberculosis and multi-drug resistance tuberculosis among homeless individuals in Dessie and Debre Birhan towns, Northeast Ethiopia.

### **4.2. Specific objectives**

- To determine the prevalence of smear positive pulmonary tuberculosis among homeless individuals.
- To determine the prevalence of multi-drug resistance tuberculosis among homeless individuals.
- To identify factors associated with smear positive pulmonary tuberculosis among homeless individuals.

## **5. Materials and Methods**

### **5.1. Study area**

Dessie town is located in the Northeast part of Ethiopia in the South Wollo administrative zone of the Amhara Region, which is 400 km away from Addis Ababa, the capital city of Ethiopia. The town has a total area of 15.08 km<sup>2</sup> with 10 kifleketema and a total population of 279,423. The climate is highland with an elevation of 2,470 meters. The city administration estimates the number of homeless individuals to be 2100 in the year 2014.

Debre Birhan town is located in the Northeast part of Ethiopia in the North Shewa zone of the Amhara Region, about 120 kilometers Northeast of Addis Ababa on the paved highway to Dessie. The town has a total area of 14.71 km<sup>2</sup> and a total population of 110,408. The climate is also highland with an elevation of 2,840 meters. The city administration estimates the number of homeless individuals to be 1900 in the year 2014.

### **5.2. Study design and periods**

A community based cross-sectional study design was conducted from February 10 to April 30, 2015 to assess the prevalence and associated factors of smear positive PTB and MDR-TB among homeless individuals in Dessie and Debre Birhan towns, Northeast Ethiopia.

### **5.3. Population**

#### **5.3.1. Source population**

All homeless individuals who live in Dessie and Debre Birhan towns were source population for this particular study.

#### **5.3.2. Study population**

Homeless individuals, who were aged  $\geq 15$  years and eligible during the study period were the study population.

### **5.4. Inclusion criteria**

Homeless individuals who had cough of two weeks and more duration were included in the study.

## 5.5. Exclusion criteria

Homeless individuals who were unable to produce sputum and/or provided incomplete information were excluded from the study.

## 5.6. Variables

### 5.6.1. Dependent variable

Smear positive pulmonary tuberculosis

Multi- drug resistance tuberculosis

### 5.6.2. Independent variable

**Socio demographic factors:** - age, sex, marital status, religion, educational status

**Behavioral factors:** - smoking, duration of smoking, alcohol drinking, duration of alcohol drinking, khat chewing, duration of khat chewing, drug using, duration of drug using

**Environmental factors:-** duration of being homelessness, number of homeless individuals slept/live together in one restricted place, close contact with known TB patients, close contact with chronically cougher patients

**Morbidity history and status:** - current TB suggestive symptoms, past TB history, past TB treatment starting, completion of TB treatment, BMI, HIV infection

## 5.7. Operational definition

**Homeless individuals:-** people who lack fixed, regular, and adequate night-time residence or who are street dwellers and living in emergency and temporary accommodation such as night shelters for more than one month(29).

**AFB smear positive** – any positive with FM smear microscopy(44).

## 5.8. Sample size determination and sampling technique

### 5.8.1. Sample size

The sample size was determined using a single population proportion formula with the assumption of 5% margin of error, 95% CI and 50% proportion

$$n = z^2_{\alpha/2} p (1-p) / d^2$$

Where, n = the minimum sample size required

$Z_{\alpha/2}$  = critical value for normal distribution at the 95% CI, which is 1.96

d= relative precision

P = the prevalence of smear positive TB and MDR-TB among homeless individuals was taken as 0.5 since no previous similar study conducted in Ethiopia

Substituting from the formula:

$$n = \frac{(1.96)^2 * 0.5(1-0.5)}{(0.05)^2} = 384$$

Hence, the total population is less than 10,000: sample size correction formula was used

$$nf = n_o / (1 + (n_o/N))$$

Where, nf = final sample size

$n_o$  = initial sample size

N = total population

Substituting from the formula:

$$nf = 384 / (1 + (384/4000))$$

$$nf = 350$$

The final sample size will be 385 after taking into account a 10% non-response rate.

### **5.8.2. Sampling technique**

Using an active screening strategies to identify PTB suspects about 1,780 homeless individuals were screened during the study period for symptoms considered suggestive for TB, cough of two weeks or more duration according to the National TB manual(5). Out of the total screened, 385 homeless individuals were had cough of  $\geq 2$  weeks duration, were included in to the study.

## **5.9. Data collection and laboratory methods**

### **5.9.1. Data collection**

A structured and pre-tested questionnaire was completed by 4 trained data collectors (2 laboratory technologists and 2 nurses) by face-to-face interview. The questionnaire had four parts; socio demographic characteristics, behavioral characteristics, environmental factors and morbidity history and status of the study participants. A questionnaire was first developed in English and then translated in to Amharic language for appropriateness and clarity so; the participants were interviewed with their mother languages and finally retranslated to English by another language expert to check its consistency.

### **5.9.2. Sputum sample collection and florescence microscopy examination**

A bout 3 to 10 ml of spot- morning-spot sputum samples were collected using coded and new, translucent, screw-capped specimen collection containers by laboratory technologist from the study participants. The sputum samples were placed in cold boxes immediately upon receipt and delivered to Dessie and Debre Birhan referral hospitals laboratory on the day of collection. Sputum-smear microscopy was performed using Primo Star iLED, light emitting diode (LED)- florescence microscopy (FM) by using Auramine O staining procedure as follows; a smear was prepared and dried, then heat-fixed. Stained the smear with filtered 0.1% Auramine O solution and kept the staining reagent for 20 minutes and washed well. Decolorized with 0.5% acid-alcohol and kept for 3 minutes and gently rinsed with water. Counterstained with 0.5% potassium permanganate solution for 1 minute then gently rinsed with water and drained. Finally, wept the back of the slide cleaned, air-dried and the stained slides were observed under 20x, 40x magnifications of FM for AFB. The AFB was appeared bright yellow against dark back ground materials(45).

### **5.9.3. GeneXpert examination**

All AFB positive sputum samples were subjected to Cepheid GeneXpert MTB/RIF system in Dissie and Debre Birhan referral hospitals laboratory. The system is a fully automated nested real-time polymerase chain reaction (PCR), which simultaneously detects *Mycobacterium tuberculosis* complex (MTBC) and mutations in the ribonucleic acid polymerase Beta subunit gene (*rpoB*), which are responsible for the resistance to RIF(46).

To perform the test first, the GeneXpert assay sample reagent was added in a 2:1 ratio to the sample tubes to kill mycobacteria and liquefy the sputum sample. Then the mixture were vigorously shaken and allowed to sit for 10 minute and again shaken and allowed to sit for another 5 minute. Finally, 2 ml were pipetted into the GeneXpert cartridge and inserted into the GeneXpert instrument for PCR testing. By starting the test on the system software, the GeneXpert automates all the subsequent steps, including sample work-up, nucleic acid amplification, detection of the target sequence and result interpretation.

The primers in the GeneXpert assay amplify a portion of the *rpoB* gene containing the 81 base pair “core” region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with resistance to RIF. The MTB/RIF assay as an entirely self-contained test with quality control incorporated. The assay includes a sample

processing control to control adequate processing of the target bacteria and to monitor the presence of inhibitors. In addition, a probe check control to validate reagent rehydration, PCR tube filling in the cartridge, probe integrity and dye stability(47). The detection of resistance to RIF can be used as a surrogate marker for MDR-TB with a high level of accuracy, since resistance to RIF in most instances (greater than 90%) co-exists with resistance to INH(48).

#### **5.9.4. Rapid HIV test**

To determine the HIV status of the study participants, pre-test counseling was provided by trained nurse. Then whole blood was collected by finger stick. The presence of HIV-1 and HIV-2 antibodies was determined by using rapid test kit, HIV (1 + 2) antibody Colloidal Gold (KHB, Shanghai Kehua Bio-engineering Co Ltd, China) as a screening test, followed by HIV 1/2 STAT-PAK® (Chembio Diagnostics, USA), when KHB result was reactive. Where the result of STAT-PAK® was discordant with KHB, a third test, Unigold™ HIV (Trinity Biotech, Ireland), was also used as a tiebreaker to determine the test result following the manufacturers' instruction. After testing, post test counseling was provided for all participants.

