ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

SCHOOL OF MEDICINE

DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

Prevalence of *Mycobacterium tuberculosis*, its Rifampicin-resistance pattern and associated factors among presumptive Pulmonary and Extra Pulmonary Tuberculosis patients at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia

By: Wakuman Taye (Bsc)

July, 2021

Addis Ababa, Ethiopia

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

SCHOOL OF MEDICINE, DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND PARASITOLOGY

Prevalence of *Mycobacterium tuberculosis*, its Rifampicin-resistance pattern and associated factors among presumptive Pulmonary and Extra Pulmonary Tuberculosis patients at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia

A thesis submitted to Addis Ababa University, School of Medicine, Department of Microbiology, Immunology and Parasitology in the partial fulfillment of the requirements for the Degree of Master of Science in Medical Microbiology

By: Wakuman Taye (BSc, MSc candidate in Medical Microbiology)
Advisor: Dr. Solomon G/Selassie (MD, MSc, Associate Professor of Medical Microbiology, Addis Ababa University)
Co-advisor: Mr. Mesay Mitiku (MSc, PhD candidate in Medical Microbiology, Addis Ababa University)

July, 2021

Addis Ababa, Ethiopia

Acknowledgements

I would like to express my sincere and heartfelt thanks to my advisors Dr. Solomon G/Selassie (MD, MSc, Associate Professor of Medical Microbiology) and Mr. Mesay Mitiku (PhD candidate) for their advice and scholarly guidance during preparation of the research proposal to the final thesis.

I would like also acknowledge the Department of Microbiology, Immunology and Parasitology for giving me the opportunity to conduct the study and Addis Ababa University for financing this research. I am also very grateful, and would like to extend my heartfelt thanks and the appreciation to the study participants for their willingness to be part of the study.

My special thanks and appreciation goes to the staff members of MWU GRH, especially Dr. Abdi Fite (FNA samples collected by him), Dr. Hirko and his colleagues who work in TB/ HIV clinic and all laboratories staff for supporting me during data collection.

List of abbreviations

ATDs……Ant-tuberculosis drugs

CBNAAT……..Cartridge based nucleic acid amplification test

DST……Drug susceptibility testing

EPTB ……Extra pulmonary tuberculosis

GRH……...Goba referral hospital

HIV……Human immunodeficiency virus

MDR-TB……...Multi-drug resistant Tuberculosis

MTB……..*Mycobacterium tuberculosis*

MWU…….Madda Walabu university

PCC……..Probe check control

PCR……...Polymerase chain reaction

PTB……....Pulmonary Tuberculosis

RIF…….Rifampicin

RR-TB…….……..Rifampicin resistance tuberculosis

RRDR……..……..Rifampicin resistance determining region

SPC……....Sample processing control

TB ……....Tuberculosis

WHO…….World health organization

XDR-TB……Extensively drug resistance tuberculosis

Table of Contents

List of tables

List of figures

Abstract

Background: Drug resistant *Mycobacterium tuberculosis* is one of the serious public health trouble that intimidating progress made in tuberculosis cases and control in several countries including Ethiopia. Rifampicin resistance is an indicator for drug-resistant *Mycobacterium tuberculosis*, because it disclose the existence of more than 90% Isoniazid resistance. Early detection of drug-resistant tuberculosis is crucial for patient management and infection control.

Objective: This study was designed to assess the prevalence of *Mycobacterium tuberculosis*, its Rifampicin-resistance pattern and associated factors among presumptive Pulmonary and Extra Pulmonary Tuberculosis patients at Madda Walabu University, Goba Referral Hospital, Southeast Ethiopia.

Methods: Hospital based cross-sectional study design was carried out from October 22, 2020 to February 7, 2021. Detection of *Mycobacterium tuberculosis* and resistance to Rifampicin pattern was determined by using GeneXpert MTB/RIF assay. Data were entered and analyzed by SPSS version 23.0. Bivariate and multivariate analyses were used to examine the relationship between dependent and independent variables. Less than 0.05 P-value was used to show significance.

Results: A total of 301 presumptive tuberculosis patients were included in the study; of these, 46 (15.3%) of them were identified as having *Mycobacterium tuberculosis* by the GeneXpert MTB/RIF assay, 2/46 (4.35%) were resistant to Rifampicin and 4/46 (14.8%) patients were TB/HIV co-infected. From the total of *M. tuberculosis* detected 40 (16.7%) were identified in pulmonary and 6 (9.8%) were in extra-pulmonary presumptive patients. Rifampicin-resistant *M. tuberculosis* was detected in 2 patients who had a history of taking Anti-tuberculosis drugs and no in new patients.

Conclusion and recommendation: Previous history of tuberculosis treatment and having close contact history with tuberculosis patients were found as an important associated factors that enhance the prevalence of tuberculosis. This indicates the mandate to make better and oversee the treatment protocol to control the burden of tuberculosis.

Keywords: Rifampicin resistant *M. tuberculosis*, GeneXpert MTB/RIF assay, Risk factors

1. Introduction

1.1. Background

Tuberculosis (TB) is potentially fatal contagious bacterial disease caused by *Mycobacterium tuberculosis* (most common cause) that can affect any part of the body, but principally associated with lung diseases (Pulmonary TB), and it can also affect other parts of the body, extra pulmonary tuberculosis (EPTB) and remains as an important infectious disease and public health concern worldwide (1).

The bacteria are passed through the air via close contact with a person who is sick with tuberculosis disease who is actively spreading the bacteria through coughing, sneezing and singing (2). Once inhaled, the infection remains latent for decades in 90 to 95% in healthy adult (3, 4). However, the illness of latent tuberculosis is apprehensible only when the bacteria become active. Human immunodeficiency virus (HIV), older age, diabetics, close contact with an active case of tuberculosis disease and other immune-compromising illness conditions are contributory factors that enable the development of latent tuberculosis bacteria to an active form (2-4).

Drug resistance is defined as the ability of bacteria and other microorganisms to withstand a drug that killed them. When mutation confers resistance to a certain antibiotic, all sensitive bacteria are killed and the resistant ones will grow and become the dominant variant in the population. Generally when one percent or more of organisms in an isolate are found resistant to an anti-tuberculosis drug therapeutic success is less likely to occur and then that the strain is considered resistant to the drug (5).

Rifampicin resistant tuberculosis is caused by tubercle bacilli that display in vitro resistance to Rifampicin, one of the most effective anti-tubercular drugs (ATDs), requiring longer treatment regimens, higher incidences of adverse effects and decreased compliance than patients with Rifampicin-susceptible tuberculosis (6).

Rifampicin resistant tuberculosis is a good indicator of Multi-drug resistance tuberculosis (MDR-TB). MDR-TB is caused by *Mycobacterium tuberculosis* which is resistant to at least Isoniazid and Rifampicin (7).

Drug-resistant tuberculosis arises due to improper and irrational use of anti-tubercular drugs (ATDs) in treatment of drug-susceptible TB patients , a genetic mutation of bacilli, an inadequate

administered treatment regimen and weak services program that lead to delay detection and ineffective treatment of drug resistance and unequipped to support patients to keep adherence to treatment (7, 8).

The prevalence of Drug Resistant tuberculosis (DR-TB) is increasing throughout the world both among new tuberculosis cases as well as among previously treated ones. Different studies indicated that a previous history of TB treatment as the strongest risk factor for the development of DR-TB (9), and treatment-naive patients are also at risk due to spontaneous mutations and transmission of resistant *Mycobacterium tuberculosis*. Currently there is an alarming growing burden of DR-TB, because of excessive transmission risk of *Mycobacterium tuberculosis* from close contacts (10).

Different research indicated that the disclosure of DR-TB presents a notable warning to tuberculosis-control programs worldwide. Rifampicin Resistance is an indicator and alternative method for detection of MDR-TB, because >90% rifampicin resistant *Mycobacterium tuberculosis* also resistant to Isoniazid (11). Therefore, WHO endorsed that patients with Rifampicin resistant *Mycobacterium tuberculosis* should be take a medical care for tuberculosis as patients with MDR-TB (12).

Studies showed that for better patient management and infection control in tuberculosis cases, early detection of drug resistance TB is the most crucial approach. Accordingly, different TB diagnosis methods like Acid-fast staining, culture and molecular techniques are used. Of those techniques Acid-fast staining is the main diagnostic method in resource limited settings despite its low sensitivity in the detection of *Mycobacterium tuberculosis.* Mycobacterium culture is the most sensitive and remains the gold standard method of TB diagnosis, but time-consuming and technically challenging (13, 14).

WHO introduced the wide use of GeneXpert MTB/RIF assay. This assay has the capability to detect *M. tuberculosis* and Rifampicin resistance associated mutations at the same time in the RNA polymerase of β gene (rpoβ) from clinical samples both in pulmonary and extra pulmonary TB (15-17). Rifampicin resistant *M. tuberculosis* possess mutations at 81-base-pair region of the RNA polymerase of β gene and it is a major target to perform a molecular diagnosis for Rifampin resistant *M.tuberculosis* (18, 19).

1.2. Statement of the problem

The burden of TB continues to present a public health problem and remains one of the most significant causes of morbidity worldwide. Globally, an estimated 10.4 million people fell ill with TB in 2019, (10% among HIV co-infected individuals), 1.67 million deaths and 490,000 MDR-TB plus an additional 110,000 RR-TB cases was reported by World Health Organization. Geographically, most people who developed TB in 2019 were from south-East Asia which shared (44%), Africa accounts for (25%) and the Western Pacific shared (18%). The smaller percentages were shared by others WHO tuberculosis regions (4).

WHO African Region TB report showed that the total TB incidence was 231 per 100, 000 population and MDR/RR-TB incidence was 58 per 100, 000 population. According to this report, the estimated proportion of TB case with MDR/RR-TB was 2.5% among new presumptive TB patients and 12% among patients who had history of taking anti-tuberculosis drugs (20).

Our country Ethiopia is still the member of top thirty high TB burden countries having an incidence of all forms of TB 140 per 100,000 populations with mortality rate of 22 per 100,000 populations and 2 per 100,000 populations in HIV-negative TB and HIV-positive TB patients respectively. The incidence of MDR/RR-TB was 1.4 per 100,000 populations. The estimated proportion of TB cases with MDR/RR-TB among new cases was 0.71% and 16% among previously treated cases (21).

The development of drug-resistant TB commonly associated with a human-made trouble, predominately as a consequence of poor supply management, limited coverage of rapid laboratory diagnosis for rifampicin resistant *M. tuberculosis*, quality of anti-TB drugs and improper treatment of drug-susceptible TB has been identified as a major contributing factor for the high burden of drug-resistant TB (22-25).

The challenge to the control of TB starts with a delay in diagnosis and the low rate of detection of TB by sputum microscopy is an even bigger challenge (16, 26). Diagnostic delay due to the high proportion of smear-negative pulmonary TB, especially in HIV-associated TB leads to increased mortality, secondary resistance, and ongoing transmission (27). In the study area, there appears to be no study on the prevalence of tuberculosis infections and associated risk factors. Therefore, this study aimed to fill this gap by using GeneXpert MTB/RIF assay.

1.3. Significance of the study

Determining the burden of Rifampicin-resistant *Mycobacterium tuberculosis* with modern and recently developed technology is essential to control Multidrug-resistance tuberculosis and extensively drug resistant tuberculosis TB as Rifampicin resistance is an important indicator for drug resistant TB. In countries with a high burden of TB, rapid diagnosis of tuberculosis, detection of Rifampicin (RIF) resistance, uninterrupted surveillance and systematic observe and checking the progress of drug resistance TB is crucial for tuberculosis control and on time treatment starting. These measures, in turn, improve patients' outcomes, enable to know the magnitude of the problem and allow taking effective public health measures.

It is conceived that the data generated from this study will provide an information on the current burden and factors associated with the prevalence and rifampicin resistant pattern of *M. tuberculosis* .So that the findings obtained from this study is hoped to provide additional data source for policy makers, health care workers and local authorities to understand the problem and burden of tuberculosis at the study site. In addition, the resulting data will serve as base-line for further future similar but larger studies.

2. Literature review

2.1. Basic characteristics of *Mycobacterium tuberculosis*

It is one of the Members of the *Mycobacterium tuberculosis complex* (MTBC). MTBC is characterized by 99.9% or greater similarity at the nucleotide level and possess identical 16S r RNA sequence. MTBC includes: *Mycobacterium tuberculosis* (MTB), the etiologic agent of TB in humans; *M. africanum*, causes tuberculosis in humans in certain regions of Africa; *M. bovis*, *M. caprae*, and *M. pinnipedii*, able to cause tuberculosis in wild and domesticated mammals; *M. microti*, that causes TB only in voles (28).

Mycobacterium tuberculosis is a rod-shaped, non-motile and an obligate aerobe related to the Actinomycetes. *Mycobacterium tuberculosis* is a facultative intracellular parasite and this physiological characteristic may contribute to its virulence. A unique feature of *Mycobacterium tuberculosis* is the peculiar cell wall structure, which provides strong impermeable barrier to noxious compounds and drugs and that plays a fundamental role in virulence (29).

2.2. Tuberculosis Pathogenesis

Tubercle bacilli are inhaled in aerosol droplets, enter into the lungs and, when the host innate immune defenses fail to eliminate the bacteria, *Mycobacterium tuberculosis* start multiplying inside alveolar macrophages and then spreads to other tissues and organs through the bloodstream and lymphatic. Once the cell-mediated immune response kicks in, bacterial replication is usually controlled and in 90-95% of cases no overt signs or symptoms of disease ensue (Latent TB). During latent infection for unknown reasons, bacilli would start replicating, a dynamic equilibrium between the bacilli and host immune responses is established and any event that weakens cell-mediated immunity may lead to active bacterial replication, tissue damage and occurs of active TB (30, 31).

In the infection success and the development of the pulmonary form of tuberculosis (lungs are the main target of this bacterium) four successive steps like phagocytosis of the bacilli, their intracellular multiplication, the latent contained phase of infection and finally the development active lung infection are needed. These successive steps can progress to towards different clinical outcomes like: spontaneous cure, disease, latent infection and re-activation, or re-infection (30).

