MEKELLE UNIVERSITY

COLLEGE OF HEALTH SCIENCES,

DIVISION OF BIOMEDICAL SCIENCES,

DEPARTMENT OF MEDICAL MICROBIOLOGY AND IMMUNOLOGY

PREVALENCE AND ASSOCIATED RISK FACTORS OF PLUMONARYTUBERCULOSIS AMONG PRISONERS IN SHIRE, AXUM AND ADWA PRISON CENTERS.

BY:- GIRMASLASIE FISEHA (B.Sc.)

A THESIS SUBMITTED TO DEPARTMENT OF MEDICAL MICROBIOLOGY AND IMMUNOLOGY COLLEGE OF HEALTH SCIENCES, MEKELLE UNIVERSITY IN THE PARTIAL FULFILMENTS FOR REQUIREMENTS FOR THE MASTERS DEGREE OF MEDICAL MICROBIOLOGY

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ADVISOR'S APPROVAL SHEET

This is to certify the thesis entitled the prevalence and associated risk factors of Pulmonary Tuberculosis among prisoners in shire, Axum and Adwa prison centers in Tigray, Ethiopia 2024/2025 study. It is submitted in partial fulfillment of the requirements for the degree of MSc with the specialization of Medical Microbiology department of Medical Microbiology and Immunology has been carried out by Girmaslasie fiseha, ID NO/CHS/MBM/006/13 under our supervision. Therefore, we recommend that the student has fulfilled the requirements and can submit the thesis to the department.

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ABBREVIATIONS AND ACRONYMS

AFB	Acid Fast Bacilli
AIDS	Acquired Immune Deficiency Syndrome
ART	Anti retro viral Therapy
DOT	Directly Observed Treatment
EQA	External quality control
FMOH	Federal Ministry of Health
HIV	Human Immune Deficiency Virus
IGRA	Interferon Gamma Release Assay
IQC	Internal quality control
MDGs	Millennium Development Goals
MDR	Multi Drug Resistant
MTB	Mycobacterium Tuberculosis
QA	Quality assurance
RIF	Rifampicin
RR	Rifampicin Resistant
SOP	Standard Operating Procedure
SPC	Sample Processing Control
SR	Sample Reagent
TB	Tuberculosis
TST	Tuberculin Skin Test
WHO	World Health Organization
XDR	Extensively Drug Resistant

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ABSRACT

Background: Pulmonary Tuberculosis is a contagious airborne disease caused by *Mycobacterium tuberculosis* and remains a significant global public health problem. Prisons, due to overcrowding, poor ventilation, and limited healthcare services, present a high-risk environment for TB transmission and progression. In the Tigray region of Ethiopia, challenges such as inadequate diagnostic capacity and insufficient data hinder effective TB control among incarcerated populations.

Objective: This study was aimed to determine the prevalence of pulmonary tuberculosis and identify its associated risk factors among prisoners in Shire, Axum, and Adwa correctional facilities in Tigray, Ethiopia, over the period from December, 2024 to May, 2025.

Methodology: An institutional-based cross-sectional design was employed to enroll 265 prisoners presenting with TB symptoms. Structured questionnaires were collected socio-demographic and clinical information, including HIV status, history of smoking, and incarceration-related exposures. Sputum samples were collected and analyzed using Ziehl-Neelsen staining and the Gen-expert MTB/RIF assay.

Results: A total of 265 prisoners were enrolled in this study, drawn from a total incarcerated population of 3,180 inmates across the three prison centers in Shire, Axum, and Adwa, located in northern Ethiopia. The study identified 5 bacteriologically confirmed TB cases, yielding a point prevalence of 1.9% (95% CI: 0.6%–4.3%) among the study participants. Occupation prior to imprisonment was significantly related to TB status: prisoners who had been government-employed or self-employed had significantly lower odds of TB infection compared to those who had been daily laborers or unemployed (exact AOR = 0.119, 95% CI: 0.000–0.946, p = 0.0432). Prisoners who were HIV-negative or whose HIV status was unknown had significantly lower odds of TB compared to HIV-positive individuals (exact AOR = 0.033, 95% CI: 0.001–0.722, p = 0.0281).

Conclusions and Recommendations: The prevalence of PTB was high in these prison centers. There should be an intensified implementation of TB screening among high-risk inmates, particularly those with a history of unemployment, HIV infection, smoking, or TB symptoms.

***Keywords**: Tuberculosis, pulmonary tuberculosis, prevalence, risk factors, prison population, Gen-expert MTB/RIF, rifampicin resistance, Tigray, Ethiopia.*

1. INTRODUCTION

1.1 Background

Pulmonary Tuberculosis is a contagious disease caused by *Mycobacterium tuberculosis*, primarily transmitted through airborne particles and typically affecting the pulmonary system [1]. Although it is preventable and treatable, TB continues to be one of the leading causes of morbidity and mortality worldwide. Its burden remains particularly high in regions with limited healthcare infrastructure, yet it poses a threat across all geographic boundaries and population groups, making it a persistent global health concern [2].PTB remains a major global health concern, ranking among the top ten causes of death worldwide and standing as the leading cause of mortality from a single infectious pathogen. Alarmingly, Pulmonary Tuberculosis claims the lives of nearly three individuals every minute [3]. In 2019 alone, an estimated 10 million people contracted the disease, resulting in approximately 1.2 million deaths. Despite intensified global efforts, the decline in TB incidence and mortality rates has been modest, with annual reductions of only about 2% and 3%, respectively falling short of global elimination targets [4].