#### **5.9.5. Nutritional assessment**

The participants body weight and height was measured by digital scale to the nearest 0.1 kg and 0.1 cm respectively. Body mass index is defined as the weight in kilogram of the individual divided by the square of the height in meter. It is used to determine the nutritional status of study participants into malnutrition (BMI = less than 18.5 kg/m<sup>2</sup>), normal (BMI = 18.5-24.9 kg/m<sup>2</sup>) and overweight (BMI = 25.0 – 29.9 kg/m<sup>2</sup>) as recommended by CDC(49).

#### **5.9.6. Quality assurance**

A pre-test was done in Kombolcha town on 20 (5%) homeless individuals who were similar with study participants prior to the data collection to check the clarity and consistency of the questionnaires and acceptability of laboratory procedure. Necessary correction was taken before the actual data collected. The data collectors, who can speak the local language (Amharic), were trained for one day on data collection procedures for this study to attain standardization and maximize interview reliability.

The purpose of the study was informed to study participants for the quality of the data. In addition the study participants were instructed on how to produce appropriate sputum specimen. Instruments and reagents were checked for reliability and reproducibility of the test before any



test started. All new lots of reagents were tested with known positive and negative control. All positive microscopy slides and 10% percent of negative slides were double checked by second experienced laboratory technologists for confirmation.

The data collections, application of standard laboratory test procedure and test result were checked by senior laboratory technologist and principal investigator. Filled questionnaire and laboratory test result were collected after checking consistency and completeness. The overall data collection process was supervised by principal investigator.

#### **5.10. Data processing and analysis**

Following the data collection, data were checked, coded and entered using EPI-INFO version 3.5 and exported to SPSS version 20 for analysis. Both descriptive and analytical statistical procedures were utilized. Descriptive statistics like percentage, mean and standard deviation were used for presentation of data and prevalence of smear positive PTB and MDR-TB. Table and graphs were also used for data presentation. All variables of the study were initially tested for association with smear positive PTB by using binary logistic regression model. Those variables which have p-value less than 0.2 by binary logistic regression were put in the multivariable analysis model to control the possible effect of confounders. Finally the variable which has independent association with smear positive PTB was identified on the basis of odd ratio (OR) with 95% confidence interval (CI) and P-value less than 0.05. The variable was entered to multivariate model using the forward stepwise (likelihood ratio) regression method. Model fitness was checked using Hosmer and Lemeshow goodness of a fit test (0.70).

#### **5.11. Ethical clearance**

Primarily, ethical clearance was obtained from ethical review board of School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar. Then official permission letters were obtained from Dessie and Debre Birhan towns' administrative body. Written informed consent was obtained from all study participants after adequate information about the potential benefits and risks of the study had been provided. Those participants with positive results for smear positive PTB and /or HIV infection were referred to the nearby health facility. Health education was also given for positive participants to prevent spread of infection to others. The collected data were kept confidentially by using anonymous instead of any personal identifiers.

## **5.12. Dissemination of results**

The finding of this study will be communicated to the School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Dessie and Debre Birhan towns' administrative body, Amhara Regional Health Bureau and Federal Ministry of Health. The research finding will be presented at different scientific conference and finally the manuscript will be prepared for publication in a standard journal.

## 6. Results

### 6.1. Socio-demographic characteristics of the study participants

A total of 351 study participants were participated in this study giving a response rate of 91.17%. Thirty four (8.83%) participants were unable to produce sputum and/or provided incomplete information. Out of the total study participants 190(175 males and 15 females) were from Dessie town that constituted 54.13% of the total participants and 161 (139 males and 12 females) were from Debre Birhan town that comprised 45.87% of the total participants. Among the total study participants, majority 324(92.7%) were male and 333(95.9%) were between 15-44 years of old. The mean age of the participants were 26.70 (SD  $\pm$  7.96). About 163 (46.4%) of the study participants were illiterate and 180 (51.3%) of the study participants had primary school (1-8 grade) education level. Most of the participants 308 (87.7%) were single and 301(85.7%) of the participants were follow Orthodox Christians religion (Table1).

Table 1. Socio-demographic characteristics of homeless individuals with smear positive PTB prevalence, Dessie and Debre Birhan towns, Northeast Ethiopia, 2015(N = 351).

Variables	Label	Smear positive PTB		Frequency N (%)
		Negative n (%)	Positive n (%)	
Age	15-24	158(98.1)	3 (1.9%)	161 (45.9)
	25-34	138 (97.2)	4 (2.8)	142 (40.5)
	35-44	28(93.3)	2 (6.7)	30 (8.5)
	45-60	18 (100)	0 (0)	18 (5.1)
Sex	Male	316(97.5)	8 (2.5)	324 (92.3)
	Female	26(96.3)	1 (3.7)	27 (7.7)
Marital status	Single	300(97.4)	8 (2.6)	308 (87.7)
	Married	11(91.7)	1 (8.3)	12 (3.4)
	Divorced	17(100)	0 (0)	17 (4.8)
	Widowed	14(100)	0 (0)	14 (4.0)
Educational status	Illiterate	158(96.9)	5 (3.1)	163 (46.4)
	Primary	176(97.8)	4 (2.2)	180 (51.3)
	Secondary	8(100)	0 (0)	8 (2.3)
Religion	Orthodox	293(97.3)	8 (2.7)	301 (85.8)
	Muslim	47(97.9)	1 (2.1)	48 (13.7)
	Protestant	2(100)	0 (0)	2 (0.6)

## 6.2. Behavioral characteristics of the study participants

Out of the total study participants 169 (48.1%) were smoke cigarette during the study periods, of these, 67 (39.6%) were smoke regularly for greater than 5 years. The mean smoking periods of the participants were 64.70 (SD  $\pm$  43.59) months. Two hundred sixty three (74.9%) participants were drunk alcohol during the study periods of whom 164 (62.4%) were drunk regularly for greater than 5 years. The mean alcohol drinking periods of the participants were 81.86 (SD  $\pm$  56.85) months. More than half of the respondents 195(55.6%) were chew khat during the study periods, of whom 89 (45.6%) were chew regularly for greater than 5 years. The mean khat chewing periods of the participants were 64.07 (SD  $\pm$  46.22) months. About 10 (2.8%) of the participants were use drug during the study periods (Figure 1).

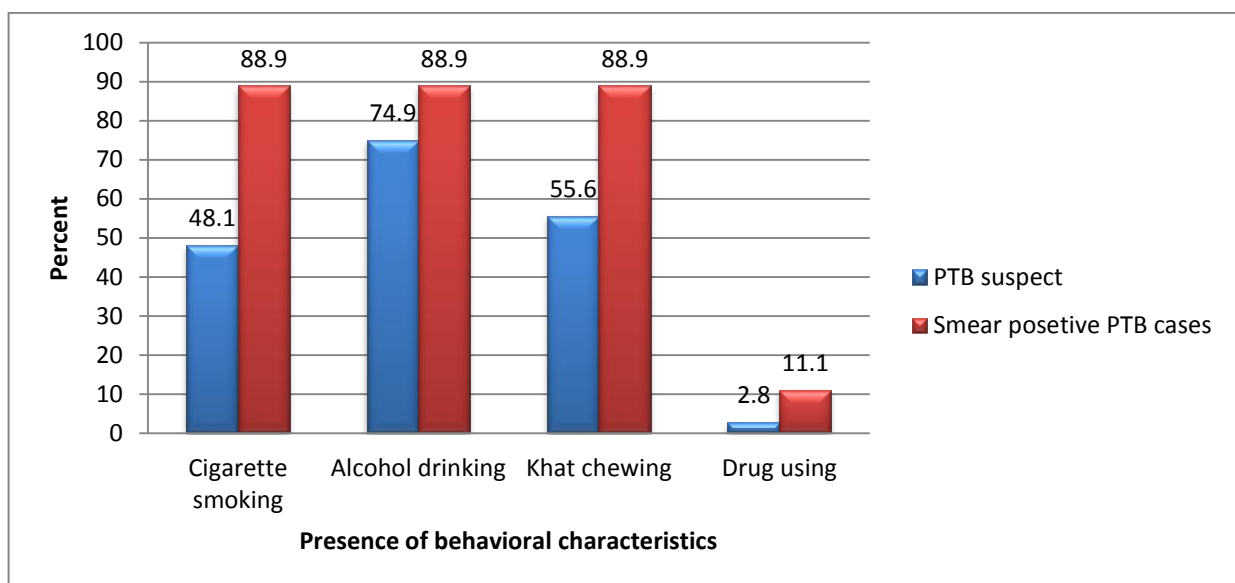


Figure 1. Presence of behavioral characteristics of homeless individuals with smear positive PTB prevalence, Dessie and Debre Birhan towns, Northeast Ethiopia, 2015 (N=351).