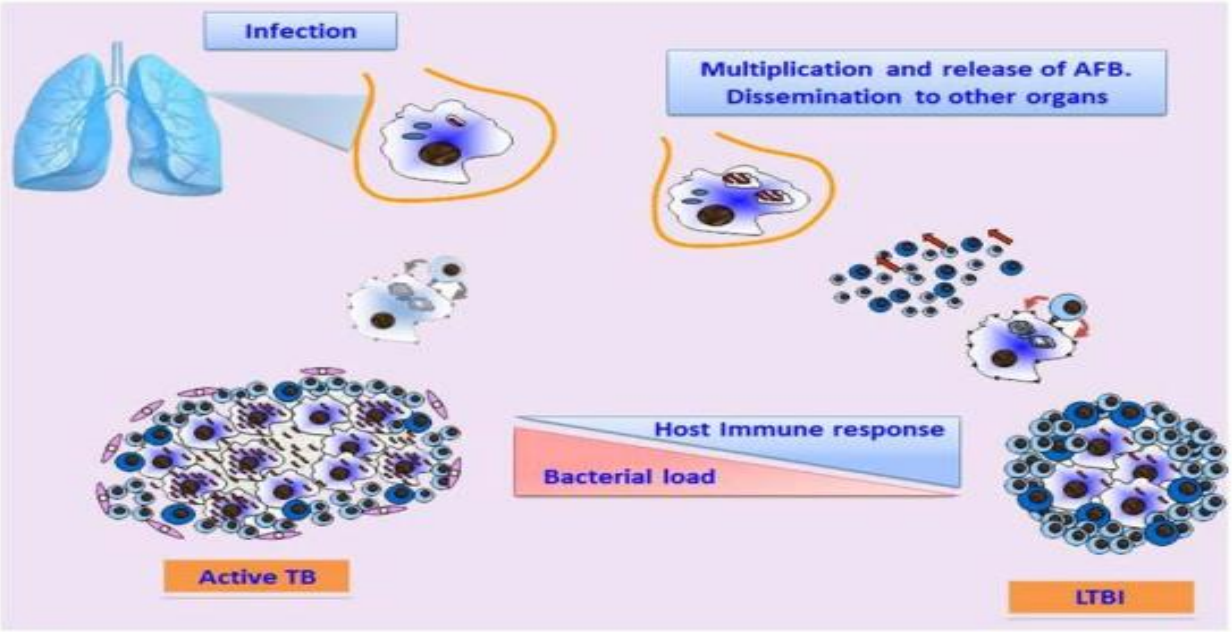

Figure 1 -Tuberculosis pathogenesis (30)

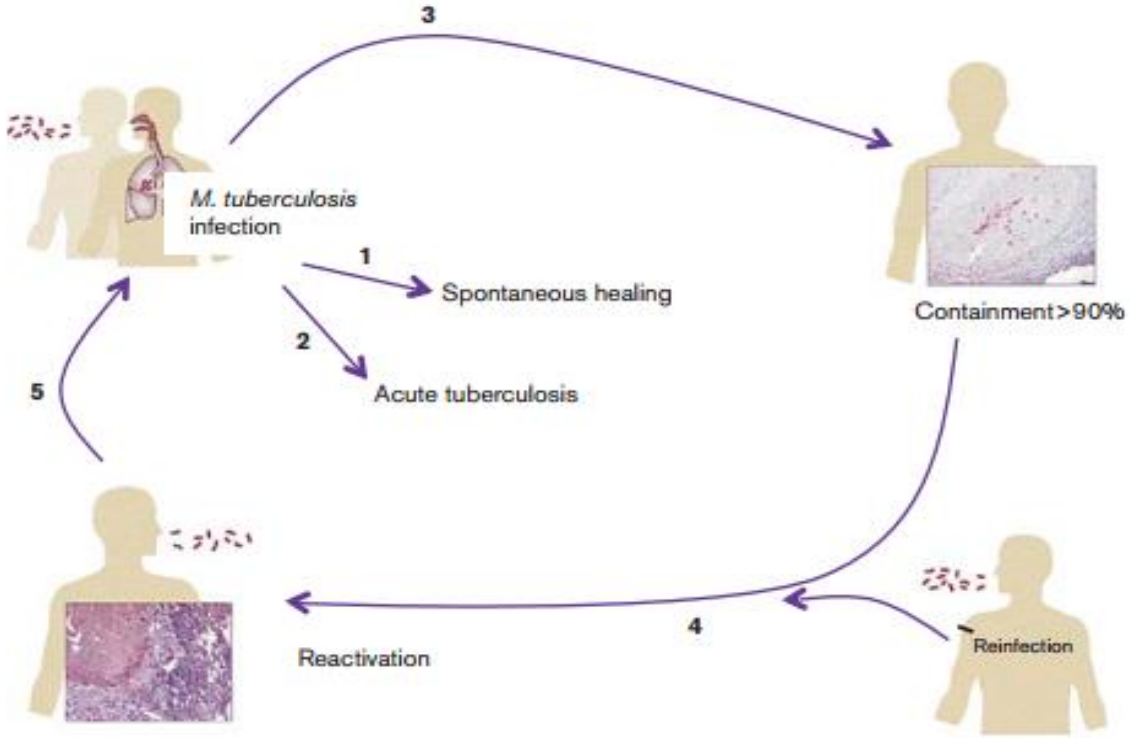

Figure 2- Different tuberculosis clinical scenarios after *M. tuberculosis* enters the host (31)

2.3. Basic concepts in the development of drug-resistant tuberculosis

Genetic resistance and phenotypic resistance are two types of drug resistance in *M. tuberculosis*. Genetic associated drug resistance is happened due to mutations on chromosomal genes in undergoing natural development bacteria, while phenotypic resistance or it can be called drug tolerance is formed due to changes in gene expression (epigenetics) and modification associated with protein which able to cause tolerance to drugs in non-growing perseverance bacteria (32). These two types of resistance can overlap and interconvert. Prior stress or sub-inhibitory concentration of drugs may induce efflux pump expression,(33,34) which causes phenotypic resistance and may, in turn, facilitate the development of more stable genetic drug resistance,(34) while genetic resistance in growing organisms can develop persistence or phenotypic resistance.

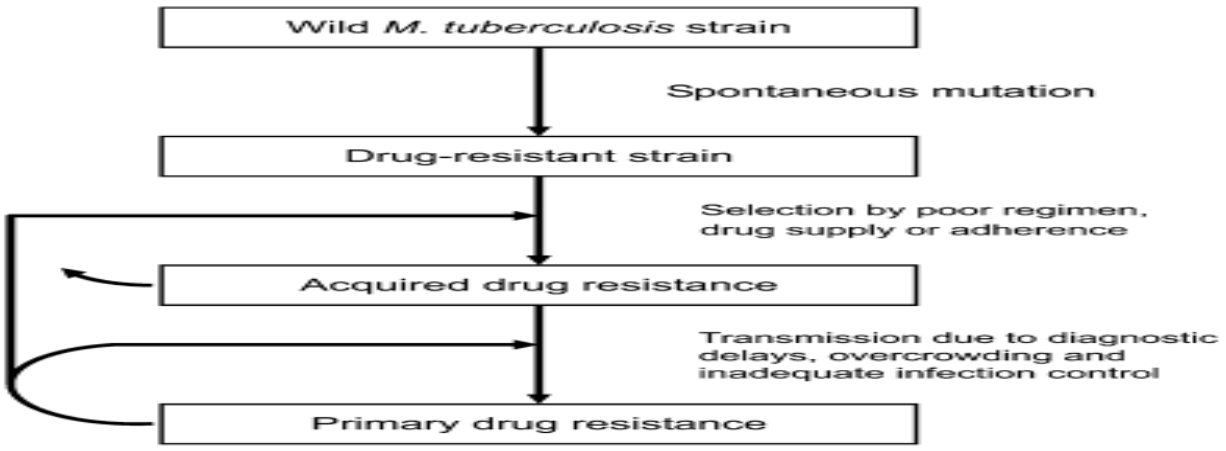

Figure 3: Concepts in the development of drug-resistant TB (32)

2.4. Rifampicin resistance mechanism in *M. tuberculosis*

Rifampicin is highly bactericidal for *M. tuberculosis*. Rifampicin interferes with RNA synthesis by binding to the rpoβ target and able to induces hydroxyl radical formation in susceptible but not in a resistant bacilli and may contribute to its killing effect. The RIF-binding site is located upstream of the catalytic center and physically blocks the elongation of the RNA chain (35).

In *M. tuberculosis*, resistance to RIF occurs at a frequency of 10^{-7} to 10^{-8} and mutation leading to resistance of *M. tuberculosis* to RIF is rare. However, High mutation frequency is intriguing and may be related to the lower RIF concentration used or previous exposure to RIF before selection for RIF's resistance. Mutation in *rpo*β (defined region of the 81 base pair (bp) results in

resistance by decreasing RIF binding affinity and found in about 96% of RIF-resistant *M. tuberculosis* isolates (36).

Mutations at positions 531, 526 and 516 are among the most frequent mutations in RIF-resistant *Mycobacterium tuberculosis*. Mutations at the region of the 81 base pair in general contribute to high form of resistance and cross-resistance to other drugs like rifamycins. In contrast, mutations at codons 511, 518 and 522 are related with lower form of resistance to Rifampicin. Not all mutations in rpoβ are associated with rifampicin's resistance (37, 38).

2.5. Risk factors for Tuberculosis

Both exogenous and endogenous risk factors govern the risk of progression from exposure to the tuberculosis bacilli to the development of the active disease. Exogenous factors play a key role in emphasizing the progression of an individual from exposure to infectious TB cases. Similarly, endogenous factors: (HIV), malnutrition, young age and elderly) lead in progression from infection to active TB disease (39).

Yetunde et al., 2017, in his research showed that Rifampicin resistance was higher among patients who were contacts of known DR-TB patients (35.9%) than patients who have no history of contact with known DR-TB patients (21.7%) (40).

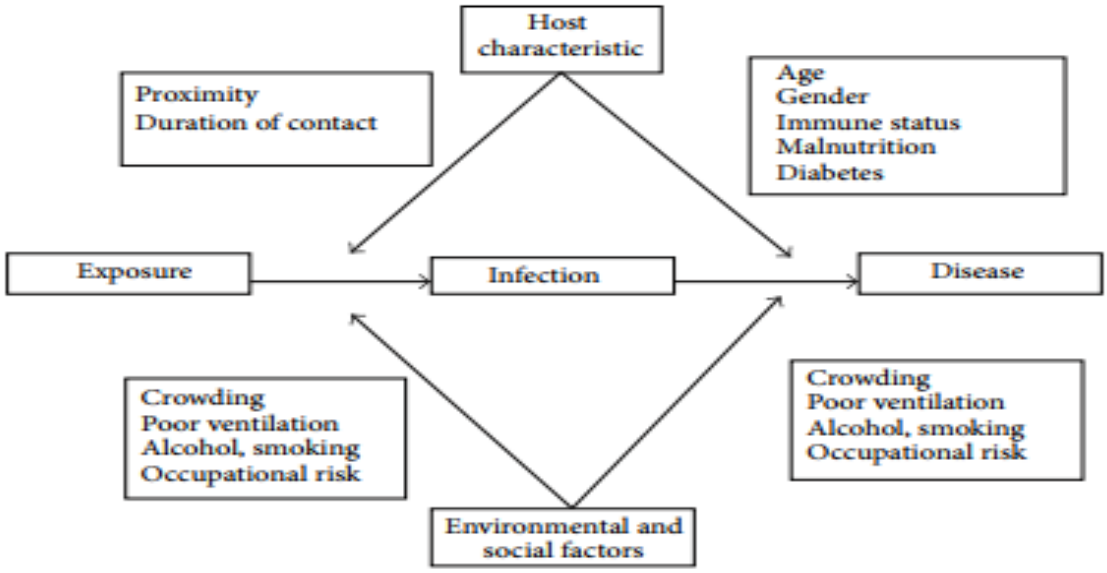

Figure 4: Associated factors for Tuberculosis infection and disease (39).

2.6. Burden of Tuberculosis

The burden of drug-resistant TB is the major interest and concern at global, regional and country levels. In 2019, there were approximately close to half a million people developed rifampicin-resistant TB (RR-TB), of which 78% had multidrug-resistant TB (MDR-TB). According to WHO report of 2019; India (27%), China (14%) and the Russian Federation (8%) shared the highest global TB burden. Accordingly, 3.3% new tuberculosis patients and 17.7% previously tuberculosis treated patients had MDR/RR-TB with the highest proportions (>50% in previously treated cases) in countries of the former Soviet Union (41).

The study conducted by Siddique *et al.*, 2019 at Provincial TB Reference Laboratory, Pakistan showed that out of 500 samples, GeneXpert MTB/RIF assay detected MTB in 211 (42.2%) samples, from these positive pulmonary cases were 48.8% (206/422) and extra-pulmonary cases were 6.4% (5/78). Among these 211 cases, RIF-resistance was detected in 11 cases (5.2 %). This study also demonstrated that most of MTB cases (55.5%) were between 21-40 years of age and a statistically significant difference in the TB prevalence was observed between different age groups ($p<0.05$) (42).

A study conducted by Gautama *et al.*, 2018 to find out the magnitude of drug resistant *tuberculosis* and related factors in eastern Uttar Pradesh, India showed that from a total 510 presumptive TB and presumptive DR-TB 168 (32.9%) MTB were detected. From 168 confirmed *M. tuberculosis*, 44 (26.1%) were resistant to rifampicin. From 44 (26.1%) Rifampicin resistant *M .tuberculosis*, 39 (37.8%) were detected in previously tuberculosis treated Patients and 5 (7.6%) *M. tuberculosis* were detected in patients with no treatment history of TB treatment. According to this study high prevalence of MTB was observed among male study participant 38 (30.6%) than female 6 (13.6%). This study also showed that high prevalence of Rifampicin resistant *M. tuberculosis* was observed 36 (27.6%) in pulmonary presumptive than extra pulmonary presumptive patients 8 (21%) (7).

A cross sectional study carried out at National Tuberculosis Center, of Nepal to determine Prevalence of Rifampicin resistant TB by GeneXpert MTB/RIF/ assay in patients with presumptive tuberculosis. The overall prevalence of MTB was found to be 13.8% and the prevalence of RR-MTB was 10.2%. The risk of acquiring tuberculosis was significantly

associated with history of taking Ant-tuberculosis drugs. This study also showed the burden MTB was somewhat higher in male 14.08% than female 13.35% TB presumptive patients. The burden of MTB detected was significantly higher in patients who had a history of TB treatment 50% compared with a patients who previously not taken Anti-tuberculosis drugs 9.13%. According to this study the prevalence of RR-MTB was also largely higher in previously treated TB patients 13.79%, in contrast to treatment new patients 7.50% (43).

Similar study done by Etim *et al*., 2017 at Bayelsa state, Nigeria to determine Prevalence of MTB and Rifampicin-resistant *M. tuberculosis* among TB suspected Patients showed that from a total of 450 study participants, the overall prevalence of tuberculosis detected were 88 (19.6%) and from detected cases 11 (2.4%) were showed resistance to rifampicin. The result of this study also indicated that the prevalence of TB was highest among age group 21 –40 years (44).