Transmission of Pulmonary Tuberculosis occurs primarily through inhalation of airborne droplets expelled by individuals with active pulmonary Tuberculosis. Everyday actions such as coughing, sneezing, talking, or even singing can release thousands of fine aerosol particles, typically ranging from 0.5 to 5 micrometers in diameter. These particles can remain suspended in the air and be inhaled by others, making Tuberculosis highly contagious in poorly ventilated or crowded environments. Notably, the infectious dose of Pulmonary Tuberculosis is remarkably low; in some cases, inhaling fewer than 10 bacilli may be sufficient to establish infection [5]. Certain populations such as migrants, people who inject drugs, individuals with low socioeconomic status, and especially incarcerated persons face a disproportionately high burden of Pulmonary Tuberculosis. Prisons create an ideal environment for Tuberculosis transmission due to factors like overcrowding, poor ventilation, delayed case detection, and inadequate medical infrastructure. These conditions amplify Tuberculosis risk in correctional settings worldwide, regardless of a country's economic status [6].

Substandard healthcare infrastructure, inadequate nutrition, and overcrowded living arrangements significantly contribute to the spread of tuberculosis. Correctional facilities are high-risk settings where these conditions often intersect, thereby facilitating Pulmonary Tuberculosis transmission. Factors such as insufficient air circulation, cramped quarters, limited food quality, and restricted access to timely medical care further heighten the risk. Men aged 15 to 45 years are especially vulnerable, accounting for a substantial share of TB cases in prison populations [7].

Studies consistently indicated that Pulmonary Tuberculosis prevalence is significantly higher among incarcerated populations compared to the general public. However, prison health systems are frequently overlooked in public health policies and are often excluded from national disease surveillance systems [8]. These settings commonly suffer from inadequate resources to manage Tuberculosis effectively, making them reservoirs for persistent transmission both within correctional facilities and in surrounding communities. The situation is further complicated by the emergence of drug-resistant Tuberculosis strains, limited healthcare infrastructure, ineffective disease control strategies, and a high prevalence of HIV co-infection.

According to the World Health Organization (WHO), TB incidence in prisons can be up to 100 times greater than in the general population [9]. Alarmingly, it is estimated that around 8.5% of community Tuberculosis cases are attributable to exposure originating in correctional settings. It is estimated that roughly 8.5% of Tuberculosis cases in the broader community are linked to transmission originating from prison environments [10].

Globally, millions of Pulmonary Tuberculosis cases go undiagnosed each year, with nearly three million individuals missed by existing healthcare systems [9]. This diagnostic gap is particularly evident within prison populations, especially in sub-Saharan Africa [8]. The lack of comprehensive screening programs, limited surveillance, and under-resourced diagnostic facilities significantly impair Tuberculosis detection among incarcerated individuals [11].

The impact of pulmonary tuberculosis within prison systems extends far beyond prison walls, posing significant public health risks to surrounding communities. When TB control measures in correctional facilities are insufficient, they contribute to increased transmission in the general population, including the emergence and spread of drug-resistant Pulmonary Tuberculosis strains. This makes addressing Pulmonary Tuberculosis in prisons a public health imperative [12].

Despite growing global attention to Pulmonary Tuberculosis, significant knowledge gaps persist regarding its prevalence and transmission dynamics within African prisons. Research indicates that the Tuberculosis rate among incarcerated individuals in Africa can be 10 to 35 times higher than in the general population. In many low- and middle-income countries (LMICs) burdened by Pulmonary Tuberculosis, prison systems often lack comprehensive control programs [9].

Ethiopia, classified among high Pulmonary Tuberculosis burden countries, reported an estimated Tuberculosis incidence of 192 cases per 100,000 population in recent years. The nation has six federal and over 120 regional prison and detention facilities, many of which suffer from overcrowding and poor ventilation conditions that facilitate Tuberculosis transmission. Moreover, prison healthcare infrastructure remains underfunded and inadequately staffed, with limited access to diagnostic services. While some initiatives have been launched to enhance prison health systems, their reach and effectiveness remain restricted [11].

Poor adherence to Tuberculosis treatment among incarcerated individuals significantly elevates the risk of developing drug-resistant strains, including multidrug-resistant TB (MDR-TB), which can easily spread within correctional facilities. The presence of HIV co-infection further complicates the situation, as individuals living with both HIV and TB are at a heightened risk of progressing to MDR-TB [13]. The absence of effective infection prevention and control measures, coupled with limited access to reliable diagnostic tools and appropriate treatment services, continues to be a major challenge in managing TB within prison systems, particularly in resource-limited settings [11].