## 6.3. Environmental factors of the study participants

The participants mean duration of being homelessness was 65.92(SD  $\pm$  49.16) months and about 148 (42.2%) of the participants were homelessness for greater than 5 years. The average number of homeless individuals slept/ live together in one restricted homeless shelter were 5 (SD  $\pm$  2.99) and almost half 163 (46.4%) of the participants were slept/ lived together in one restricted homeless shelter by being more than 5 persons. Among the study participants 17 (4.8%) and 114 (32.5%) had close contact with known TB patients and chronically cougher patients respectively (Table 2).

Table 2. Environmental factors of homeless individuals with smear positive PTB prevalence, Dessie and Debre Birhan towns, Northeast Ethiopia, 2015(N=351).

Variables	Label	Smear positive PTB		Frequency N (%)
		Negative n (%)	Positive n(%)	
Duration of being homelessness	≥ 5 years	141(41.2)	7(77.8)	148 (42.2)
	≤ 5 years	201(58.8)	2(22.2)	203 (57.8)
Average number of homeless individuals slept/ lived together	≥5 persons	157(45.9)	6(66.7)	163 (46.4)
	≤ 5 persons	185(54.1)	3(33.3)	188 (53.6)
Close contact with known TB patients	Yes	15(4.4)	2(22.2)	17 (4.8)
	No	327(95.6)	7(77.8)	334 (95.2)
Close contact with chronically cougher	Yes	108(31.6)	6(66.7)	114 (32.5)
	No	234(68.4)	3(33.3)	237 (67.5)

#### 6.4. Morbidity history and status of the study participants

Out of the total study participants 85(24.2%) participants were had chest pain, followed by difficulty of breathing 79(22.5%) and night sweating 67(19.1%) (Figure 2. ).

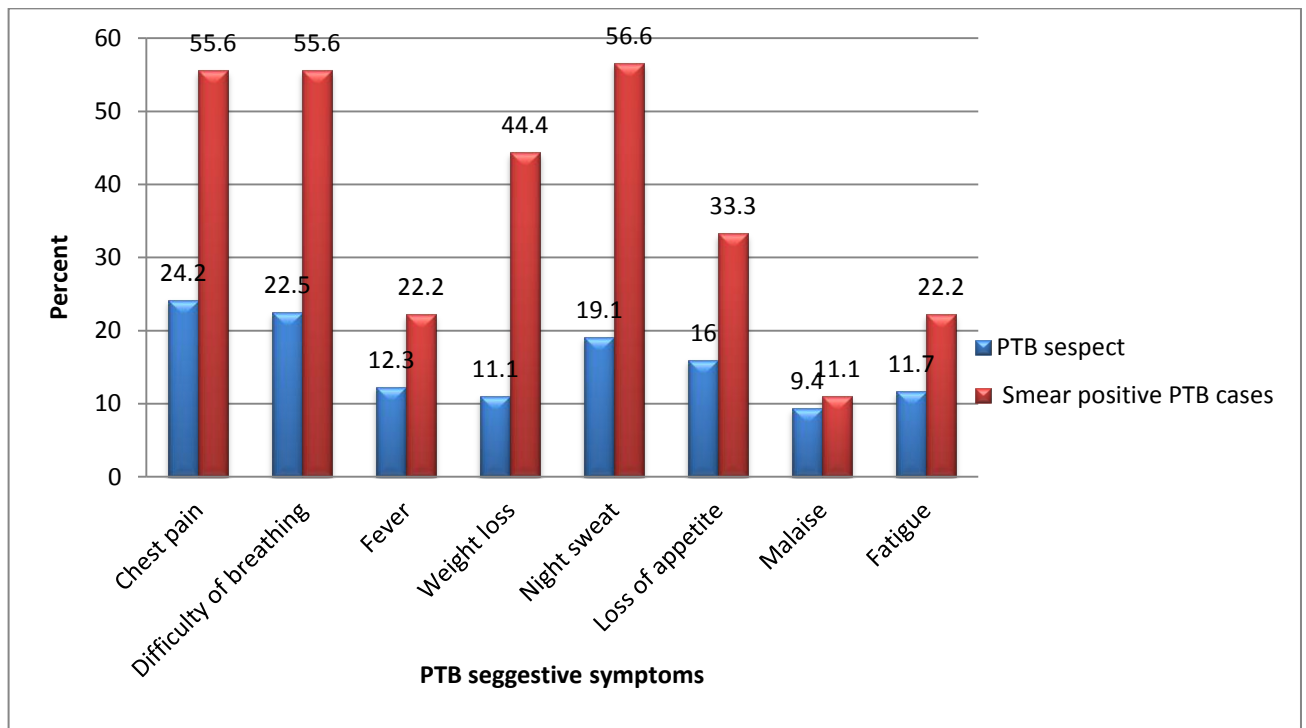


Figure 2. Presence of PTB suggestive symptoms among homeless individuals with smear positive PTB prevalence, Dessie and Debre Birhan towns, Northeast Ethiopia, 2015 (N=351).

Out of the total study participants 10(2.8%) participants had past history of TB disease, all of these were diagnosed during being homelessness and all were start anti-TB treatment but more than half of them 6 (60%) were defaulted anti-TB treatment. Out of the total study participants 25 (7.1%) participants were malnourished, BMI less than 18.5. Out of the total study participants 22 (6.3%) participants were HIV infected (Table 3).

Table 3. Morbidity history and status of homeless individuals with smear positive PTB prevalence , Dessie and Debre Birhan towns, Northeast Ethiopia, 2015(N = 351)

Variables	Label	<u>Smear positive PTB</u>		Frequency N (%)
		Negative (%)	Positive (%)	
<b>Past history of TB</b>	Yes	9(2.6)	1(11.1)	10 (2.8)
	No	333(97.4)	8(88.9)	341 (97.2)
<b>Past anti- TB treatment</b>	Defaulted	5(55.6)	1(100)	6 (60)
	Completed	4(44.4)	0(0)	4 (40)
<b>BMI (kg/m<sup>2</sup>)</b>	≤18.5	20(5.8)	5(55.6)	25(7.1)
	≥ 18.5	322(94.2)	4(44.4)	326 (92.9)
<b>HIV antibody</b>	Reactive	17(5)	5 (55.6)	22 (6.3)
	Non reactive	325(95)	4(44.4)	329 (93.7)

### 6.5. Prevalence of smear positive pulmonary tuberculosis and multi-drug resistant tuberculosis in homeless individuals

Out of the total study participants, smear positive PTB was detected in 9 of the participants (8 males and 1 female) by LED-FM. All smear positive PTB sputum specimen were further analyzed by GeneXpert assay, the assay confirmed that all were positive for MTBC but none were resistant to RIF. Therefore, the prevalence of smear positive PTB was 2.6% (95% CI: 1.3%, 5%) among the study participants. The point prevalence of smear positive PTB was extrapolated to be 505/100,000 homeless individuals. All smear positive PTB cases are found in the age group (17 – 44 years) of the participants. About 8(88.9%) smear positive PTB cases were found in the study participants who smoke cigarettes, drink alcohol and chew khat during the study periods. Five (55.5%) smear positive PTB cases are found in the study participants who were malnourished during the study periods. In addition among the total smear positive PTB participants, 5(55.5%) were co-infected with HIV infection.