Cross sectional study conducted in India to determine the factors associated with tuberculosis and rifampicin-resistant tuberculosis among tuberculosis suspected patients showed that the overall prevalence of tuberculosis detected was 21.8%; from total MTB detected 2.7 % were resistance for Rifampicin. According to this study, having history of previous tuberculosis treatment, being male and adult age range were identified as a risk factors associated with high tuberculosis burden d among suspected patients This study also showed that previous history of taking Anti-tuberculosis was identified as a significant risk factor for burden rifampicin-resistant *M. tuberculosis* (45).

Cross sectional study conducted in Egyptian population for diagnosis of Pulmonary, Extra-pulmonary Tuberculosis and Rifampicin Resistance pattern by GeneXpert showed that the overall prevalence of tuberculosis was found to be 19%; of these 3.75% were resistance for Rifampicin .The prevalence of TB was 22.7% among patients suspected to have pulmonary TB and 10% among patients suspected of having extra-pulmonary TB (46).

A study conducted at selected governmental hospitals in Addis Ababa showed that the prevalence of all form of tuberculosis was 15.11% in all tuberculosis presumptive patients and 13.6% among children TB suspected. The prevalence of Rifampicin resistant *M. tuberculosis* was 9.9% in all tuberculosis detected cases, the 7.6% among new and 27.4% among patients who had a previous history of TB treatment. According to this study Sex and having previous history

of TB treatments were significantly connected with the prevalence of rifampicin resistant *M. tuberculosis* (47).

Study conducted by Bodene *et al*., 2019 at Hiwot Fana Specialized University Hospital showed that the prevalence of *Mycobacterium tuberculosis* was 15.7%. From the total *Mycobacterium tuberculosis* detected 4.1% were resistance for rifampicin. The *Mycobacterium tuberculosis* detection rate was somewhat higher in a specimen taken from the pulmonary site 15.7% when compared to specimen taken from the extra pulmonary site 13.4%. This study also showed that having history of taking Anti-tuberculosis drugs, being an HIV positive, sex and age were significantly related with the prevalence of tuberculosis (48).

Hospital -based cross-sectional Study conducted at Ataye District Hospital, North East Ethiopia showed that the prevalence of detected *Mycobacterium tuberculosis* was 8.98% and that of rifampicin resistant *Mycobacterium tuberculosis* was 5.3%. The rate of MTB /HIV co-infections was 7.89%. This study also showed that having history of imprisonment, frequent usage of crowded transportation and having close contact history with tuberculosis patients were significantly related with the burden of tuberculosis (49).

Hospital based cross sectional study design was conducted at Tigray, Northern Ethiopia to determine the prevalence of *M. tuberculosis* and rifampicin resistant *M. tuberculosis* by using GeneXpert MTB/RIF assay among suspected tuberculosis patients. According to this study the overall prevalence of *M. tuberculosis* was 7.9% and 9% of detected cases were resistant to rifampicin. This study also showed that having previous history of taking Anti-tuberculosis drugs and being the age of 18-29 years were significantly associated with high prevalence of *M. tuberculosis* (50).

A study conducted by Araya *et al*., 2020 at St. Peter Tuberculosis Specialized Hospital showed that the overall prevalence of *Mycobacterium tuberculosis* was 13.5%; of these, 9.8% were confirmed to have resistance to rifampicin. In this study, the prevalence of *M. tuberculosis* was 15.1% in males and 11.5% in females. This study also showed the prevalence of *M. tuberculosis* and Rifampicin resistant *M. tuberculosis* were significantly associated with having previous history of tuberculosis treatment (51).

Cross sectional study conducted at Gambella regional state showed that from a total presumptive tuberculosis patients, 20.0% of them had confirmed to have *M. tuberculosis* and 4.9% had rifampicin resistance. From the confirmed rifampicin resistant *M. tuberculosis,* 2.3% were detected among new patients and 14.3% were among patients having history of tuberculosis treatment. This study also showed the rate of TB/HIV co-infections was 35.5% and high prevalence of *M. tuberculosis* was detected among 15-44 year tuberculosis suspected patients (52).

The study conducted at University of Gondar Hospital showed the overall prevalence of M. tuberculosis was 24.6% and 15.8% had rifampicin resistance. The rate of /TB/HIV co-infection was 23.8% and 24.5% tuberculosis patients had previous history of tuberculosis treatment. According to this study, the high prevalence of *M. tuberculosis* (29.8%) was detected in the age group 24–30 years (53).

The study conducted at Felege Hiwot Referral Hospital and Debre Tabor General Hospital showed that the Overall GeneXpert assay diagnosed was 14.6%; of these, Rifampicin resistant detected was 9.3%. TB/HIV co-infection rate was 41.9%. Higher prevalence of *Mycobacterium tuberculosis* detected cases were observed among male patients 58.5%. According to this study, being HIV positive, sex, being presumptive drug resistance and age were significantly associated with the prevalence of tuberculosis (54). The same study conducted at Debre Berhan Referral Hospital also showed that the overall magnitude of *Mycobacterium tuberculosis* was 13% and that of rifampicin resistant *Mycobacterium tuberculosis* was 5.2%. According to this study the majority of affected presumptive tuberculosis patients were the age group 16-30 years (55).

Study conducted by Ramos *et al*., 2018 at Gambo Rural General Hospital showed that the samples detected for tuberculosis by GeneXpert MTB/RIF was 22.4%; of these, 0.3% rifampicin resistance was observed. The results of this study also showed that the prevalence of TB among children was 23.3% and 20.9% among Adults (56).

A Hospital-based cross-sectional study design carried out at Debre Markos Referral Hospital, to determine the prevalence of M. tuberculosis. Accordingly, the overall prevalence of *M. tuberculosis* was 117 (23.2%). From the total MTB detected, 12 (10.3%) were showed resistance to rifampicin. Rifampicin-resistant M. tuberculosis was observed in 7 (17.1%) patients who had a

previous history of TB treatment and 5 (6.7%) in patients with no had history of taken Anti-tuberculosis drugs. The magnitude of rifampicin-resistant M. tuberculosis was 6 (9.8%) in pulmonary and 6 (11.3%) in extra -pulmonary presumptive TB patients. This study also showed that the rate of MTB/HIV co-infection was 25.6% (57).

A study was carried out to determine the prevalence of Rifampicin resistant *M. tuberculosis* in presumptive tuberculosis patients at Dubti General Hospital, Afar. The result of this study showed that from a total of 384 presumptive TB patients the prevalence of MTB was 24.5%; from total detected MTB 4 (4.3%) were resistant to Rifampicin. The result of this study also indicated that the history of previous anti-TB treatment and close contact with TB patients were significantly associated with the prevalence of *M. tuberculosis* (58).

2.7. Diagnosis of Tuberculosis

Diagnosis of TB requires the detection of *Mycobacterium tuberculosis* from the biological sample by one of the following current microbiological techniques. These are smear microscopy, isolation in culture or molecular techniques. These assays form the basis for the microbiological diagnosis of TB and the clinicians may require detection of *Mycobacterium tuberculosis* in one or more specimens depending on the clinical symptoms (59).

2.7.1. GeneXpert for diagnosis of Tuberculosis

GeneXpert MTB/RIF has been recommended by the WHO in 2010 and approved by the Food and Drug Administration (FDA) in 2013 as an initial diagnostic tool for individuals suspected of drug-resistant tuberculosis, patients with TB/ HIV co-infections and pediatrics tuberculosis patients (26).

It is a semi quantitative nested real-time PCR designed to amplify the 81-bp hot-spot region of the rpoβ gene within Rifampin-resistance determining region (RRDR) and probing the region subsequently for RIF- resistance related mutations. The assay tests for MTB and mutations associated with Rifampicin resistance directly from clinical samples in less than 2 hours. The detection of rpoβ gene mutations is most useful for diagnosing RIF-resistance in *Mycobacterium tuberculosis*. The test has a sensitivity of 97.6% and specificity of 99.8% (16, 26).

The MTB/RIF assay blend and automates specimen processing, increasing the amount of nucleic acid and finding of the target order on the MTB/RIF test platform (GeneXpert, Cepheid). This

assay able to designates if Mycobacterium tuberculosis complex was notified or not notified in the specimen. In some cases there may be an occurrence of the result is “invalid,” test result, and the test is expected to be repeated. When *Mycobacterium tuberculosis* complex was noticed, the results also designates whether resistance to rifampicin was notified, not notified, or undefined (60).

2.8. Treatment of Tuberculosis

Effective regimens for the treatment of TB must contain multiple drugs to which the organisms are susceptible. Active, drug-susceptible TB disease is treated with 2RHZE/4RH that are provided with information and support to the patient by a health worker or trained volunteer (61). Patients with RR/MDR-TB require second-line treatment regimens. RR/MDR-TB patients may be treated using a 9–11-month MDR-TB treatment regimen (the shorter regimen) unless they have resistance to second-line anti-TB agents or meet other exclusion criteria. In these cases, a longer (individualized) prescribed course of medical treatment with a minimum five potent anti-tuberculosis drugs for 5-7 months and four anti-tuberculosis drugs in the continuation phase is recommended for ⩾20 months (62).

2.9. Tuberculosis prevention

The most principal method to control the expansion of tuberculosis and drug-resistant tuberculosis is to receive all anti-tuberculosis drugs precisely as ordered by the health care provider. No anti-tuberculosis drugs should be missed and taking anti-tuberculosis drugs should not be come to an end early by the patients. Patients who were under receiving treatment for tuberculosis should inform their health care providers if they are having any adverse effect while they are taking the anti-tuberculosis drugs. The other way to avert getting tuberculosis and drug-resistant tuberculosis is keep away from subjection to known tuberculosis patients in close contact or accommodation or spaces filled beyond what is safe such as refugee camps, prisons, hospitals, or homeless shelters (63).

3. Objectives

3.1. General objective

- To assess the prevalence of *Mycobacterium tuberculosis,* its Rifampicin-resistance pattern and associated factors among presumptive Pulmonary and Extra Pulmonary Tuberculosis patients at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia

3.2. Specific Objectives

- To determine the prevalence of *Mycobacterium tuberculosis* among presumptive Pulmonary and Extra Pulmonary Tuberculosis patients at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia
- To determine the Rifampicin resistance pattern of *Mycobacterium tuberculosis* detected from Pulmonary and Extra Pulmonary Tuberculosis presumptive patients at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia
- To identify the associated factors for the prevalence of *Mycobacterium tuberculosis* and Rifampicin resistant *M. tuberculosis* detected from presumptive Pulmonary and Extra Pulmonary Tuberculosis patients at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia

4. Methods and materials

4.1. Study area

This study was conducted at Madda Walabu University Goba Referral Hospital, which is located in Goba town, Bale zone, Southeast Ethiopia and has a distance of 446 km from Addis Ababa, the capital city of Ethiopia. According to the data obtained from the hospital, currently the hospital has more than 120 beds offering different specialized services and it serves more than 1.5 million peoples. MWU GRH accepts patients from the geographic area which studies are eligible (Goba) and comes from different district of the Bale zone. Based on the 2007 census conducted by Country Statistical Agency Bale Zone has a total population of 1.5 million. The hospital has separated room for GeneXpert, TB/HIV and Multidrug resistant tuberculosis room used for proper diagnosis and treatment of drug susceptible and Multidrug resistant tuberculosis patients.

4.2. Study design and period

Hospital based cross-sectional study design was conducted from October to February, 2021.

4.3. Population

4.3.1. Study population

All patients that attended Madda Walabu University Goba Referral Hospital who were presumptive Pulmonary and Extra Pulmonary tuberculosis and who fulfilled the inclusion basis were the study population.

4.4. Variables of the Study

4.4. 1. Dependent variables

- Prevalence of Tuberculosis
- Rifampicin-resistant *M. tuberculosis*

4.4.2. Independent variables

- Socio-demographic factors
  - ✓ Age
  - ✓ Sex
  - ✓ Marital status
  - ✓ Occupation
- HIV sero status
- Treatment history
  - ✓ New
  - ✓ Relapse
  - ✓ Defaulter
- Contact history
- Family size

4.5. Inclusion and exclusion criteria

4.5.1. Inclusion criteria

Presumptive patients of pulmonary or Extra-pulmonary tuberculosis who visited the Out Patients Departments and wards of Madda Walabu University Goba Referral Hospital and who signed consent form were involved in the study.

4.5.2. Exclusion criteria

Presumptive patients of pulmonary who provided sputum with obvious food particles and presumptive extra-pulmonary tuberculosis who provided a poor quality and insufficient volume of specimens were excluded from the study.

4.6. Sample size determination and sampling technique

4.6.1. Sample Size determination

The sample size was calculated using a single proportion formula. Accordingly, the sample size for the number of study subjects calculated by considering the prevalence of 23.2 %(P= 0.232) from a study done in Debre Markos hospital (46) and 95% level of confidence (α=0.5), with the tolerable error of 5% (d=0.05) as follows:

The sample size n= $z(\alpha/2)^2 p(1-p)/d^2$ $= (1.96)^2 0.232(1-0.232)/0.05^2 = 274$

n=sample size

z=level of confidence, 1.96

p=prevalence, 23.2 %

d=margin of error (precision), 0.05

Non response rate: 10% of 274=27

n= 274+ 27=301

the finally sample size was **301**

4.6.2. Sampling Technique

Non-probability, consecutive sampling technique was applied until the achievement of the expected sample size within the given study period. Presumptive Tuberculosis patients who accomplish the eligibility basis were allowed to take part in the study until the intended sample size was achieved.

4.7. Operational definition

The following definitions relating to Tuberculosis and drug resistance were used (8, 10, 12 and 32).

Presumptive TB: Patients with persistent and progressive cough for two or more weeks, (cough of any duration for HIV positives), fever, night sweats or loss of weight or chest X-ray abnormality suggestive of TB

Pulmonary tuberculosis: A patient with a TB case involving the lung parenchyma.

Extra pulmonary tuberculosis: A patient with a tuberculosis case involving organs other than the lungs.

New patients: A patient with a TB case who never received treatment for TB, or have taken anti-TB drugs for <1 month, may have negative or positive bacteriological specimens and may have the tuberculosis at any anatomical site.

Previously treated patients: A patient with a TB case, who has received one or greater than one month of anti-TB drugs in the past, may have negative or positive bacteriological specimens and may have the tuberculosis at any anatomical site.

Close contact: Defined as living in the same household or in frequent contact with a source case (e.g., care giver) with sputum smear-positive TB

DR-TB: TB that is resistant to any first-line anti-tuberculosis drug

Rifampicin resistance: Defined as in vitro resistance to Rifampicin only – a surrogate marker of MDR-TB.

MDR-TB: Tuberculosis which is caused by *M. tuberculosis* that show resistant to at least rifampicin and Isoniazid.