## **6.6. Factors associated with smear positive tuberculosis in homeless individuals**

Bivariate logistic regression analysis showed that there were statistically significant association between smear positive PTB and smoking, smoking cigarette regularly for greater than 5 years, chewing chat regularly for greater than 5 years, being homelessness for greater than 5 years, close contact with known TB patients, close contact with chronically cougher patients, chest pain, difficulty of breathing, weight loss, night sweating, BMI less than 18.5 and HIV infection. However, smoking cigarette regularly for greater than 5 years, BMI less than 18.5 and HIV infection were had statistically significant association with smear positive PTB in multivariable logistic regression after controlling the effect of confounder.

Multivariate analysis revealed that, participants who smoke cigarette regularly for greater than 5 years were 10.08 times more likely to have smear positive PTB than those who smoke cigarette regularly for less than 5 years [AOR =10.08, 95% CI: 1.04, 97.66]. The study also showed that statistically significant association between smear positive PTB and BMI. Participants who had BMI less than 18.5 were 6.94 times more likely to have smear positive PTB as compared to those who had BMI greater than 18.5 [AOR =6.94, 95% CI: 1.17, 41.07]. Furthermore, HIV infected homeless individuals were 6.75 times more likely to have smear positive PTB than those HIV uninfected homeless individuals [AOR =6.75, 95% CI: 1.14, 40.07] (Table 4).

Table 4. Factors associated with smear positive pulmonary tuberculosis in homeless individuals (bivariate and multivariate analysis) in Dessie and Debre Birhan towns, Northeast Ethiopia, 2015

Variables	Smear positive PTB		COR (95% CI)	AOR (95% CI)	p- value
	Negative	Positive			
<b>Smoking cigarette</b>					
Yes	161	8	8.99(1.11-72.69)*	_____	_____
No	181	1	1		
<b>Smoking duration</b>					
Greater than 5 years	60	7	11.78(1.41-98.12)*	10.08(1.04-97.66)*	0.046
Less than_5 years	101	1	1	1	
<b>Khat chewing</b>					
Yes	187	8	6.63(0.82-53.6)	_____	_____
No	155	1	1		
<b>Khat chewing duration</b>					
Greater than 5 years	82	7	8.96(1.08 -74.31)*	_____	_____
Less than_5 years	105	1	1		
<b>Drug using</b>					
Yes	8	1	4.62(0.52-40.98)	_____	_____
No	334	8	1		
<b>Duration of being homelessness</b>					
Greater than 5 years	141	7	4.99 (1.02-24.37)*	_____	_____
Less than_5 years	201	2	1		
<b>Close contact with known TB patients</b>					
Yes	15	2	6.23(1.19-32.58)*	_____	_____
No	327	7	1		
<b>Close contact with chronically cougher</b>					
Yes	108	6	4.33(1.06- 17.65)*	_____	_____
No	234	3	1		
<b>Chest pain</b>					
Yes	80	5	4.09 (1.07 – 15.61)*	_____	_____
No	262	4			
<b>Difficulty of breathing</b>					
Yes	74	5	4.53 (1.19 – 17.28)*	_____	_____
No	268	4	1		
<b>Weight loss</b>					
Yes	35	4	7.02(1.80 -27.35)*	_____	_____
No	307	5	1		
<b>Night sweating</b>					
Yes	62	5	5.64 (1.47- 21.63)*	_____	_____
No	280	4	1		
<b>Loss of appetite</b>					
Yes	53	3	2.72(0.66-11.24)	_____	_____
No	289	6	1		
<b>Past TB history</b>					
Yes	9	1	4.62(.52-40.98)	_____	_____
No	333	8	1		
<b>BMI</b>					
Less than_18.5	20	5	20.12(5.01–80.81)*	6.94 (1.17 –41.07)*	0.033
Greater than 18.5	322	4	1	1	
<b>HIV anti - body</b>					
Reactive	17	5	23.89 (5.88–97.13)*	6.75(1.14 – 40.07)*	0.036
Non reactive	325	4	1	1	

\* Statistically significant variable to smear positive PTB which, p- value less than 0.05



## 7. Discussion

This study showed that the prevalence of smear positive PTB among TB suspect homeless individuals was 2.6%. This finding is in line with studies conducted in USA (3.28%)(33), Japan(1.5%)(29) and Rome (3.86%)(39). The finding is also supported by systematic review and meta-analysis of prevalence of active TB among homeless individuals, estimated to be 0.2–7.7%(19). In this study all TB cases are found in young and productive age group (17–44 years) of the participants. This finding is similar to the developing world TB rates, highest among young adults which leads to grave socio-economic consequences in a country(7). This high TB burden may also attribute to an aggressive transmission of TB in the homeless individuals and to the surrounding community.

Moreover, TB prevalence in this study is to some extent higher than studies conducted among homeless individuals in Marseilles (1%)(37) and Iran (1.2%)(27). The relatively lower prevalence of TB in those countries could be due to high socio-economic status and low overall TB prevalence in the countries. In addition relatively lower prevalence of TB in Iran might be due to lower prevalence of HIV infection (3.4%)(27).

However, TB prevalence in this study is lower than studies conducted among homeless individuals in USA (6.1%)(32), Seoul, South Korea (24.86%)(24), North-eastern Poland (4.13%)(40) and Colombia (7.9%)(38), the difference might be due to difference in study design and setting, sample size and laboratory diagnosis method used. In USA the analysis was covered a wide geographic area with large sample size and the cases were either smear positive PTB or culture confirmed other form of TB(32). On the other hand in South Korea the prevalence of active TB was not based on sputum smear AFB but, it is based on chest radiography(24) this could be over sensitive and not specific enough as FM thus might be increase the prevalence of TB than this study. In North-eastern Poland, the participants were first screened by chest radiography, then molecular testing and culture were performed(40), the use of an advanced diagnosis technique might be not underestimated the actual prevalence. In Colombia all reported case are culture positive for MTB(38), could be revealed all types of TB in addition to smear positive PTB.

Several studies indicated that higher prevalence of TB in homeless individuals than the general population (29, 35, 41). For example, in USA compared to the general population, homeless individuals had an approximately 10-fold increase in TB prevalence(34). Extrapolation of this study finding also indicated that about 4.67 times high burden of smear positive PTB in homeless

individuals than the general population (108/100,000) in Ethiopia(42). This study point prevalence of smear positive PTB is comparable with study conducted in Greater London (780/100,000)(41). Furthermore despite the overall decline TB incidence in the general population, 28 outbreaks of TB occurred in Illinois among homeless individuals, indicating that ongoing transmission of TB to the homeless individuals and the entire community(36). These disproportionate burden of TB in homeless individuals might be due to that these groups are the most neglected and live in under-privileged social conditions such as poverty, malnutrition and overcrowd unhygienic environment with relatively limited access to health care(21).

In this study MDR-TB was not found. However, studies conducted in USA(2.7%)(32), USA(1.1%)(34), Busan Medical Center, Korea (11.5%)(21) and London(6.5%)(41) MDR-TB was found among homeless individuals. Absence of MDR-TB in this study might be due to the small number of smear positive PTB cases enrolled in the study. However well known risk factors for the development of MDR-TB, previous anti-TB treatment defaulter rate is high (60%) in this study.

In this study participants who smoke cigarette regularly for greater than 5 years were 10.08 times more likely to have smear positive PTB than those who smoke cigarette regularly for less than 5 years. Even though there was no other studies considered duration of smoking in homeless individuals, the role of smoking in the development of active TB is well established(50) either through increased susceptibility to new infection with MTB or increase the risk of developing active TB(51). Thus, increasing duration of smoking might increase the development of TB disease. Smoking cigarette by itself were reported as a risk factor for acquisition of active TB in Rome(39) and Montreal, Canada (43) but in this study it was not significantly associated. However, in this study about 88.9% smear positive PTB cases were found among cigarette smokers.