XDR-TB: Multidrug resistant tuberculosis plus resistant to any fluoroquinolone and at the minimum for one second-line injectable agent: Amikacin, Kanamycin, and/or Capreomycin.

Relapse: A patient who previously was declared cured or treatment completed and now is diagnosed with bacteriological positive (sputum smear or culture).

Defaulter: A patient interrupted treatment for 8 or more consecutive weeks after he/she had been on treatment for at least 4 weeks

Treatment failure: A patient who remains or becomes again smear-positive at the end of 5 months or later during treatment

4.8. Data collection procedures

4.8.1. Demographic characteristics and exposure to risk factors

Laboratory personnel and Nurses were identified, trained and informed to collect the data as per the pre-structured questionnaire. The purpose of the study was explained to the study participants accordingly. Demographic data and potential risk factors of tuberculosis including the presence of chronic diseases like HIV, Cigarette smoking habit and category of treatment-related risk factors including treatment failure and relapse were recorded (Annex 2).

4.8.2. Specimen collection

Each eligible patient provided clinical specimens (Sputum, Gastric lavage, Lymph node aspirates, pleural fluid, etc). From each patient presumptive of pulmonary TB, 4 ml of sputum (either morning or random sputum sample can be used) sample was collected in a 50 ml falcon

tube. For patients who were presumptive of extra pulmonary tuberculosis different extra pulmonary specimens such as, Pus, lymph node aspirate, peritoneal and pleural fluid samples were collected based on clinical presentations (Annex 1).

4.8.3. Specimen Processing

Clinical specimens were diluted and GeneXpert MTB/RIF assay was carried out as stated by the manufacturer's company and Specimen was instantly processed for GeneXpert MTB/RIF assay by Senior Laboratory technologist (52).

4.9. GeneXpert MTB/RIF Assay

Based on the manufacturer's manual, one ml sputum sample was treated with 2 mL of sample reagent containing sodium hydroxide (NaOH) and isopropanol. Then it was shaked gently, homogenized and incubated for 15 minutes at room temperature. Two mL of the treated samples were transferred into the cartridge and then loaded into the GeneXpert instrument (Cepheid, Sunnyvale, CA, USA)

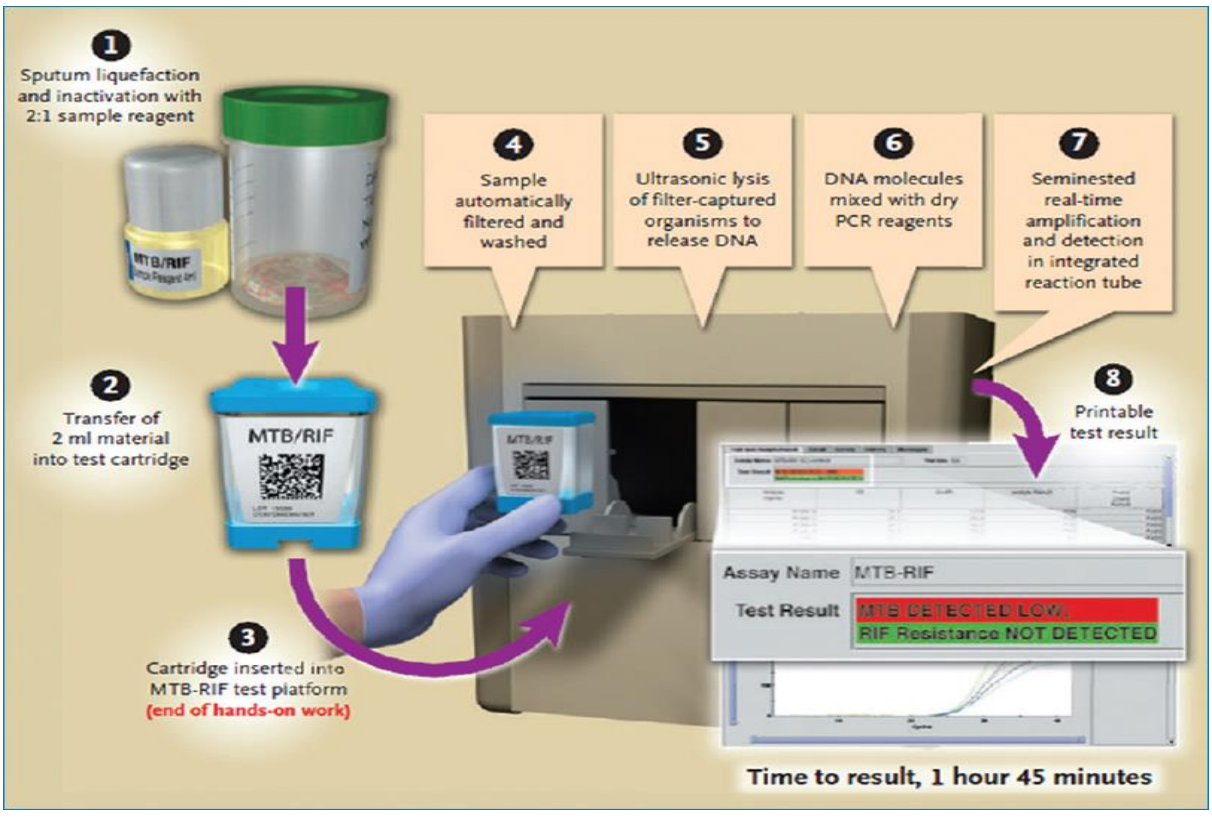

Figure 5: GeneXpert MTB/RIF Assay workflow

4.10. Data Quality Assurance

Data quality was ensured through the use of standardized data collection materials, pretesting of the questionnaires, proper training before the start of data collection and intensive supervision during data collection by the principal investigator. The patient's mouth was rinsed twice with water before sputum was collected in a sterile container (Falcon tube).

The GeneXpert MTB/RIF cartridges and reagents were stored at 2–28 °C, the cartridge was used within 4 hours after opening the cartridge lid, and powder-free glove was used because powdered gloves interfere with molecular detection.

4.11. GeneXpert MTB/RIF Quality Assurance

This was done through internal control (Probe check control (PCC) and sample processing control (SPC)). The SPC contains non-infectious spores (from *Bacillus globigii*) in the form of utilizing wet which is contained in every cartridge to demonstrate enough processing of *M. tuberculosis*. SPC ensures the sample was correctly processed**.** Sample processing control must be positive in a negative specimen and can be negative or positive in a positive specimen. The sample processing control passes if it encounters the proven acceptance basis. If the sample processing control is not notified in a negative test, the test result will not be accepted

Probe check control was carried out for each cartridge before initiation of polymerase chain reaction (PCR). The GeneXpert diagnosis system measures the fluorescence signal from the probes to monitor bead hydration, reaction-tube filling, probe integrity and dye stability are measured by the GeneXpert diagnosis system. Probe Check control get acceptance if it meets the appropriate standard acceptance basis (52).

4.12. Data analysis and interpretation

Data were analyzed by using Statistical Product and Service Solutions (SPSS) version 23.0 software. Frequency and percentage was calculated to summarize the results and presented in Tables and Graphs. . Bivariate and multivariate logistic regressions (Variables with p-value ≤ 0.25) were performed to compute the effect of independent variables on the dependent variable. Those results with p-value < 0.05 at 95% confidence interval were regarded as statistically significant.

4.13. Ethical Consideration

Ethical approval was secured from the Department of Microbiology, Immunology, and Parasitology of Addis Ababa University. Authorization and assistance letter were also secured from the Clinical director of Madda Walabu University Goba Referral Hospital. Before enrolling any of the eligible study participants; the purpose, benefits and confidential nature of the study was clarified and discussed for each participant. Volunteer Patients signed an agreement (consent form) to take part in the study. Data were collected after obtaining full informed written consent and confidentiality of the information was kept by using codes instead of any personal identifiers.

4.14. Dissemination of research findings

The finding of the study will be submitted to Addis Ababa University, DMIP and Madda Walabu University Goba Referral Hospital as well as other concerned organizations. The finding will also be disseminated to the national and international academic community through publication.

5. Results

5.1. Socio-Demographic Characteristics

A total of 301 tuberculosis presumptive patients were enrolled; of whom 165 (54.8 %) were males and 136 (45.2 %) females with age ranging from 2 months to 85 years. The majority of the study participants, 196 (65.1 %), were rural residents and 79 (25.6 %) were farmers. From the total of study participant, 240 (79.7%) patients were presumptive for pulmonary tuberculosis and 61 (20.3%) patients were presumptive for extra-pulmonary tuberculosis. Prevalence of HIV was 27 (9%) among study participants.

Table1. Frequency of Socio-demographic characteristics and risk factors of presumptive Pulmonary and EPTB patients at MWU GRH, Southeast Ethiopia, October to February 7, 2021

Variables		Frequency	Percent (%)
Sex	Male	165	54.8
	Female	136	45.2
Age	<15	63	20.9
	15-39	136	45.2
	40-59	77	25.6
	>60	25	8.3
Residence	Urban	105	34.9
	Rural	196	65.1
Family size	<4	72	23.9
	4-7	164	54.5
	>7	65	21.6
Educational level	Illiterate	145	48.2
	Elementary	104	34.6
	High school	34	11.3
	Above College	18	6
Habit of drinking milk	Raw	219	72.8
	Boiled	74	24.6
	No	8	2.7

Habit of smoking cigarette	No	285	94.7
	Yes	16	5.3
Habit of drinking alcohol	Yes	53	17.6
	No	248	82.4
Utilization of congested transportation	Yes	224	74.4
	No	77	25.6
Imprisonment history	Yes	21	7
	No	280	93
Contact with TB patient	Yes	60	19.9
	No	241	80.1
Previous history of TB treatment	Yes	54	17.9
	No	247	82.1
HIV infection	Negative	274	91
	Positive	27	9
Reason for examination	Suspected TB	248	82.4
	Suspected DR-TB	53	17.6
Presumptive drug resistant tuberculosis	Relapse	14	26.4
	Treatment failure	4	7.5
	Lost to follow-up	2	3.8
	New	31	58.5
	DRTB contact	2	3.8
Site of infection	Pulmonary	240	79.7
	Extra-pulmonary	61	20.3
Types of specimen	Sputum	204	67.8
	Gastric lavage	36	12
	Lymph node aspirate	16	5.3
	Pleural fluid	15	5
	Peritoneal fluid	12	4
	pus	18	6

5.2. Prevalence of *Mycobacterium tuberculosis*

The overall prevalence of detected *M. tuberculosis* among presumptive tuberculosis patients was 46 (15.3%). The prevalence of *M. tuberculosis* was 28 (17%) in males tuberculosis presumptive and 43 (13.2%) in females tuberculosis presumptive. *M. tuberculosis* was notified in 40 (16.7%) pulmonary suspected patients and in 6 (9.8%) extra-pulmonary suspected tuberculosis patients. The prevalence of *M. tuberculosis* was 13 (24.1%) in patients who had a previous TB treatment history and 4 (14.8%) rate of TB/HIV co-infection was detected. The overall prevalence of rifampicin resistant *Mycobacterium tuberculosis* was 2 (4.35%) and the cases were detected patients who had a previously history of tuberculosis treatment.

Table 2: Prevalence of *M. tuberculosis* among presumptive pulmonary and EPTB patients at MWU GRH by using GeneXpert MTB/RIF assay, October 22 to February 7, 2021

Characteristics		*M. tuberculosis*				Total	
		Detected (Positive)		Not detected (Negative)			
		No	%	No	%	No	%
Sex	Male	28	17	137	83	165	54.8
	Female	18	13.2	118	86.8	136	45.2
Age	<15	6	9.5	57	90.5	63	20.9
	15-39	31	22.8	105	77.2	136	45.2
	40-59	8	10.4	69	89.6	77	25.6
	>60	1	4	24	96	25	8.3
Residence	Urban	12	11.4	93	88.6	105	34.9
	Rural	34	17.3	162	82.7	196	65.1
Family size	<4	9	12.5	63	87.5	72	23.9
	4-7	22	13.4	142	86.6	164	54.5
	>7	15	23.1	50	76.9	65	21.6
Educational level	Illiterate	25	17.2	120	82.8	145	48.2
	Elementary	15	14.4	89	85.6	104	34.6
	High school	4	11.8	30	88.2	34	11.3
	Above College	2	11.1	16	88.9	18	6
Occupation	Gov. employer	1	4.3	22	95.7	23	7.6
	Unemployed	11	12.6	76	87.4	87	29
	Student	9	18	41	82	50	16.6
	Farmer	16	20.3	63	79.7	79	26.2
	House wife	9	14.5	53	85.5	62	20.6
Habit of drinking milk	Raw	39	17.8	180	82.2	219	72.8
	Boiled	7	9.5	67	90.5	74	24.6
	No	0	0	8	100	8	2.7

Habit of smoking cigarette	No	43	15.1	242	84.9	285	94.7
	Yes	3	18.8	13	81.2	16	5.3
Habit of drinking alcohol	Yes	5	9.4	48	90.6	53	17.6
	No	41	16.5	207	83.5	248	82.4
Utilization of congested transportation	Yes	42	18.8	182	81.2	224	74.4
	No	4	5.2	73	94.8	77	25.6
Imprisonment history	Yes	4	19	17	81	21	7
	No	42	15	238	85	280	93
Contact with TB patient	Yes	16	26.7	44	73.3	60	19.9
	No	30	12.4	211	87.6	241	80.1
Previous history of TB treatment	Yes	13	24.1	41	75.9	54	17.9
	No	33	13.4	214	86.6	247	82.1
HIV infection	Negative	42	15.3	232	84.7	274	91
	Positive	4	14.8	23	85.2	27	9
Reason for examination	Presumptive TB	35	14.1	213	85.9	248	82.4
	Presumptive DR-TB	11	20.8	42	79.2	53	17.6
Presumptive DR-TB	Relapse	3	21.4	11	78.6	14	26.4
	Treatment failure	4	100	0	0	4	7.5
	Lost to follow-up	0	0	2	100	2	3.8
	New	3	9.7	28	90.3	31	58.5
	MDR-TB contact	1	50	1	50	2	3.8
Site of infections	Pulmonary	40	16.7	200	83.3	240	79.7
	Extra-pulmonary	6	9.8	55	90.2	61	20.3
Types of specimen	Sputum	38	18.6	166	81.4	204	67.8
	Gastric lavage	2	5.6	34	94.4	36	12
	Lymph node aspirate	2	12.5	14	87.5	16	5.3
	Pleural fluid	1	6.7	14	93.3	15	5
	Peritoneal fluid	0	0	12	100	12	4
	Pus	3	16.7	15	83.3	18	6

5.4. Frequency of associated factors among tuberculosis presumptive

Risk factor assessment showed that 60 study subjects (19.9%) had history of contact with TB patients, 54 (17.9%) had history of anti-TB treatment, 27 (9%) were HIV positive. Bivariate analysis results showed that the prevalence of *M. tuberculosis* was significantly associated with history of contact with TB patients and previous history of TB treatment ($p<0.05$). On the other hand, rifampicin-resistant *M. tuberculosis* was also significantly associated with previous history of TB treatment ($p<0.05$). In a multivariate logistic regression analysis, participants aged between 15 and 39 years, history of previous TB treatment and history of contact with TB patients also showed significant association with the prevalence of *M. tuberculosis* ($p<0.05$).