In addition this study showed that participants who had BMI less than 18.5 were 6.94 times more likely to have smear positive PTB as compared to those who had BMI greater than 18.5. This finding also supported by studies conducted in Seoul, South Korea(24) and Rome(39). This might be due to that since malnutrition adversely affects the immune status of individuals, it makes individuals more susceptible to TB infection and progression of active TB disease (52).

This study also showed that HIV infected homeless individuals were 6.75 times more likely to have smear positive PTB than those HIV uninfected homeless individuals. This finding is in line with studies conducted in USA(32, 33) and Montreal, Canada(43). This might be due to that HIV

infection often leads to a greater rate of TB either through reactivation or increased susceptibility to new infection with MTB thus, the main driving factor which aggravates TB. The life time risk of HIV infected individuals to develop TB is 20-37 times higher than HIV uninfected individuals(5). In addition in this study HIV infection was found in 22 (6.3%) of the study participants. This high HIV burden might be due to risky behaviors of homeless individuals to acquire the disease. In this study TB - HIV co-infection were also considerably high (55.56%).

In contrast to studies conducted in USA(32), Rome (39) and Montreal, Canada(43), in this study alcohol drinking was not significantly associated with smear positive PTB. The reason might be more or less homeless individuals in this study exhibit similar alcohol drinking characteristics (about three quarter of the participants were drunk alcohol during the study periods), this might reduce the individual variation and make it difficult to see its effect on outcomes of smear positive PTB. However, about 88.9% smear positive PTB cases are found among alcohol drinker study participants during the study periods. In addition, in contrast to study conducted in Seoul, South Korea(24), in this study past history of TB disease is not significantly associated with smear positive PTB. The reason might be participants who had past history of TB disease were small in number in this study, might be not enough to show the effect of past history of TB on smear positive PTB.

## **8. Limitations of the study**

Lack of adequate published data on developing country TB prevalence among homeless individuals make the comparison limited. Furthermore, the information on socio-demographic, behavioral, environmental factors and morbidity history were self-reported; homeless individuals might be less likely to recall such information accurately. Another limitation of the study was the difficulty of addressing more mobile homeless individuals. The GeneXpert test was not done for all study participants' sputum samples, this might be underestimated the actual prevalence of MDR-TB. The cross-sectional nature of the study limits the degree to assign causality, especially with respect to temporality. It is a quantitative study which was not supported by qualitative data.

## **9. Conclusion and recommendation**

### **9.1. Conclusion**

The prevalence of smear positive PTB among TB suspect homeless individuals was high. Extrapolation of this study finding also indicates that the point prevalence of smear positive PTB in homeless individuals was 4.67 times higher than the general population in Ethiopia. It indicates that there is high transmission of TB in the homeless individuals and also become a risk to the entire community. Although MDR-TB was not found, well known risk factors for MDR-TB, previous anti-TB treatment defaulter rate is high in these study participants. Smoking cigarette regularly for greater than 5 years, malnutrition and HIV infection were significantly associated factors with smear positive PTB among homeless individuals.

### **9.2. Recommendations**

#### **For Federal Ministry of Health**

- Special emphasis is needed for homeless individuals to exert intensive effort to identify undetected TB cases to limit circulation of disease in to the community.
- Developing and implementing specific TB prevention and control strategies with integrated risk reduction approach is needed for homeless individuals.

#### **For Dessie and Debre Birhan towns' Health office**

- Conducting an active surveillance of TB is needed, to identify early infectious cases and to reduce prolonged transmission of the disease in the homeless individuals and to the community.
- Appropriate health education should be given for homeless individuals to control the spread of TB and to reduce associated risk factors that aggravate TB.
- Careful follow-up is needed for homeless individuals during anti-TB treatment to prevent the emergence of MDR-TB.
- Providing HIV testing and counseling to homeless individuals is needed.

#### **For researchers**

- Further research is needed to reinforce and explore the prevalence and associated factors of TB and MDR-TB among homeless individuals in depth with large sample sizes and advanced diagnostic techniques.
- Further studies should be conducted to assess the impact of homelessness on TB, MDR-TB and TB-HIV co-infection in different geographical area with both qualitative and quantitative approach.

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## **11. Annexes**

### **Annexes 1: Information sheet and consent form**

#### **Information sheet**

Good morning/good afternoon. My name is \_\_\_\_\_. I am working here on behalf of Tsedale Semunigus, student of Medical Microbiology in the University of Gondar. She is conducting a research for the partial fulfillment of the requirement for master's degree in Medical Microbiology. She has received permission from institutionalized research review board, School of Biomedical and Laboratory Sciences at University of Gondar and Dessie and Debre Birhan town administrative body to conduct this study. I would like to invite you to participate in this research project.

#### **Title of the research project**

Prevalence and associated factors of smear positive pulmonary tuberculosis and multi drug resistant tuberculosis among homeless people in Dessie and Debre Birhan town, Northeast Ethiopia

**Name of principal investigator:** Tsedale Semunigus

**Name of the organization:** School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar

**Name of the sponsor:** Amhara Regional Health Bureau

#### **Introduction**

This information sheet and consent form is prepared with the aim of explain the research project that you are asked to join by the group of research investigators. The main aim of this research project is to assess the prevalence and associated factors of smear positive pulmonary tuberculosis and multi drug resistant tuberculosis among homeless people in Dessie and Debre Birhan town, northeast Ethiopia. The research group includes trained two laboratory technologist, two nurse, principal investigator and two advisors from University of Gondar.

#### **Purpose of the research project**

The aim of this study is to assess the prevalence and associated factors of smear positive pulmonary tuberculosis and multi drug resistant tuberculosis among homeless people in Dessie and Debre Birhan town, northeast Ethiopia. Smear positive TB and MDR TB in homeless people is a major public health problem. Prevalence of tuberculosis is high in our country where there is overcrowding, malnutrition, past exposure to TB and other underlying health problems such as

HIV. However, the prevalence and associated factors of smear positive pulmonary tuberculosis and multi drug resistant tuberculosis among homeless people has not yet determined in Ethiopia. The result of this study will be used as a basis, especially in the study area to design appropriate intervention towards TB and MDR TB and their associated risk factors.

### **Procedure**

To assess the prevalence and associated factors of smear positive pulmonary tuberculosis and multi drug resistant tuberculosis among homeless people in Dessie and Debre Birhan town, we invite you to take part in our project. If you are willing to participate in this project, you need first understand and sign the agreement form. Then you will be asked to give your response and sputum and blood samples by the data and specimen collectors respectively. For participants who are not capable of giving response or specimen, they will be exempted. For this questionnaire and specimen based study, participant are all those homeless people living in the study town. All the response given by the participants and laboratory result obtained will be kept confidential using coding system whereby no one will have access to your response except the principal investigator.

### **Risk and discomfort**

By participating in this research project, you may feel that it has some discomfort especially on wasting your time 15 minute for questionnaire response, and for sputum specimen 10 minute for three times in two consecutive days and 30 minute for blood specimen. But these will not be too much as compared with the benefits. No risk in participating in this study project.

### **Benefits**

If you participate in this research project, you will be benefited from free diagnosis, treatment and advice depending on your result. Moreover your participation is likely to help us in identifying the gap and will have the benefit of improving strategies to prevent PTB, MDR TB and associated factors among homeless individuals.

### **Incentives**

You will not be provided any incentive or payment to participate in this project.

### **Confidentiality**

The information that we collect from this research project will be kept strictly confidential. Information about you that will be collected from the study will be handled in a file, which will

not have your name on it, but the code number assigned to it. It will not be accessible to anyone except the principal investigator.

### **Right to refuse**

You have full right to refuse from participating in this research project and you can choose not to respond to some or all questionnaire and giving specimen. If you do not wish to participate in this study, it will not affect any service on health institution.

### **Whom to contact**

This research project will be received and approved by ethical committee and School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University Of Gondar. If you have any question, contact any of the following individuals and you may ask at any time you want.

1. Tsedale Semunigus (B.Sc), mobile 09163724810, Email address [tsedalesemu@gmail.com](mailto:tsedalesemu@gmail.com)
2. Prof. Feleke Moges (PhD), mobile 0918778160, Email address [Mogesfeleke@gmail.com](mailto:Mogesfeleke@gmail.com)
3. Dr Belay Tessema (PhD), mobile 0919306918, Email address [bt1488@yahoo.com](mailto:bt1488@yahoo.com)

## Consent form

I the undersigned individual has been well informed about the objectives of the study entitled on prevalence and associated factors of smear positive pulmonary tuberculosis and multi drug resistant tuberculosis among homeless individuals in Dessie and Debre Birhan towns, Northeast Ethiopia. I am also told that all information obtained at any course of the study is to be kept confidential. More over I have also been well informed of my right to keep hold of, decline to cooperate and drop out of the study if I want. Therefore, with full understanding of the information sheet, I voluntarily consent to participate in the study.

Respondent code                      sign                      Date

\_\_\_\_\_                      \_\_\_\_\_                      \_\_\_\_\_

Data collector name                      sign                      Date

\_\_\_\_\_                      \_\_\_\_\_                      \_\_\_\_\_

**Annex 2: Amharic version of information sheet and consent form**

**የሚጃ ቅፅ**

ይህና አደራሽሁ/ዋላችሁ፡ ስሜ----- ይባላል፡፡ በአሁኑ ሰአት በጎንደር ዩንቨርሲቲ በሜዲካል ማይክሮባዮሎጂ የደህረ ምረቃ ተማሪ ከሆነችው ፀዳለ ሰማጉስ ጋር አብራራ እሰራለሁ፡፡ የደህረ ምረቃ ሂደቱን ለማግለጥ ጥናት እያካሄደች ሲሆን ከጎንደር ዩንቨርሲቲ የስነምግባር ኮሙኒኬሽንና የባዮሜዲካልና ላቦራቶሪ ሳይንስ ትምህርት ክፍል እና ከደሴ እና ደብረብርሃን ከተማ መከተዳድር አካላት ፈቃድ አግኝታለች፡፡

**የምርምር/ጥናቱ ርዕስ**

በአክታ ምርመራ ምርመራ ምርመራ የሆነ የሳንባ ቲቢና ብዙ መድሃኒቶችን የተላመደ ቲቢ በሽታ ስርጭትና ተያያዥነት ያላቸው ነገሮች በደሴና ደብረብርሃን ከተማ ሰሜን-ምዕራብ ኢትዮጵያ በሜዲካል የጎዳና ተዳዳሪዎች

**የዋና ተመራማሪው ስም፡ ፀዳለ ሰማጉስ**

**የድርጅቱ ስም፡ ጎንደር ዩንቨርሲቲ የባዮሜዲካል እና ላቦራቶሪ ሳይንስ ትምህርት ክፍል**

**የሰጋገሰር ድርጅቱ ስም፡ የአሜሪካ ክልላዊ መንግስት ጤ ጥበቃ ቢሮ**

**መግቢያ**

ይህ የሚጃና የስምምነት ቅፅ ወል የተዘጋጀው እርስዎ ተሳታፊ እንዲሆኑ ስለተጋበዘብት በምርምር ቡድኑ የሚካሄደውን ጥናት በተመለከተ መግለጫ ለመስጠት ነው፡፡ የምርምር ፕሮጀክቱ ዋና አላማ በአክታ ምርመራ ምርመራ የሆነ የሳንባ ቲቢና ብዙ መድሃኒቶችን የተላመደ ቲቢ በሽታ ስርጭትና ተያያዥነት ያላቸው ነገሮች በደሴና ደብረብርሃን ከተማ ሰሜን-ምዕራብ ኢትዮጵያ በሜዲካል የጎዳና ተዳዳሪዎች ላይ ማጥናት ነው፡፡ የምርምር ቡድኑ ለዚህ ጥናት የሰለጠኑ ሁለት የድግሪ ላቦራቶሪ ሳይንስ ባለሙያዎች፣ ሁለት ነርሶች፣ ዋና ተመራማሪዎችና ሁለት አሜሪካውያን ከጎንደር ዩንቨርሲቲ ያካተተ ነው፡፡

**የጥናቱ አላማ**

የዚህ ጥናት አላማ በአክታ ምርመራ ምርመራ የሆነ የሳንባ ቲቢና ብዙ መድሃኒቶችን የተላመደ ቲቢ በሽታ ስርጭትና ተያያዥነት ያላቸው ነገሮች በደሴና ደብረብርሃን ከተማ ሰሜን-ምዕራብ ኢትዮጵያ በሜዲካል የጎዳና ተዳዳሪዎች ላይ ማጥናት ነው፡፡ በአክታ ምርመራ ምርመራ የሆነ የሳንባ ቲቢና ብዙ መድሃኒቶችን የተላመደ ቲቢ በጎዳና ተዳዳሪዎች ላይ መኖር የህብረተሰብ የጤና ችግር ነው፡፡ የበሽታው ስርጭት በሀገራችን፣ በተለይም መተፋፈን፣ የምግብ እጥረት፣ ከዚህ በፊት ለቲቢ የመጋለጥ ሁኔታና ሌሎች በሽታዎችን የሚያባብሱ የጤና ችግሮች እንደ ኤች አይቪ ያሉ ሁኔታዎች በሚኖሩበት ጊዜ ከፍተኛ ነው፡፡ ይህንን በኢትዮጵያ በጎዳና ተዳዳሪዎች ላይ የበሽታው ስርጭትና ተያያዥነት ያላቸው ነገሮች ዙሪያ እስከአሁን የተጠናቀቀ ጥናት የለም፡፡ የዚህ ጥናት ወጠክ የሳንባ ቲቢና ብዙ መድሃኒቶችን የተላመደ ቲቢና ተያያዥነት ያላቸው ነገሮች ላይ ተገቢውን የመከላከል ስራ አቅዶ ለመስራት በተለይም ጥናቱ በሚካሄደበት ቦታ እንደሚሻ ሃሳብ ሊጠቅመው ይችላል ፡፡

**የአሰራር ሂደት**

የአክታ ምርመራ ምርመራ የሆነ የሳንባ ቲቢና ብዙ መድሃኒቶችን የተላመደ ቲቢ በሽታ ስርጭትና ተያያዥነት ያላቸው ነገሮች በጎዳና ተዳዳሪዎች ላይ ያለውን ችግር ለመወቅ ይረዳ ዘንድ በዚህ ጥናት እንዲሳተፉ ጋብዘንዎታል፡፡ በዚህ ጥናት እንዲሳተፉ ከተስማሙ ስምምነቱን መረጋገጥና መረጋገጥ ይኖርብዎታል፡፡ በዚህ ጥናት ሲሳተፉ መረጃ ሰብሳቢው የሚጠይቀውን ጥያቄና የአክታና የደም ናሙና እንዲሰጡ ፈቃደኝነትዎ ይጠየቃል፡፡ በህመም ወይም በሌላ ምክንያት መሳተፍ ካለቻሉ አይገደዱም፡፡ በዚህ ጥናት የሚከተሉት በደሴና ደብረብርሃን ከተማ የሚኖሩ የጎዳና ተዳዳሪዎች ሲሆኑ የሚሰጡት መልስም ሆነ የአክታና የደም ናሙና ወጠክ በሚሰጥበት ደብዳቤ፣ ከዋና ተመራማሪው በስተቀር ማንም በሚይደርስበት ቦታ በኩል ይቀመጣል፡፡

**አደጋዎችና አለመሆኑ**

በዚህ ጥናት መሳተፍዎ ምናልባት የተወሰነ ያለመሆኑ ሊሰማዎት ይችላል፡፡ በተለይም ጊዜዎትን ጥያቄዎችን ለመወለስ 20 ደቂቃ፣ አክታ ለመስጠት 10 ደቂቃ ለሶስት ጊዜ በሁለት ተከታታይ ቀናትና የደም ናሙና ለመስጠት 30 ደቂቃ ሊሻሰም