Table 3: Bivariate and Multivariate analysis that shows the relationship between associated factors and Prevalence of *M. tuberculosis* at MWU GRH, Southeast Ethiopia, October to February, 2021

Variables		**COR (95% CI)**	**P-V**	**AOR (95% CI)**	**P-V**
Sex	Male	0.746 (0.39-1.41)	0.371		
	Female (Ref)				
Age	<15 (Ref)				
	15-39	2.53 (0.28-22.12)	0.128	6.67 (0.54-82.0)	**0.038**
	40-59	7.09 (0.92-54.49)	0.060	4.04 (0.44-37.12	0.217
	>60	2.78 (0.33-23.41)	0.146	1.20 (0.12-11.8)	0.874
Residence	Urban	1.62 (0.80-3.29)	0.177	0.75 (0.25-2.20)	0.605
	Rural (Ref)				
Family size	<4 (Ref)				
	4-7	0.476 (0.19-1.17)	0.108	0.69 (0.22-2.16)	0.534
	>7	0.51 (0.24-1.07)	0.076	0.55 (0.24-1.25)	0.157
Educational Level	Illiterate (Re)				
	Elementary	1.66 (0.36-7.71)	0.513		
	High school	1.34 (0.28-6.47)	0.709		
	Above Collage	1.06	0.944		

		(0.17-6.47)			
Habit of drinking milk	Raw	NA	0.999		
	Boiled	NA	NA		
	No (Ref)				
Habit of smoking cigarette	No (Ref)				
	Yes	0.77 (0.21-2.81)	0.693		
Habit of drinking alcohol	Yes	1.90 (0.71-5.06)	0.199	1.46 (0.44-4.84)	0.528
	No (Ref)				
Use of congested transportation	Yes	1.23 (0.08-0.68)	**0.008**	1.09 (0.01-0.52)	**0.007**
	No (Ref)				
Imprisonment history	Yes	0.75 (0.24-2.33)	0.62		
	No (Ref)				
Contact with TB patient	Yes	2.39 (0.19-0.77)	**0.007**	3.38 (0.17-.85)	**0.020**
	No (Ref)				
Previous history of TB treatment	Yes	2.48 (0.23-1.0)	**0.005**	3.09 (0-1.29)	**0.007**
	No (Ref)				
HIV infection	Negative (R)				
	Positive	1.04 (0.34-3.16)	0.074	0.57 (0.16-2.06)	0.398
Reason for examination	Presumptive TB (Ref)				
	Presumptive DR-TB	0.62 (0.29-1.33)	0.20	11.63 (0.78-1.72)	0.075
Site of infections	Pulmonary	1.83 (0.73-4.54)	0.191	1.63 (0.59-4.5)	0.338
	EPTB (Ref)				

AOR: Adjusted Odds ratio, **COR**: Crude Odds ratio, **CI**: Confidence interval, **Ref**: Reference category, **Bold p-value**: Significant association

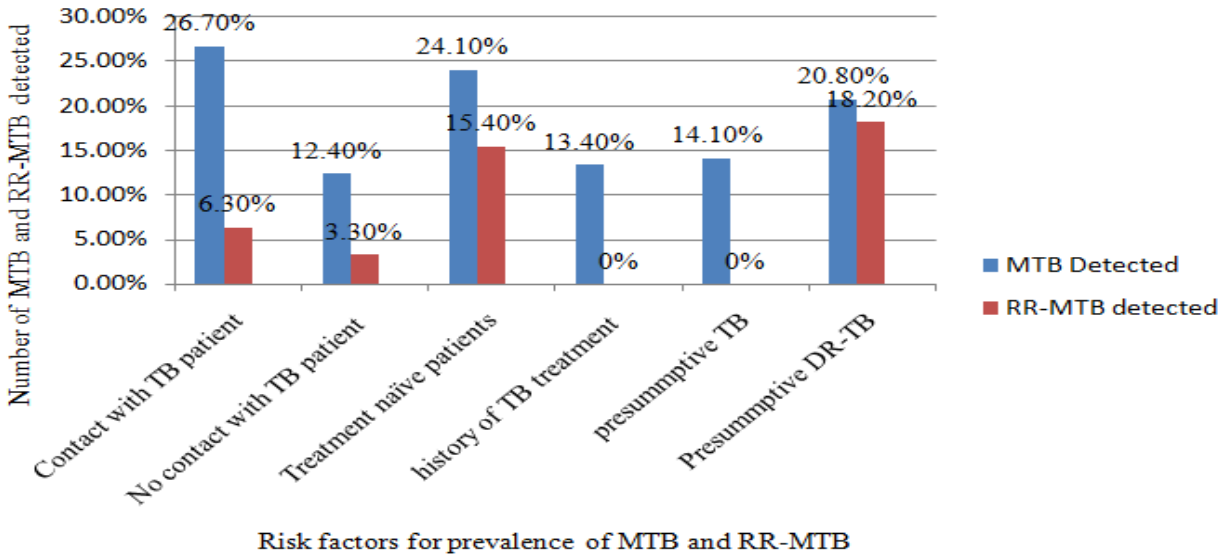

Figure 6: Frequency of MTB and RR-MTB detected in presumptive TB patients with regards of contact with TB patients, previous history of TB treatment and reason for diagnosis at MWU GRH, October to February, 2021

6. Discussion

Ethiopia is among top 30 high TB burden countries having an incidence of all forms of TB 140 per 100,000 populations, 0.71% and 16% of the estimated proportion of TB cases with MDR/RR-TB among new and previously treated cases respectively (21). This study assessed the current burden and factors associated with the prevalence of drug susceptible TB and RR-TB among TB presumptive patients at MWU GRH by using the GeneXpert MTB/RIF assay from October 2020 to January 2021.

6.1. The overall prevalence of *Mycobacterium tuberculosis*

In the present study, the overall prevalence of *M. tuberculosis* among presumptive TB patients was 15.3%, which is in agreement with studies conducted in Addis Ababa, 15.1% (47), Hiwot Fana Specialized University Hospital 15.7% (48), Felege Hiwot Referral Hospital and Debre Tabor General Hospital 14.6% (54). However, result from our study was lower than other similar studies done in Gambo Rural General Hospital 22.4% (56), Debre Markos Referral hospital 23.2% (57), India 32.9% (7) and Egyptian population 19% (46).The possible reasons attributable for such like inconsistency were variation in subset of targeted population, setting and locations where the data was collected, and framework of study design. In contrast, it is higher than studies conducted in other parts of Ethiopia like Ataye District Hospital, 8.98% (49) and Tigray 7.9% (50). This lower prevalence and discrepancy in estimation with the current study might be due to dissimilarities in nature of study subjects and geographical area.

6.2. Prevalence of Rifampicin resistant *Mycobacterium tuberculosis*

In this study, the prevalence of rifampicin-resistant *M. tuberculosis* was 4.35%. This result is congruent with studies done in Dubti Hospital, Afar, Ethiopia 4.3% (58), Hiwot Fana Specialized University Hospital 4.1% (48), and Debre Berhan referral hospital 5.2% (55). However, it was higher than studies conducted in India 2.7 % (45), Nigeria 2.4% (44) and Gambo general rural hospital 0.3% (56). This might be due to majority of TB suspected were new cases and differences in TB control and prevention program among countries. To the contrary, there was a lower reported prevalence of rifampicin-resistant *M. tuberculosis* from Eastern Uttar Pradesh, India 26.1% (7) and other parts of Ethiopia like Debre Markos Referral hospital 10.3% (57), University of Gondar Hospital 15.8% (53), Tigray, Northern Ethiopia 9% (50), and St. Peter

Tuberculosis Specialized Hospital 9.8% (51). This lower prevalence and inconsistency could be due to difference in history of taking Anti-tuberculosis drugs, possibility of HIV/AIDS acquisition and TB, TB/HIV and MDR-TB prevention and control program.

6.3. Associated factors of tuberculosis infections among TB presumptive

To achieve the global targets in TB control and prevention, there is a need for a better understanding of specific factors that enhance burden of tuberculosis. Some factors are identified by this analysis as consistent with what is already understood about TB burden:

In our study, the prevalence of *M. tuberculosis* in relation to gender among TB presumptive showed difference between male and female. The prevalence of *M. tuberculosis* was 17% in males and 13.2% in females but no statistically significant association was observed. WHO 2020 Global Tuberculosis Report, showed that tuberculosis exert an influence on both males and female and in all people of age groups, however the inflated burden was in men of productive ages, who shared 56 % of all tuberculosis cases in 2019 (41). Similar higher prevalence MTB in Males than Females were reported in Eastern Uttar Pradesh India (7), Bayelsa state, Nigeria (44), St. Peter Tuberculosis Specialized Hospital (51), and Felege Hiwot Referral Hospital and Debre Tabor General Hospital (54). This Variation in burden of tuberculosis among male and female could be due to difference in community cultural expectations on attempt to get healthcare services, having habit of smoking and alcoholism, high level exposure of males to surrounding or outer environment.

The results of our study showed that the assessed TB/HIV co-infection rate was 8.7%, which is comparable with studies done at Ataye District Hospital, 7.89% (49) and Addis Ababa 6.7% (47). It is also comparable with WHO estimation on TB/HIV co-infection report of 2019 which shows 8.2% of new HIV patients were confirmed to develop tuberculosis (41). In contrary to the present study, high rate of TB/ HIV co-infection was reported from Gambella regional state, southwest Ethiopia 35.5% (52), Felege Hiwot Referral Hospital and Debre Tabor General Hospital 41.9 % (54), and University of Gondar Hospital 23.8 % (53). This inconsistency could be due to difference in the high burden of HIV setting area, Lack of awareness of TB/HIV co-morbidity among communities and the extent of performed HIV testing.

The prevalence of *M. tuberculosis* in relation to ages among TB presumptive patients showed significance differences between age groups, with age group 15-39 years having the highest prevalence (22.8%) followed by 40-59 years (10.4%) and <15 years (9.5%) while the least prevalence was recorded within the age group >60 (4%). The chance of getting *M. tuberculosis* was 6.67 fold in patients between ages 15-39 when compared to those age >60 years, the difference being statistically significant with p value 0.038. This significance difference may be due to the extent contact of productive ages to the surrounding for different activities, and high movability of those productive ages group from one location to another may be results in chance of getting tuberculosis bacilli. Our result is in agreement with similar studies in Pakistan (42), Nigeria (44), Debre Berhan referral hospital (55), and Gambella regional state (52).

In this study, 248 (82.4%) study participants were presumptive TB and 53 (17.6 %) study participants were identified to have had presumptive of DRTB. The prevalence of *M. tuberculosis* was higher (20.8%) in presumptive of DRTB patients than presumptive TB patients (14.1%), but no statistically significant association was observed (P>0.05) (Table 4). The reasons for this high prevalence of *M. tuberculosis* in suspected drug resistance tuberculosis patients could be due not succeeded tuberculosis treatment regimen and chance of getting of bacilli from suspected drug resistant pulmonary tuberculosis close contacts. Our finding agrees with other studies which reported higher prevalence among presumptive DRTB patients compared to presumptive TB patients: 36.7% presumptive DRTB versus 15.1% presumptive TB in Debre Markos Referral hospital (57).

With respect to previous history of TB treatment, of the total 301 study participants majority of them were treatment naive patients accounting 247 (82.1%), whereas the remaining 54 (17.9%) had a history of taking anti-TB drugs. However, the prevalence of *M. tuberculosis* among previously treated patients was higher 24.1% than treatment new patients 13.4%, with statistical significant difference (P<0.050) (Table 4). Our finding agrees with other studies which reported higher prevalence *M. tuberculosis* among previously treated tuberculosis patients compared to treatment new patients: 50% previously treated TB patients versus 19.3% treatment new patients in National Tuberculosis Center, Nepal (43); 27.6% previously treated TB patients versus 15.5% treatment new patients in Hiwot Fana Specialized University Hospital (48); 17%

previously treated TB patients versus 13.1% treatment new patients in St. Peter Tuberculosis Specialized Hospital (51).

WHO 2020 report showed that an estimated 18% of previously tuberculosis treated patients had chance of developing MDR/RRTB. In this study, high prevalence of rifampicin resistant *M. tuberculosis* was detected among patients who had a history of previously taking anti-tuberculosis drugs (15.4 %) which is comparable with studies from Eastern Uttar Pradesh (7), National Tuberculosis Center, Nepal 13.79% (43) and selected governmental hospitals in Addis Ababa 27.4% (47).This might be due to lack of success from previous tuberculosis treatment history and patients with previously history of taking anti-tuberculosis drugs were more likely to harbor drug-resistant bacilli because of previous exposure of bacilli to TB agents.

In this study, high prevalence of pulmonary tuberculosis 16.7 % was detected when compared with extra-pulmonary tuberculosis which was 9.8%. This result agrees with a study done at Provincial TB Reference Laboratory, Pakistan, pulmonary tuberculosis (48.8%) and extra-pulmonary tuberculosis (6.8%) (42), in Egyptian population (46) pulmonary tuberculosis (22.7%) and extra-pulmonary tuberculosis (10%). The current study also agrees with the study done at Hiwot Fana Specialized University Hospital (48), 15.7% pulmonary tuberculosis and 13.4%the extra pulmonary tuberculosis. This shows that pulmonary tuberculosis infection has inflated chance of transmission from pulmonary tuberculosis patients during coughing, sneezing and others way of bacilli transmission.

In present study the rifampicin resistant *M. tuberculosis*, 2 (4.35%) were detected among rural residents and this study agrees with the findings from Dubti General Hospital, Afar (58). The most likely reason might be due to low socioeconomic status and far distance from health facilities which may affect direct observation therapy (DOT) and the treatment adherence. The result of this study also showed frequent utilization of congested transportation had a significant association ($p<0.05$) with the prevalence of drug susceptible tuberculosis and drug resistance tuberculosis. The possible reason for such significance could be there is chance of acquiring bacilli from the index cases because of denied of opening window for different reasons and lack of ventilation during voyage. This study agrees with study done at Ataye district hospital (49).