ይችላል :: ነገር ግን ጥናቱ ከሚከተለው ጥቅም አንጻር ይህን ያህል አይደለም፡፡ በዚህ ጥናት መሳተፍ ምንም አይነት አደጋ የለውም፡፡

**ጥቅሞች**

በዚህ ጥናት በመሳተፍ በነፃ ለመረጃና በምረጃ ወጠኛ መሰረት ሊታከሙ ምክር ለሰጥቶት ይችላል፡፡ ከዚህ በበለጠ የእርስዎ ተሳትፎ ለእኛ በጎዳና ተዳዳሪዎች ላይ በአከታ ምርመራ ምክርብ የሆነ የሳንባ ቲቢና ብዙ መደሃኒቶችን የተላመደ ቲቢ በሽታ ስርጭትና ተያያዥነት ያላቸው ነገሮች ላይ ያሉትን ክፍት-ቶች ለመለየትና ለማሻሻል ይጠቅማል፡፡

**የተሳትፎ ማካካሻ**

በዚህ ጥናት በመሳተፍ የሚጠየቁ ማካካሻ ክፍያ የለም፡፡

**ሚጠየቁ ስለመጠበቅ**

በዚህ ጥናት የሚጠበሰበው መረጃ በትክክል ሚጠራዊነቱ ይጠበቃል፡፡ የሚጠበሰበው መረጃ የእርስዎ ለመሆኑ መለያ አይኖረውም፡፡ መረጃው በዋና ተራራው ፋይል ተደርጎ የሚቆይበት ለሌላ ሰው ሊያገኘው አይችልም፡፡

**በጥናቱ ያለመሳተፍ ወይም እራስዎን ከጥናቱ የማገለል መብት**

በጥናቱ ያለመሳተፍ እና የሚመቻቸውን ጥያቄ በሙሉ ወይም በከፊል መርጠው ያለመላስና ናመቻ ያለመስጠት መብት አለዎት፡፡ በጥናቱ ባለመሳተፍ የሚጠየቁ የጠፍ ድርጅት አገልግሎት አይኖርም፡፡

**መረጃ ስለማግኘት**

ይህ ጥናት በጎንደር ዩኒቨርሲቲ የሰነድ ጥናት ኮሌጅና የባዮሜዲካልና ላቦራቶሪ ሳይንስ ትምህርት ክፍል ተከፈሎ የሚደቀ ይሆናል፡፡ ማንኛውም ጥያቄ ካለዎት በፊልጉት ሰአት በሚከተሉት አድራሻዎች መግናኘት ይችላሉ፡፡

- 1. ፀዳለ ሰማጉስ ስ. ቁ . 0963724810 ኢ.ሜል tseadalesemu@gmail.com
- 2. ፕሮፌሰር ፊለቀ ሞስ ስ. ቁ 0918778160 ኢ.ሜል Mogesfeleke@gmail.com
- 3. ዶ/ር በላይ ተሰማ ስ.ቁ. 0919306918 ኢ.ሜል bt1488@yahoo.com

**የሰምጥነት ቅፅ**

እኔ ከዚህ በታች የፈረምኩት ግለሰብ የጥናቱ ርዕሰ ለገጽ ምርመራ ምክርብ የሆነ የሳንባ ቲቢና ብዙ መደሃኒቶችን የተላመደ ቲቢ በሽታ ስርጭትና ተያያዥነት ያላቸው ነገሮች በደሴና ደብረብርሃን ከተማ ሰሜን ሰሜን ሰሜን ሰሜን ኢትዮጵያ በሚኖሩ የጎዳና ተዳዳሪዎች ላይ ስለሚከራው ጥናት አላሞኝ ጥቅም በሚገባ ተረድቻለሁ፡፡ በተጨማሪም በጥናቱ የመሳተፍና ከጥናቱ በፊልግሁት ጊዜ የመወጣት መብት እንዳለኝ ተረድቻለሁ፡፡ ስለሆነም የመረጃ ቅፁን በሚገባ ተረድኜ በሚከራው ጥናት ላይ በፈቃደኝነት ለመሳተፍ መወሰኔን ከዚህ በታች በፈረምኩት ፈርማዬ አረጋግጣለሁ፡፡

የተሳታፊው  
 ኮድ \_\_\_\_\_  
 ፊርማ \_\_\_\_\_  
 ቀን \_\_\_\_\_

የመረጃ ሰብሳቢው  
 ስም \_\_\_\_\_  
 ፊርማ \_\_\_\_\_  
 ቀን \_\_\_\_\_

### Annex 3: Questionnaires

Code number \_\_\_\_\_ Town \_\_\_\_\_ Date \_\_\_\_\_

#### Part I. Socio-demography characteristics

No	Questions	Coding categories
101	Sex	0. Male 1. Female
102	Age	_____ years
103	Marital status	0. Single 1. Married 2. Divorced/Separated 3. Widowed
104	Educational status	0. Illiterate 1. Primary (1-8 grade) 2. Secondary(9-10 or 12 grade) 3. College (10+ or 12+)



105	Religion	0. Orthodox 1. Muslim 2. Protestant 3. Others_____
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**Part II. Behavioral factors**

201	Do you smoke cigarette currently?	0. Yes 1. No
202	If say yes for how long smoke regularly?	_____ Months
203	Do you drink alcohol currently?	0. Yes 1. No
204	If say yes for how long drink regularly?	_____ Months
205	Do you chew khat currently?	0. Yes 1. No
206	If say yes for how long chew regularly?	_____ Months
207	Do you use drugs currently?	0. Yes 1. No
208	If say yes for how long use drugs regularly?	_____ Months

**Part III. Environmental factors**

301	How long did you live on the streets?	_____ Months
302	In average how many people live/sleep together in one restricted homeless shelter?	_____ Persons
303	Do you have close contact with known TB patient in same homeless shelter?	0. Yes 1. No
304	Do you have close contact with chronically coughing person in same homeless shelter?	0. Yes 1. No

**Part IV. Morbidity history and status**

401	Currently, what kind of TB suggestive symptoms (illness) do you have?  N.B. Multiple choices possible.	0. Cough $\geq$ 2 weeks 1. Chest pain 2. Difficulty of breathing 3. Fever 4. Weight loss 5. Night sweating 6. Loss of appetite 7. Malaise 8. Fatigue
402	Have you ever had TB disease?	0. Yes 1. No
403	If yes to Q.402, When have you been diagnosed for TB?	0. During being homelessness 1. Before being homelessness
404	If yes to Q.402, did you take treatment?	0. No 1. Yes
405	If yes to Q.402, did you complete the full course of TB treatment?	0. No 1. Yes
406	Weight (to be measured by data collector)	_____kg
407	Height(to be measured by data collector)	_____meter
408	Collected sputum (to will be filled by data collector): make mark if taken.	0. Spot 1. Morning 2. Spot
409	Blood sample collected make mark if taken.	0. Collected 1. Not collected

**Annex 4: Amharic version of the questionnaires**

ከዚህ በታች ላሉት ጥያቄዎች መልስዎን ይስጡ

ከተማ ----- ቀን ----- ከድ-----

ክፍል አንድ : -ስለሚሰበራዎት አካላዎቹ ስነ ህዝብ ሁኔታን የሚጥለኩ ጥያቄዎች

ተ.ቁ	ጥያቄዎች	ምርመራ
101	የታ	0. መንድ 1. ሴት
102	ዕድሜ	_____ አመት

103	የጋብቻ ሁኔታ	0. ያላገባ /ች 1. ያገባ /ች 2. የፈታ/ች 3. የሞተባት/በት
104	የትምህርት ደረጃ	0. ያልተማረ/ች 1. የመጀመሪያ ደረጃ የተማረ/ች (1-8ኛ ክፍል ) 2. ሁለተኛ ደረጃ የተማረ/ች (9-10 / 12ኛ ክፍል) 3. ኮሌጅ የተማረ/ች (10+ / 12+)
105	ሀይማኖት	0. ኦርቶዶክስ 1. ሙስሊም 2. ፕሮቴስታንት 3. ሌላ _____