The prevalence of detected *M. tuberculosis* in relation to contact history with TB patients among TB presumptive was analyzed and showed significance difference. In this study, 241 (80.1%) study participants had no history of contact with TB patients and 60 (19.9 %) study participants were identified to have a history of contact with TB patients. The prevalence of *M. tuberculosis* was higher 26.7% in patients who had history of contact with TB patients than who had no history of contact with TB patients 12.4%, the difference being statistically significant with p value 0.02 (Table 4). The reasons for this high prevalence of *M. tuberculosis* in patients who had history of contact with TB patients might be due to the patients have poor adherence and acquiring of bacilli from TB patients. Our finding is supported by similar study from Ataye District Hospital (49) and Dubti Hospital, Afar (58), which showed that patients who had history of contact with TB patients had high prevalence of *M. tuberculosis.*

7. Limitation of the study

This study was conducted in a single institution. It would have been better if we incorporated a number of health institutions for a better representation of study subjects. This study also lacks any other diagnostic tool that can be used as gold standard like culture (LJ or MGIT).

8. Conclusion and recommendation

Conclusion

Our study showed that high rate of *Mycobacterium tuberculosis* at Madda Walabu University Goba Referral Hospital in presumptive Tuberculosis patients. This indicated that TB is still a serious public health problem that needs to be addressed urgently particularly in the study area and generally in Ethiopia. The finding obtained from this study has showed that having history of taking anti-tuberculosis drugs, close contacts with tuberculosis patients and frequent use of congested transportation were significantly associated with the prevalence of *M. tuberculosis* at the study area.

Recommendations:

- Improving tuberculosis case notification through early identification of presumptive tuberculosis case is mandatory.
- Early diagnosis of tuberculosis and drug resistant tuberculosis should be practiced for prompt treatment and better outcome.
- There is an implication for broad-scale investigation on the prevalence of extra pulmonary tuberculosis by using more sensitive laboratory techniques
- Further study also needed to establish a clear association between GeneXpert test and MTB culture with microscopic examination of TB.

9. References

1. World Health Organization. Definitions and reporting framework for tuberculosis-2013 *Revision.* 2013
2. Sanchez JL, Sanchez JL, Cooper MJ, Hiser MJ, Mancuso JD. Tuberculosis as a force health protection threat to the United States military. *A Review Military Medicine*. 2015; 180:276–84
3. World Health Organization. Tuberculosis fact sheet N°104. *Revised* March 2006
4. World Health Organization. Global tuberculosis report. Geneva: *World Health*; 2019
5. Porter JD, McAdam KP. Tuberculosis in Africa in the AIDS era—the role of chemoprophylaxis. Transactions of the *Royal Society of Tropical Medicine and Hygiene*. 1992 Sep 1; 86(5):467-9.
6. Ormerod LP. Multi-drug resistant tuberculosis (MDR-TB) epidemiology, prevention and treatment. *British Medical Bulletin*. 2005; 73 and 74:17-24
7. Gautama PB, Mishra A, Kumar S. Prevalence of rifampicin resistant Mycobacterium tuberculosis and associated factors among presumptive tuberculosis patients in eastern Uttar Pradesh: a cross sectional study. *International Journal of Community Medicine and Public Health*. 2018 May 22; 5(6):2271-6
8. Guidelines on programmatic management of drug resistant tuberculosis in India, *Revised National Tuberculosis Control Program*. 2017
9. Sharma SK, Mohan A. Multidrug-resistant tuberculosis: a menace that threatens to destabilize tuberculosis control. *Chest* 2006; 130: 261-72
10. WHO Global Project on Anti-tuberculosis Drug Resistance Surveillance. Anti-Tuberculosis Drug Resistance in the World. *Report No.4.* Geneva, Switzerland: WHO; WHO/HTM/TB/2008.394.
11. Riordan P, Schwab U, Logan S, et al. Rapid molecular detection of rifampicin resistance facilitates early diagnosis and treatment of multi-drug resistant tuberculosis. *PLoS ONE*. 2008; 3:1–7
12. WHO: treatment guidelines for drug-resistant tuberculosis, 2016 update. October 2016 *Revision*

13. Pinyopornpanish K, Chaiwarith R, Pantip C, Keawvichit R, Wongworapat K, Khamnoi P, et al. Comparison of GeneXpert MTB/RIF assay and the conventional sputum microscopy in detecting *Mycobacterium tuberculosis* in Northern Thailand. *Tuberculosis Research and Treatment*. 2015; 2015:571782
14. Blakemore R, Story E, Helb D, Kop J, Banada P, Owens MR, *et al.* Evaluation of the analytical performance of the GeneXpert MTB/RIF assay. *Journal of Clinical Microbiology*.2010; 48:2495-501
15. Chang K, Lu W, Wang J. Rapid and effective diagnosis of tuberculosis and rifampicin resistance with Xpert MTB/RIF assay: a meta-analysis". *Journal of infection.*2012; 64(6):580–8
16. Boehme CC, Nabeta P, Hillemann D. Rapiad molecular detection of tuberculosis and rifampin resistance". *New England Journal of Medicine*.2010; 363(11):1005–15
17. Story BE, Helb D. Evaluation of the analytical performance of the Xpert MTB/RIF assay. *Journal of Clinical Microbiology*. 2010; 48(7):2495–501
18. Sam IC, Drobniewski F, More P, Kemp M, Brown T. *Mycobacterium tuberculosis* and rifampin resistance, United Kingdom. *Emerging Infectious Diseases*.2006; 12:752–75
19. El-Hajj HH, Marras SA, Tyagi S, Kramer FR, Alland D. Detection of rifampin resistance in *Mycobacterium tuberculosis* in a single tube with molecular beacons. *Journal of Clinical Microbiology*. 2001; 39:4131–4137.
20. Ministry of Health. Annual Report of National Tuberculosis and Leprosy Control Program. Addis Ababa; 2018
21. World Health Organization. Global tuberculosis report. *World Health Organization;* 2018
22. Biadglegne F, Sack U, Rodloff AC. Multidrug-resistant tuberculosis in Ethiopia: efforts to expand diagnostic services, treatment and care. *Antimicrobial Resistance and Infection Control*.2014 Dec; 3(1):31.
23. Mekonnen F, Tessema B, Moges F, Gelaw A, Eshetie S, Kumera G. Multidrug resistant tuberculosis: prevalence and risk factors in districts of Metema and west Armachiho, Northwest Ethiopia. *BMC infectious diseases*. 2015 Dec; 15(1):461.
24. Shenoi S, Friedland G. Extensively drug-resistant tuberculosis: a new face to an old pathogen. *Annual Review of Medicine*. 2009; 60:307–20

25. Zignol M, Dean AS, Falzon D, et al. Twenty Years of Global Surveillance of Anti tuberculosis-Drug Resistance. *New England Journal of Medicine*. 2016; 375:11
26. Walusimbi S, Bwanga F, De Costa A, Haile M, Joloba M, Hoffner S. Meta-analysis to compare the accuracy of Gene Xpert, MODS and the WHO 2007 algorithm for diagnosis of smear negative pulmonary tuberculosis. *BMC infectious diseases*. 2013; 13:507
27. Espinal MA, Laszlo A, Simonsen L, Boulahbal F, Kim SJ, Reniero A, *et al*. Global trends in resistance to antituberculosis drugs. World Health Organization-International Union against Tuberculosis and Lung Disease Working Group on Anti-Tuberculosis Drug Resistance Surveillance. *New England Journal of Medicine.*2001; 344:1294-303.
28. Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K, Garnier T, Gutierrez C, Hewinson G, Kremer K, Parsons LM. A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. *Proceedings of the national academy of Sciences*. 2002 Mar 19; 99(6):3684-9.
29. Brennan PJ. Structure, function, and biogenesis of the cell wall of *Mycobacterium tuberculosis.Tuberculosis*.2003 Feb 1; 83(1-3):91-7.
30. Urdahl KB, Shafiani S, Ernst JD. Initiation and regulation of T-cell responses in tuberculosis. *Mucosal immunology*. 2011 May; 4(3):288.
31. Wolf AJ, Desvignes L, Linas B, Banaiee N, Tamura T, Takatsu K, Ernst JD. Initiation of the adaptive immune response to *Mycobacterium tuberculosis* depends on antigen production in the local lymph node, not the lungs. *Journal of Experimental Medicine*.2008 Jan 21; 205(1):105-15.
32. Zhang Y, Yew W W. Mechanisms of drug resistance in *Mycobacterium tuberculosis*. *International Journal of Tuberculosis and LungDisease.*2009; 13: 1320–1330.
33. Zhang Y. Persisters, persistent infections and the Yin–Yang model. *Emerging Microbes & Infections.*2014 Jan 1; 3(1):1-0.
34. Jiang X, Zhang W, Zhang Y, Gao F, Lu C, Zhang X, Wang H. Assessment of efflux pump gene expression in a clinical isolate *Mycobacterium tuberculosis* by real-time reverse transcription PCR. *Microbial Drug Resistance*. 2008 Mar 1; 14(1):7-11.
35. Piccaro G, Pietraforte D, Giannoni F, Mustazzolu A, Fattorini L. Rifampin induces hydroxyl radical formation in *Mycobacterium tuberculosis*. *Antimicrobial Agents and Chemotherapy*.2014; 58: 7527–7533

36. Telenti A, Imboden P, Marchesi F, et al. Detection of rifampicin resistance mutations in *Mycobacterium tuberculosis. Lancet.* 1993; 341: 647–650
37. Bodmer T, Zurcher G, Imboden P, et al. Mutation position and type of substitution in the beta-subunit of the RNA polymerase influence in-vitro activity of rifamycins in rifampicin-resistant *Mycobacterium tuberculosis. Journal of Antimicrobial Chemotherapy.*1995; 35: 345–348.
38. Williams D L, Spring L, Collins L, et al. Contribution of *rpo*B mutations to development of rifamycin cross-resistance in *Mycobacterium tuberculosis* . *Antimicrobial Agents and Chemotherapy.* 1998;42: 1853–1857
39. Narasimhan P, Wood J, MacIntyre CR, Mathai D. Risk factors for tuberculosis. *Pulmonary Medicine*.2013
40. Kuyinu YA, Odugbemi BA, Salisu-Olatunji SO, Adepoju FO, Odusanya OO. Characteristics of *Mycobacterium tuberculosis* Positive Patients Screened for Drug-Resistant Tuberculosis at a Tertiary Health Facility in Lagos, Nigeria. *Journal of the National Medical Association*. 2018 Feb 1; 110(1):88-91.
41. World Health Organization. Global tuberculosis report 2019. Geneva: *World Health Organization Press*; 2020.
42. Siddique G, Shafee M, Abbas F, Naeem M, Raziq A, Ahmad S, Babar Km. *Mycobacterium tuberculosis* ; Frequency of rifampin resistance mutations in 81-bp rrdr of rpob gene in *Mycobacterium tuberculosis* isolates in quetta, pakistan. *ProfessionalMedical Journal*. 2019 June 1; 26(5).
43. Ikuabe PO, Ebuenyi ID. Prevalence of rifampicin resistance by automated Genexpert rifampicin assay in patients with pulmonary tuberculosis in Yenagoa, Nigeria. *The Pan African Medical Journal.* 2018; 29.
44. Etim NG, Briyai FO. Prevalence of Pulmonary and Rifampicin-resistant tuberculosis among patients attending Federal Medical Centre, Yenagoa, Bayelsa state, Nigeria. *International Journal of Healthcare and Medical Sciences.* 2017; 3(11):85-92.
45. Nair SA, Raizada N, Sachdeva KS, Denkinger C, Schumacher S, Dewan P, Kulsange S, Boehme C, Paramsivan CN, Arinaminpathy N. Factors associated with tuberculosis and rifampicin-resistant tuberculosis amongst symptomatic patients in India: *Public Library of Science One*. 2016 Feb 26; 11(2).

46. Ali SA, Hassan RM, Shehata MM. GeneXpert MTB/RIF as a Rapid Diagnostic tool for Diagnosis of Pulmonary, Extra-pulmonary Tuberculosis and Rifampicin Resistance in the Egyptian Population. *Journal of Advances in Microbiology*. 2018 Dec 8:1-4
47. Arega B, Menbere F, Getachew Y. Prevalence of rifampicin resistant Mycobacterium tuberculosis among presumptive tuberculosis patients in selected governmental hospitals in Addis Ababa, Ethiopia. *BMC infectious diseases*. 2019 Dec 1;19(1):307
48. Bodena D, Ataro Z, Tesfa T. Trend Analysis And Seasonality Of Tuberculosis Among Patients At The Hiwot Fana Specialized University Hospital, Eastern Ethiopia: A Retrospective Study. *Risk Management and Healthcare Policy*. 2019;12:297
49. Gebretsadik D, Ahmed N, Kebede E, Mohammed M, Belete MA. Prevalence of tuberculosis by automated GeneXpert rifampicin assay and associated risk factors among presumptive pulmonary tuberculosis patients at Ataye District Hospital, North East Ethiopia. *Infection and drug resistance*. 2020; 13:1507.
50. Wasihun AG, Dejene TA, Hailu GG. Frequency of MTB and rifampicin resistance MTB using Xpert-MTB/RIF assay among adult presumptive tuberculosis patients in Tigray, Northern Ethiopia: A cross sectional study. *Public Library of Science one*. 2020 Nov 4;15(11)
51. Araya S, Negesso AE, Tamir Z. Rifampicin-Resistant Mycobacterium tuberculosis Among Patients with Presumptive Tuberculosis in Addis Ababa, Ethiopia. *Infection and Drug Resistance.* 2020;13:3451
52. Ejeta E, Beyene G, Bonsa Z, Abebe G. Xpert MTB/RIF assay for the diagnosis of Mycobacterium tuberculosis and Rifampicin resistance in high human immunodeficiency virus setting in Gambella regional state, southwest Ethiopia. *Journal of clinical tuberculosis and other Mycobacterial diseases*. 2018 Aug 1;12:14-20
53. Jaleta KN, Gizachew M, Gelaw B, Tesfa H, Getaneh A, Biadgo B. Rifampicin-resistant Mycobacterium tuberculosis among tuberculosis-presumptive cases at University of Gondar Hospital, Northwest Ethiopia. *Infection and Drug Resistance*. 2017;10:185
54. Derbie A, Seble W, Daniel M, et al. Xpert MTB/RIF assay for the diagnosis of Mycobacterium tuberculosis and its Rifampicin resistance at Felege Hiwot and Debre Tabor Hospitals, Northwest Ethiopia: a preliminary implementation research. *Ethiopian Journal of Health Development*. 2016; 30(2):61–63