ክፍል ሁለት: - ከስነ ምግባር ጋር የተያያዙ ጉዳዮች

201	በአሁኑ ሰዓት ሲጋራ ያጨሰሉ ?	0. አጨሰሁ 1. አላጨሰም
202	አዎ ካሉ ለምን ያህል ጊዜ በመደበኛ ሁኔታ አጨሰዋል ?	_____ ወር
203	በአሁኑ ሰዓት አልኮል ማጠጥ ይጠጥሉ ?	0. አጠጥሁ 1. አልጠጥም
204	አዎ ካሉ ለምን ያህል ጊዜ በመደበኛ ሁኔታ ጠጥተዋል ?	_____ ወር
205	በአሁኑ ሰዓት ጫካ ይቅማሉ ?	0. አቅማለሁ 1. አልቅምም
206	አዎ ካሉ ለምን ያህል ጊዜ በመደበኛ ሁኔታ ቅመጠዎ ?	_____ ወር
207	አደንዛኝ እፅ ይጠቅማሉ	0. አዎ 1. አልጠቅምም
208	አዎ ካሉ ለምን ያህል ጊዜ በመደበኛ ሁኔታ ተጠቅመዋል ?	_____ ወር

ክፍል ሶስት: - ከአካባቢያዊ ሁኔታዎች ጋር የተያያዙ ጉዳዮች

301	ለምን ያህል ጊዜ በጎዳና ላይ ኖረዋል ?	_____ ወር
302	በጎዳና ላይ በሚኖሩበት ጊዜ በአንድ በተወሰነ ቦታ በአሜሪካ ምን ያህል ሰዎች በአንድነት ይኖራሉ/ይተኛሉ?	_____ ሰዎች
303	ከታወቀ የቲቢ ህመም ጋር የቅርብ ግንኙነት አለዎት በጎዳና ላይ ?	0. አለኝ 1. የለኝም
304	ለረጅም ጊዜ ከሙከራ ሰው ጋር የቅርብ ግንኙነት አለዎት በጎዳና ላይ?	0. አለኝ 1. የለኝም

ክፍል አራት: የህመም ታሪክና ሁኔታ

401	በአሁኑ ሰዓት ምን አይነት የህመም ምልክት ወይም ስሜት አለብዎት/ ይስማሙታል?  ማስታወሻ: ብዙ ምርመራዎች ይቻላል	0. ሁለት ሳምንትና ከዚያ በላይ የቆየ ሳል 1. የደረረት ህመም 2. የመተንፈስ ችግር 3. ትኩሳት 4. ክብደት መካነ ስ 5. በለሊት ማለብ 6. የምግብ ፍላጎት ምክነ ስ 7. ማቅለሽለሽ 8. ደካም
402	የቲቢ በሽታ ታመው ያወቃሉ?	0. አዎ 1. አላወቅም
403	መልስዎ አዎ ከሆነ (በጥያቄ ተ.ቁ 402) መቼ ነው ቲቢ የታመመት?	0. የጎዳና ተዳዳሪ ሆነው እያሉ 1. የጎዳና ተዳዳሪ ከመሆንም በፊት
404	መልስዎ አዎ ከሆነ (ለጥያቄ ተ.ቁ 402) የቲቢ መድሃኒት ወስደዋል ?	0. አልወሰድኩም 1. አዎ
405	መልስዎ አዎ ከሆነ (ለጥያቄ ተ.ቁ 404) መድሃኒቱን ሙሉ በሙሉ አጠናቀው ወስደዋል?	0. አልወሰድኩም 1. አዎ
406	ክብደት በመረጃ ሰበሳቢው ይለካ	_____ ኪ.ግራም
407	ቁመት በመረጃ ሰበሳቢው ይለካ	_____ ሜትር
408	አክታ መወሰዱን ምልክት ያደርጉ	0. ወዲያውኑ 1. ጠዋት 2. ወዲያውኑ
409	የደም ናመፍ መወሰዱን ምልክት ያደርጉ	0. ተወስዷል 1. አልተወሰደም

**Annex 5: Procedure for sputum smear microscopy using Auramine O staining**

- Prepare the smears, let air dry and fix it by passing three times slowly through the flame
- Arrange slides in serial order on staining rack, with smear side up, at a distance of at least 1cm between every slide.
- Flood slides with filtered 0.1% Auramine O solution, without heating.
- Keep the staining reagent for 20 minutes.
- Rinse with water and drain.
- Apply decolorizing solution, 0.5% Acid alcohol, for 3 minutes.
- Gently rinse with water until the macroscopically visible stain has been washed away and drained.
- Flood smear with 0.5% potassium permanganate solution for 1 minute.

- Gently rinse with water and drain then air dry on a slide rack.
- Keep stained smears in the box and read as soon as possible.
- Using the 20x objective of fluorescence microscope to scan the stained smear systematically and 40x objective for confirmation of AFB. Acid-fast bacilli appear bright yellow against the dark background material(45).

#### **Annex 6: GeneXpert MTB/RIF assay procedure**

- Sputum sample liquefaction and inactivation with 2:1 sample reagent buffer to sample ratio
- Shake and incubate for 10 minutes at room temperature
- Shake it again and let it stand further for 5 minutes
- Remove Xpert cartridge from its wrapper.
- Label the side of the Xpert cartridge with the sample identification number.
- Open the lid of the cartridge
- Open the lid of the collection container containing the mixed sputum specimen
- Transfer of 2ml sample into the open port of the cartridge
- Close the cartridge lid firmly.

- Insert the cartridge and switch on the GeneXpert instrument and start the test, End of hand work
- Sample is automatically filtered and washed
- Ultrasonic lysis of filter captured organism to release deoxyribo nucleic acid (DNA)
- DNA molecules are mixed with dry PCR reagent
- Semi nested real time amplification and detection in integrated reaction tube
- Print test result and interpretation of the result

## **Annex 7: Rapid HIV tests procedures**

### **KHB**

1. Wear gloves
2. Remove a test cassette from protective foil pouch, and place it on flat surface
3. Label the test device with participant identification number.
4. Collect 40 µl of blood specimen using a capillary tube by finger stick.
5. Apply the specimen to the sample well on the device.
6. Add 1 drop of buffer to the specimen.
7. Read within 30 minutes.

### **STAT-PAK**

1. Wear gloves.
2. Remove a test cassette from protective foil pouch, and place it on flat surface.

3. Label device with participant identification number.
4. Collect 5  $\mu\text{l}$  of specimen using a new disposable loop.
5. Dispense the sample in the center of sample well.
6. Add 3 drops of buffer, holding vial vertically over sample well.
7. Wait for 10 minutes and read the result.

### **UNI-GOLD**

1. Wear gloves.
2. Remove a test cassette from protective foil pouch, and place it on flat surface.
3. Label device with participant identification
4. Collect specimen using the disposable pipette.
5. Add 2 drops of specimen to the sample port in the device.
6. Add 2 drops of the appropriate wash reagent to sample port.
7. Wait for 10 minutes before reading the result.

### **Declaration**

I, the undersigned, Senior Medical Microbiology student declare that this thesis is my original study for partial fulfillment of the requirements for degree of Master of Science in Medical Microbiology.

Name Tsedale Semunigus

Signature \_\_\_\_\_

Place of submission: School of Biomedical and Laboratory sciences, College of Medicine and Health Sciences, University of Gondar.

Date of submission: \_\_\_\_\_

This thesis was submitted for examination with my/ our approval as university advisors

Advisors	Signature	Date
1. Prof. Feleke Moges (PhD, Professor)	_____	_____
2. Dr Belay Tessema (PhD, Assistant Professor)	_____	_____

Examiners

1. External examiners _____	Signature _____	Date _____
2. Internal examiners _____	Signature _____	Date _____