55. Asfaw T, Terefe A, Nigus M. Patterns and Trends of Rifampicin-Resistance Mycobacterium tuberculosis and Associated Factors among Presumptive Tuberculosis Patients at Debre Berhan Referral Hospital. *Journal of Bioprocess and Biotechnology*. 2018; 8(339):2.
56. Ramos JM, Fernández-Muñoz M, Tisiano G, Fano H, Yohannes T, Gosa A, Tesfamariam A, Verdu-Expositvo C, Romanyk J, Reyes F, Gorgolas M. Use of Xpert MTB/RIF assay in rural health facilities in southern Ethiopia. *Archives of Clinical Microbiology*. 2017; 8:2.
57. Mulu W, Abera B, Yimer M, Hailu T, Ayele H, Abate D. Rifampicin-resistance pattern of Mycobacterium tuberculosis and associated factors among presumptive tuberculosis patients referred to Debre Markos Referral Hospital, Ethiopia: a cross-sectional study. *BMC research notes*. 2017 Dec;10(1):8
58. Gebrehiwet GB, Kahsay AG, Welekidan LN, Hagos AK, Abay GK, Hagos DG. Rifampicin resistant tuberculosis in presumptive pulmonary tuberculosis cases in Dubti Hospital, Afar, Ethiopia. *The Journal of Infection in Developing Countries*. 2019 Jan 31; 13(01):21-7
59. Cornaglia G, Courcol R, Herrmann JL, Kahlmeter G, Peigue-Lafeuille H, Jordi V, editors. European manual of clinical microbiology. *European Society for Clinical Microbiology and Infectious Diseases.* 2012.
60. Lawn SD, Nicol MP. Xpert® MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future microbiology*. 2011 Sep; 6(9):1067-82.
61. World Health Organization. Guidelines for treatment of drug susceptible tuberculosis and patient care, 2017 update. Geneva, Switzerland: *World Health Organization*; 2017.
62. Falzon D, Schünemann HJ, Harausz E, *et al.* World Health Organization treatment guidelines for drug-resistant tuberculosis, 2016 update. *European Respiratory Journal* 2017; 49: 1602308
63. Centers for Disease Control and Prevention. Guidelines for preventing the transmission of Mycobacterium tuberculosis in health-care settings. *Morbidity and Mortality Weekly Report.* 2005; 54

64. World Health Organization. Policy Statement: Automated Real-time Nucleic Acid Amplification Technology for rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: GeneXpert MTB/RIF System. Geneva, Switzerland: *World Health Organization;* 2011.

Annex I: Procedure for specimen collection and processing

Sputum collection

Sputum was obtained by expectoration method from patients greater than 5 years of age who able to cooperate to provide purulent sputum by instructing to clean their mouth with water before giving the sputum. Then, the patient was instructed to have two deep inhalation, keeping the inhalation for a few seconds after every breath and then breathing out slowly. He or she was informed to inhale or breathe in the third time and then forcefully blow the air out by asking the patient to breathe in again and then cough to produce purulent sputum from deep in the lungs into leak-proof container.

Extrapulmonary samples collection

Fine needle aspirate (FNA) specimens were collected by a pathologist, but others body fluid specimens were collected by physicians during patient examination and sent to the tuberculosis laboratory for GeneXpert MTB/RIF assay test.

Gastric Lavage Collection

Gastric lavage was performed in the early morning after an overnight fast of at least 4 hours. A nasogastric tube was passed and normal saline 20 Ml inserted, left for 3 minutes, and then aspirated. An additional 5–10 Ml of normal saline was introduced and aspirated, until a minimum of 20 Ml of aspirate was obtained. Then the gastric content was transferred into a sterile container, and the specimen container was wiped with alcohol to prevent cross contamination, and transported to the laboratory as soon as possible within 4 hours.

Preparation of Sputum Specimens

The Sample Reagent (SR) was added to untreated sputum specimen in a 2:1 ratio and to each decontaminated sputum specimen pellets in a 3:1 ratio. The lid was tightly replaced to avoid any spill or leakage. Briefly vortex the closed sputum containers and keep at room temperature for ten minutes. After 10 min incubation, the container again re-vortexes and incubate for an additional 5 minutes at room temperature. After the incubation, the sputum specimens were completely liquefied, inactivated and ready for testing with GeneXpert® MTB/RIF assay.

Preparation of Specimens other than Sputum

The specimens other than sputum (bronchoalveolar lavage, gastric aspirate, pleural fluid, ascitic fluid, cerebrospinal fluid, and pericardial fluid) were first concentrated by centrifugation for 15 minutes at 3000×g because of their high volumes (10-30 ml). The supernatant was carefully decanted and the concentrated pellets were processed further similarly like sputum specimens by adding Sample reagent (SR) in a 3:1 ratio to each concentrated pallet.

Preparing the Cartridge

Diluted specimen was transferred into the sterile transfer pipette up to the predetermined mark (=2ml). Then, the specimen was slightly transferred into the open part of the GeneXpert MTB/RIF cartridge. The cartridge cover was closed and the cover snaps securely into place. The test was begin within 30 minutes of adding the specimen to the cartridge.

Reading, Recording and Reporting

View the results by clicking the menu bar in the GeneXpert Dx System window. Then the view window activated and become visible. In case of error, invalid or no result was reported by the software, the test was repeated using the readymade specimen and a new cartridge. When there was repeated error, Invalid or no result, manual troubleshooting was done to exclude technical problems.

Interpretation of the Results

The software reported MTB detected or not MTB detected. While the Rifampicin resistance result indicated Rifampicin resistance not detected or Rifampicin resistance detected. Accordingly:

- **Rifampicin resistance detected**: A mutation in the rpoβ gene has been detected that falls within the valid delta Ct setting.
- **Rifampicin resistance not detected**: No mutation in the rpoβ has been detected.
- **rifampicin resistance indeterminate**: The MTB concentration was very low and resistance could not be detected

Annex II: Information sheet, Assent and Consent form

Information Sheet for Adults (≥18 years) (English Versions)

Name of Organization:-Department of Microbiology. Immunology and Parasitology, College of Health Sciences, Addis Ababa University

Principal Investigator: Wakuman Taye

Title: Prevalence of *Mycobacterium tuberculosis*, its Rifampicin-resistance pattern and associated factors among presumptive Pulmonary and Extra Pulmonary Tuberculosis patients at MWU GRH, Southeast Ethiopia

Introduction

I am asking you to take part in a research to be conducted by Master of Science candidate in Medical Microbiology, from Addis Ababa University.The team of this research include principal investigator and advisors from Addis Ababa University Microbiology, Immunology and Parasitology department. I want to be sure that you understand the purpose and your responsibilities in the research before you decide if you want to be part of the study. Please ask us to explain any words or information that you may not understand.

Purpose of the study

The purpose of the study is to determine the Rifampicin-resistance pattern of *Mycobacterium tuberculosis* and associated risk factors among presumptive Pulmonary and Extra Pulmonary Tuberculosis patients at MWU GRH, Southeast Ethiopia

Possible Risks (During sample collection)

No risks for patients who provide sputum samples and there may be minimal pain for patients who provide samples other than sputum. Generally, it is very unlikely that participation in this research will expose you to any physical, social or psychological risks.

Possible Benefits

Participation in this research may not benefit you directly. But results from this study will be used to inform decisions in implementation and strengthening of programs aimed at controlling, rapid diagnosis and detection of Rifampicin (RIF) resistance *M. tuberculosis*, regular monitoring

of drug resistance TB as essential for tuberculosis management and earlier treatment initiation in Ethiopia.

Participation in the Research

You are free to decide if you want to be part of this research or not.

Confidentiality

I will protect information about you taking part in this research to the best of our ability. I will neither use your name in any reports nor discuss your participation with anyone outside the research team.

Payment

No payments will be made for participation.

Leaving the Research

You may end your participation at any time with no negative consequence to you.

Your rights as a Participant

This research has been reviewed and approved by the Addis Ababa University ethical review board and permission from Madda Walabu University Goba Referral Hospital. If you have any questions about how you are being treated by the study or your rights as a participant you may contact through email: tayewakuma21@gmail.com.

Telephone number: 0913922965/0946934471

Thank you for Participation and cooperation!

Information sheet for Adults (≥18 years) (Afaan Oromoo version)

(Waraqaa odeeffannoo hirmaannaa Ga'eessotaa)

Maqaa Dhaabbatichaa: Department of Microbiology. Immunology and Parasitology, College of Health Sciences, Addis Ababa University

Gaggeessaa qorannoo: Waaqumaan Taayyee

Mata duree qorannoo: Prevalence of *Mycobacterium tuberculosis*, its Rifampicin-resistance pattern and associated factors among presumptive Pulmonary and Extra Pulmonary Tuberculosis patients at MWU GRH, Southeast Ethiopia

Seensa

Ani kanan isin gaafadhu qorannoo kaadhimamaa digirii 2ffaa Univeersiitii Finfinnee kanaan taasifamu irratti fedhii keessaniin qooda akka fudhattanii dha. Gareen qorannoo kana gaggeessu kaadhimamaa digirii 2ffaa kanaa fi gorsaa isaa Univeersiitii Finfinneetti barsiisaa Medical Microbiology kan ta'anii dha. Wantin akkan isini hubattan barbaadu kaayyoo fi itti gaafatamumaam qorannnoo kanaa qaama qorannoo kanaa ta'uu yoo barbaaddan dursee kan isinitti himamu ta'uu isaati. Gaaffilee fi wanta isiniif ifa ta'u kan barbaaddan yoo jiraate gaafattanii hubachuu dandeessu.

Kaayyoo qorannichaa

Kaayyoon qorannoo kanaa Baayina dhukkubsattoota 'Tiibii'/daranyoo sombaa, qoricha Rifampicin jedhamuun wal baruu fi wal baruu dhabuu Bacteria *M. tuberculosis* jedhamuu fi sababoota/ka'umsa dhukkubni irraa nama qabuu shakkamtoota dhukkuba 'Tiibii'/daranyoo sombaa irratti qorannoo adeemsisuu dhafi.

Miidhaa Qorannichaa

Dhukkubbiin xiqqaan yeroo dhangala'oo duguggurruu dugdaa keessatti argamuu fi dhangala'oo kanneen biro fudhatan dhagahamuu mala. Sodaanis akkasuma isinitti dhagahamuu mala.

Faayidaa Qorannichaa

Qorannoon kun kallattiidhumaan faayidaa isinii kennuu dhabuu danda'a, garuu bu'aan qorannicha irraa argamuu qorannoo si'ataa dhukkuba sombaa qorichaa wal bare dafanii adda baasuu hojiirra oolchuu fi yaaluun du'a sababa dhukkuba kanaan dhufuu gadi xiqqeessuu ni danda'a**.**

Yoo qaama qorannichaa tahuu yookan dhiisuu barbaadde

Qaama qoranichaa tahuus yookan dhiisuuu ni dandeessa.

Icciitii Eeguu

Ichiitii keessan eeguun dhirqama keennaa. Qaama qorattootatin alatti maqaa keessan barreeffama kamiinuu irratti hin fayyadamnuu.

Kafaltii

kaffaltiin qaama qoratamaaf kennamu kan hin jirre ta'uu isiin beeksifna.

Mirga akka qaama hirmaataa

Qorannoon kun Universiitii Finfinneetti, boordii seera qorannoo ilaaluu irraa ilaalamee, mirkanaayee hospitaala rifaraalii Gobbaa irraa heeyyamameera. yoo gaaffii faayidaa qorannichaafi mirgaa akka qaama hirmaatatti qabaattee gaafatuu dandeessa.

Email: tayewakuma21@gmail.com.

Bilbila: 0913922965/0946934471.

Hirmaannaa fi gargaarsa keessaniif galata argadhaa!

Consent Form for Adult Participant's (≥18 years) (English Version)

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that I would participate in this study to give the specimen required for laboratory analysis and I understand that I have the right to withdraw from the study at any time.

Patient Name _____signature _____Date_____

Investigator name _____signature _____Date_____

Consent Form for Adult Participant's (≥18 years) (Afaan Oromoo Version)

Ani odeeffannoo hirmaannaa qorannoo taasifamu kana dubbise ykn naaf dubbisamee jira. Akkasumas carraan gaaffii gaaffachuu fi gaaffiin gaafadheef deebii quubsaanis naaf kennamee jira. Kanaaf qaama qorannoo kanaa ta'uuf fedhii koon kanan walii gale ta'uu, saamuda qorannoo kanaaf barbaachisu kennuufis walii galuu koo fi qorannoo kana yeroon barbaadettis adda kutuu akkan danda'u hubadheera.

Maqaa dhukkubsataa…….……Mallattoo……Guyyaa……

Gaggeessaa qorannoo……Mallattoo……Guyyaa……

Information sheet for parents/guardians (English version)

Name of Organization:-Department of Microbiology. Immunology and Parasitology, College of Health Sciences, Addis Ababa University

Principal Investigator: Wakuman Taye

Title: Prevalence of Mycobacterium tuberculosis its Rifampicin-resistance pattern and associated factors among presumptive Pulmonary and Extra Pulmonary Tuberculosis patients at MWU GRH, Southeast Ethiopia

Introduction

I would like to conduct a research entitled with the Prevalence of Mycobacterium tuberculosis its Rifampicin-resistance pattern and associated factors among presumptive Pulmonary and Extra Pulmonary Tuberculosis patients at MWU GRH, Southeast Ethiopia. I am requesting you to allow your child to voluntarily participate in this study, and inform you about the purpose, responsibility of investigators or data collectors to keep confidentiality and how I am going to use the data. Please take as much time as you need to read or listen to the information provided here.

Purpose of the study

The purpose of the study is to determine the Rifampicin-resistance pattern of *Mycobacterium tuberculosis* and associated risk factors among presumptive Pulmonary and Extra Pulmonary Tuberculosis patients at MWU GRH, Southeast Ethiopia

Possible Risks (During sample collection)

No risks for your child when providing sputum samples and there may be minimal pain when providing samples other than sputum. Generally, it is very unlikely that participation in this research will expose your child to any physical, social or psychological risks.

Possible Benefits

Participation in this research may not benefit your child directly. But results from this study will be used to inform decisions in implementation and strengthening of programs aimed at controlling, rapid diagnosis and detection of Rifampicin (RIF) resistance *M. tuberculosis*, regular

monitoring of drug resistance TB as essential for tuberculosis management and earlier treatment initiation in Ethiopia.

Participation in the Research

You are free to decide if your child wants to be part of this research or not.

Confidentiality

I will protect information about your child taking part in this research to the best of our ability. I will neither use your child name in any reports nor discuss your child participation with anyone outside the research team.

Payment

No payments will be made for participation.

Leaving the Research

You may end your child participation at any time with no negative consequence to your child.

Your child rights as a Participant

This research has been reviewed and approved by the Addis Ababa University ethical review board and permission from MWU GRH. If you have any questions about how your child are being treated by the study or your child rights as a participant you may contact through:

Email: tayewakuma21@gmail.com.

Telephone number: 0913922965/0946934471

Thank you for Participation and cooperation!

Information sheet for parents/guardians (Afaan Oromoo version)

(Waraqaa odeeffannoo Warra/Guddiftoota daa'immaniif)

Maqaa Dhaabbatichaa:-Department of Microbiology, Immunology and Parasitology, College of Health Sciences, Addis Ababa University

Gaggeessaa qorannoo: Waaqumaan Taayyee

Mata duree: Prevalence of Mycobacterium tuberculosis, its Rifampicin-resistance pattern and associated factors among presumptive Pulmonary and Extra Pulmonary Tuberculosis patients at MWU GRH, Southeast Ethiopia

Seensa

Ani kanan isin gaafadhu qorannoo kaadhimamaa digirii 2ffaa Universiitii Finfinnee kanaan shakkamtoota dhukkuba sombaa irratti taasifamu irratti fedhii keessaniin Mucaan/ Intalli keessan qooda akka fudhattanii dha. Gareen qorannoo kana gaggeessu kaadhimamaa digirii 2ffaa kanaa fi gorsaa isaa Univeersiitii Finfinneetti barsiisaa Medical Microbiology kan ta'anii dha. Gaaffilee fi wanta isiniif ifa ta'u kan barbaaddan yoo jiraate gaafattanii hubachuu dandeessu.

Kaayyoo qorannichaa

Kaayyoon qorannoo kanaa Baayina dhukkubsattoota 'Tiibii'/daranyoo sombaa, qoricha Rifampicin jedhamuun wal baruu fi wal baruu dhabuu Bacteria *M. tuberculosis* jedhamuu fi sababoota/ka'umsa dhukkubni irraa nama qabuu shakkamtoota dhukkuba 'Tiibii'/daranyoo sombaa irratti qorannoo adeemsisuu dhafi.

Miidhaa Qorannichaa

Dhukkubbiin xiqqaan yeroo dhangala'oo duguggurruu dugdaa keessatti argamuu fi dhangala'oo kanneen biro fudhatan Mucaa/Intala keessanitti dhagahamuu mala. Sodaanis akkasuma itti dhagahamuu mala.

Faayidaa Qorannichaa

Qorannoon kun kallattiidhumaan faayidaa Ilma/ Intala keessaniif kennuu dhabuu danda'a, garuu bu'aan qorannicha irraa argamuu qorannoo si'ataa dhukkuba sombaa qorichaa wal bare dafanii adda baasuu hojiirra oolchuu fi yaaluun du'a sababa dhukkuba kanaan dhufuu gadi xiqqeessuu ni danda'a**.**

Yoo qaama qorannichaa tahuu yookan dhiisuu barbaadde

Qaama qoranichaa tahuus yookan dhiisuuu ni danda'ama.

Icciitii Eeguu

Ichiitii Mucaa/Intala keessanii eeguun dhirqama keennaa. Qaama qorattootatin alatti maqaa Mucaa/Intala keessanii barreeffama kamiinuu irratti hin fayyadamnu.

Kafaltii

kaffaltiin qaama qoratamaaf kennamu kan hin jirre ta'uu isiin beeksifna.

Mirga Mucaa/Intala keetii

Qorannoon Kun Universiitii Finfinneetti, boordii seera qorannoo ilaaluu irraa ilaalamee, mirkanaayee hospitaala rifaraalii Gobbaa irraa heeyyamameera. yoo gaaffii faayidaa qorannichaafi mirgaa akka qaama hirmaatatti qabaattee gaafatuu daandeessa.

Email: tayewakuma21@gmail.com.

Bilbila: 0913922965/0946934471

Hirmaannaa fi gargaarsa keessaniif galata argadhaa!

Consent form for parents/guardians (English Version)

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that my child participates in this study provided he/she gives assent to give specimen for laboratory investigation, be a participant in this study and understand that I have the right to withdraw my child from the study at any time .

Participant name _____Signature _____Date_____

Investigator name _____Signature _____Date_____

Consent form for parents/guardians (Afaan Oromoo Version)

(Guca walii galtee Maatii/Guddiftoota Daa'immaniif)

Ani odeeffannoo hirmaannaa qorannoo taasifamu kana dubbise ykn naaf dubbisamee jira. Akkasumas carraan gaaffii gaaffachuu fi gaaffiin gaafadheef deebii quubsaanis naaf kennamee jira. Kanaaf Mucaan/ Intalli koo qaama qorannoo kanaa akka ta'uuf/taatuuf fedhii koon kanan walii gale ta'uu, saamuda qorannoo kanaaf barbaachisus daa'ima koo irraa akka fuudhamus walii galuu koo fi qorannoo kana yeroon barbaadettis adda kutuun akkan danda'u hubadheera.

Maqaa Maatii…….……..Mallattoo……Guyyaa……

Gaggeessaa qorannoo……Mallattoo……Guyyaa……

Assent form for children aged 12-17 years (English version)

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily assent that I would participate in this study provided my parents/guardians give their consent to give specimen for laboratory investigation, be a participant in this study and understand that I have the right to withdraw from the study at any time.

Participant name _____Signature _____Date_____

Investigator name _____Signature _____Date_____

Assent form for children aged 12-17 years (Afaan Oromoo version)

(Guca walii galtee ijoollee umrii waggaa 12 -17 jiraniif)

Ani odeeffannoo hirmaannaa qorannoo taasifamu kana dubbise ykn naaf dubbisamee jira. Akkasumas carraan gaaffii gaaffachuu fi gaaffiin gaafadheef deebii quubsaanis naaf kennamee jira. Kanaaf heyyama maatiin koon qaama qorannoo kanaa akkan ta'u walii galuu koo, saamuda qorannoo kanaaf barbaachisus akka narraa fuudhamus walii galuu koo fi qorannoo kana yeroon barbaadettis adda kutuun akkan danda'u hubadheera.

Maqaa hirmaataa…….……Mallattoo……Guyyaa……

Gaggeessaa qorannoo……Mallattoo……Guyyaa……

Annex III: Questionnaire

Protocol Title: Prevalence of Mycobacterium tuberculosis, its Rifampicin-resistance pattern and associated factors among presumptive Pulmonary and Extra Pulmonary Tuberculosis patients at Madda Walabu University Goba Referral Hospital, Southeast Ethiopia

Principal Investigator: Wakuman Taye

Patient's Card No-_____Date of Interview_____

Introduction

Thanks so much for your permission. I am **Wakuman Taye**, a student pursuing a Master of Medical microbiology Program at Addis Ababa University School of medicine, Addis Ababa, Ethiopia. This interview is being conducted as part of a research on the prevalence of *Mycobacterium tuberculosis*, its Rifampicin-resistance pattern and associated factors among presumptive Pulmonary and Extra Pulmonary tuberculosis patients. I would be very much grateful if you would kindly find some time to answer these questions. Your views, opinions and contributions are very valuable and important and would go a long way to help me to assess the Rifampicin-resistance pattern of *Mycobacterium tuberculosis* and associated factors among presumptive Pulmonary and EPTB patients. This study is strictly for academic purpose and I can assure you of the confidentiality on any information that you would provide.

Thanks for your cooperation.

Please tick [√] where appropriate and give the appropriate response to each item as presented.

Part I	**Study participant address**
	Zone:
	Woreda/Town:
	Kebele:
Part II	**Socio- demographic characteristics of the study participants**
	1. What is your age? (Years) <15 ☐ 15-39 ☐ 40-59 ☐ ≥60 ☐
	2. Sex? Male ☐ Female ☐

	3. Marital Status? Single ☐ Married ☐ Separate ☐ Widowed ☐ Divorced ☐
	4. Educational Level? Illiterate ☐ Elementary ☐ High School ☐ College and above ☐
	5. Religion? Orthodox ☐ Muslim ☐ Protestant ☐ Catholic ☐ Waaqefannaa ☐ Other _____
	6. Residence? Urban ☐ Rural ☐
	7. Family size? <4 ☐ 4 – 7 ☐ >7 ☐ 8. Occupation? Gov. employed ☐ Self-employed ☐ Daily laborer ☐ Student ☐ Merchant ☐ Farmer ☐ House Wife ☐ Other _____
Part III	**Predisposition for Tuberculosis**
	9. Do you know about TB and how it is transmitted from one another? Yes ☐ No ☐
	10. Is there someone who is TB patient in your household? Yes ☐ No ☐ Don't know ☐
	11. Have you ever had a friend, neighbor, or colleague with TB? Yes ☐ No ☐ Don't know ☐
	12. Habit of Drinking Milk: Raw ☐ Boiled ☐ Both ☐ I do not drink ☐
	13. Habit of Drinking Alcohol: Sometimes ☐ Always ☐ No ☐
	14. Habit of Smoking Cigarettes: Sometimes ☐ Always ☐ No ☐
	15. Frequent utilization of congested transportation? Yes ☐ No ☐
	16. Imprisonment history? Yes ☐ No ☐
	17. Do you know someone who is treated for TB more than one times? Yes ☐ No ☐
	18. If yes (question 14) what relationship do you have with that person? Family member ☐ Close friend ☐

	Noncontact person ☐
	19. Do you have contact with MDR-TB Patients? Yes ☐ No ☐
	20. Previous history of TB treatment: Yes ☐ No/New
	21. If yes (question 14), **(Presumptive DRTB):** New ☐ Relapse ☐ Default ☐ Failure ☐ lost to follow up ☐
	22. Do you know your HIV test result? Yes ☐ No ☐ ➢ If yes, what was your HIV Test Result? Positive ☐ Negative ☐ On ART ☐
	23. Site of presumptive TB: Pulmonary ☐ Extra pulmonary ☐
	24. Types of specimens: Respiratory (sputum) ☐ Non respiratory ☐ ➢ If Non respiratory: Pus ☐ Peritoneal ☐ Pleural fluid Lymph node aspirates ☐ Other……

	GeneXpert MTB/RIF test result		
	Detected	Not detected	Invalid/Indeterminate
M.tuberculosis			
Rifampicin –resistance			

Name and Signature of the Data Collector/Interviewer……

Questionnaire Afaan Oromoo Version

Prevalence of *Mycobacterium tuberculosis, its* Rifampicin-resistance pattern and associated factors among presumptive Pulmonary and Extra Pulmonary Tuberculosis patients at MWU GRH, Southeast Ethiopia

Lakk. Addaa dhukkubsataa _____Guyyaa_____

Kutaa I. Iddoo jireenyaa hirmaattota qorannoo

1. Godina_____ 3. Aanaa_____4. Ganda_____Lakk bilb_____

Kutaa II. Ibsituuwwan hawaasummaa hirmaattota qorannoo

1. Ummurii ? (Waggaa) < 15 ☐ 15-39 ☐ 40-39 ☐ >60 ☐

2. Saala? Dhiira ☐ Dhalaa ☐

3. Haala fuudhaa ? Hin fuune/heerumne ☐ Fuudhe/heerumte ☐Adda bahan ☐

Gursumeettii ☐ Hiike/hiikte ☐

4. Sadarkaa barnootaa? Hin baranne☐Sad 1 ffaa☐Sad 2ffaa☐College fi isaa ol☐

5. Amantaa? Orthodox ☐ Musliima☐ Protestaantii☐ Catholic ☐ Other ☐_____

6. Iddoo jireenyaa? Magaala ☐Baadiyyaa☐

6. Heeddumina maatii? <4 ☐ 4 – 7 ☐>7 ☐

7. Hojii? Hojjetaa mootummaa ☐ Hojii dhuunfaa ☐ Hojjetaa guyyaa ☐ Barataa/ttuu ☐

Kutaa II: Ka'umsa sababoota dhukkuba sombaa

8. Waa'ee dhukkuba sombaa fi akkaataa daddarbiinsaa ni beektaa? Eeyyee ☐ Lakki ☐

9. Maatii keessaa dhukkubsataan dhukkuba sombaa ni jiraa? Eeyyee ☐ Lakki ☐ Hin beeku ☐

10. Hiriyyaa, Ollaa ykn hiriyyaa mana barnootaatti dhukkubsataa dhukkuba sombaa ta'e ni jiraa? Eeyyee ☐ Lakki ☐ Hin beeku ☐

11. Aannan ni dhugdaa?: Kan danfe ☐Kan hin danfine☐ Lameenuu ☐ Hin dhugu ☐

12. Dhugaatii alkoolii ni dhugdaa?: Darbee darbee ☐Yeroo hunda ☐ Hin dhugu ☐

13. Tamboo ni aarsitaa?: Darbee darbee ☐ Yeroo hunda ☐ Hin aarsu ☐

14. Nama dhukkuba sombaa si'a tokkoo ol yaalame ni beektaa? Eeyyee ☐ Hin beeku ☐

15. yoo gaaffiin 14ffaan eeyyee ta'e , hariiroon isin gidduu jiru maali? Miseensa maatii ☐

Hiriyyaa dhihoo ☐ Hariiroo dhihoo hin qabnu ☐

16. Dhukkubsataa dhukkuba sombaa qorichaan wal bare waliin haariiroo qabda turee?

Eeyyee ☐ Hin qabu ☐

17. Dhukkuba sombaa yaalamtee beektaa? Eeyyee ☐ lakki/Miti ☐

18. Yoo gaaffii 17ffaan eeyyee ta'ee Relapse ☐ Default ☐ Failure ☐ lost to follow up ☐

19. Qorannoo HIV/AIDS taasistee beektaa? Eeyyee ☐ lakki/Miti ☐

20. Gaaffii 19 ffaan eeyyee yoo ta'e, firiin qorannoo maal ture? Positive ☐ Negative ☐ Yaala

Irraa/on ART ☐ Hin beeku ☐

21. Yeroo hunda geejjiba imaltoonni itti heeddummaatan ni fayyadamtaa? Eeyyee ☐ lakki ☐

22. Seenaa mana sirreessaa qabdaa? Eeyyee ☐ lakki ☐

Maqaa fi mallattoo odeeffannoo funaanaa _____

DECLARATION SHEET

I, the undersigned, Medical Microbiology student declare that this thesis is my original work in partial fulfillment of the requirement for the degree of Master of Medical Microbiology.

Name of principal investigator: _____Signature: _____

Place of submission: Addis Ababa University, College of Health Science, School of Medicine, Department of Microbiology, Immunology and Parasitology

Date of submission: _____

Advisors:

This thesis paper has been submitted for examination with our approval as advisors.

Advisor	**Co-advisor**
Dr. Solomon G/Selassie	Mr. Mesay Mitiku
(MD, MSc, Associate professor)	(MSc, PhD Candidate)
Signature: _____	Signature: _____
Date: _____	Date**:** _____