Sputum smear microscopy remains one of the most commonly used methods for diagnosing *Mycobacterium tuberculosis*, particularly in resource-limited settings due to its affordability, simplicity, and rapid results. This technique is especially effective in identifying patients with a high bacterial load. The process involves smearing a sample of purulent sputum onto a clean glass slide, heat-fixing it, and then staining it using the Ziehl-Neelsen technique. The prepared slides are subsequently examined under a light microscope to detect acid-fast bacilli (AFB) [9]. In regions such as Tigray, northern Ethiopia, where Tuberculosis continues to pose a significant public health threat, smear microscopy remains a cornerstone of diagnostic efforts despite its limitations in sensitivity, especially among individuals with low bacterial loads or HIV co-infection [14].

The Gene X pert MTB/RIF assay is a widely adopted molecular diagnostic tool for tuberculosis offering rapid and accurate detection of *Mycobacterium tuberculosis* and rifampicin resistance. This fully automated, cartridge-based nucleic acid amplification test analyzes sputum samples and delivers results in approximately two hours. Each cartridge is preloaded with reagents necessary for sample preparation, DNA extraction, amplification, and detection of mutations in the rpo B gene, which is associated with rifampicin resistance. A major advantage of the Gene X pert system is its ease of use, requiring minimal technical expertise and manual handling. The assay has shown excellent sensitivity and specificity, making it a critical component in Tuberculosis diagnosis, particularly in high-burden and resource-limited settings [15].

1.2. Statement of the Problem

Pulmonary Tuberculosis remains a major global public health challenge, especially in environments with limited healthcare infrastructure, such as correctional facilities. Incarcerated individuals are among the most vulnerable populations, facing a significantly increased risk of acquiring Tuberculosis, including its drug-resistant forms, particularly in resource-limited regions like sub-Saharan Africa. Tuberculosis continues to be one of the leading causes of morbidity and mortality among prison populations [8]. According to the World Health Organization (WHO), Tuberculosis is responsible for over a million deaths annually worldwide, despite sustained global efforts and interventions to control its spread. The disease continues to disproportionately affect marginalized groups and remains a persistent burden across all age groups [9].

The burden of Tuberculosis in prison settings is intensified by a combination of environmental and behavioral risk factors. Conditions such as overcrowding, insufficient ventilation, and inadequate nutrition—commonly observed in correctional facilities, create an ideal environment for TB transmission. In addition, individual behaviors including tobacco use, alcohol consumption, khat chewing, and exposure to indoor air pollution significantly heighten vulnerability to infection [16]. People living with chronic illnesses such as HIV/AIDS, diabetes, and cancer—especially those undergoing immunosuppressive treatments, face an even greater risk of developing active TB. Moreover, individuals in close and prolonged contact with TB patients, such as healthcare workers and cellmates, are at increased risk of contracting the disease

Tuberculosis is one of the most pressing public health concerns worldwide. According to the World Health Organization an estimated 10.6 million people developed TB in 2022, leading to approximately 1.3 million deaths among HIV-negative individuals and an additional 167,000 deaths among those living with HIV. The disease continues to disproportionately affect low- and middle-income countries, with 30 high TB burden countries accounting for nearly 87% of global Tuberculosis cases. Drug-resistant Tuberculosis also remains a critical challenge, with about 410,000 cases of multidrug- or rifampicin-resistant Tuberculosis reported globally [9].

In the African region, the Pulmonary Tuberculosis burden is particularly high, with an estimated incidence rate of 237 cases per 100,000 population in 2022. The continent faces compounded challenges such as high rates of TB/HIV co-infection, inadequate diagnostic infrastructure, and under-resourced healthcare systems. Countries in southern and eastern Africa including South Africa, Lesotho, and Mozambique record some of the highest Tuberculosis incidence rates globally. Delays in diagnosis and treatment, coupled with the emergence of drug-resistant Tuberculosis strains, continue to hinder Tuberculosis control efforts in the region [9].

Ethiopia is recognized as one of the 30 countries with the highest Tuberculosis burden globally. The estimated Tuberculosis incidence in Ethiopia was 132 per 100,000 population in 2022, with roughly 143,000 new cases reported annually. It remains a leading cause of death among people living with HIV in the country. Although the national Tuberculosis control program has made significant progress over the past two decades, challenges persist, including low case detection rates, limited access to quality laboratory services, and the rising burden of multidrug-resistant Tuberculosis estimated to affect 2.7% of new cases and 14% of previously treated patients [34].

In the Tigray region of northern Ethiopia, the Tuberculosis situation has been further complicated by the ongoing conflict since 2020. Prior to the conflict, its incidence in Tigray ranged between 140 and 170 cases per 100,000 population, slightly higher than the national average. However, widespread destruction of health infrastructure, mass displacement, and interruptions in health service delivery have significantly disrupted Tuberculosis prevention, diagnosis, and treatment services. As a result, the actual burden of Tuberculosis in the region is likely underestimated, and the risk of increased transmission and poor treatment outcomes is substantial particularly among vulnerable groups such as prisoners, internally displaced persons, and malnourished populations [9].

1.3. Significance of the Study

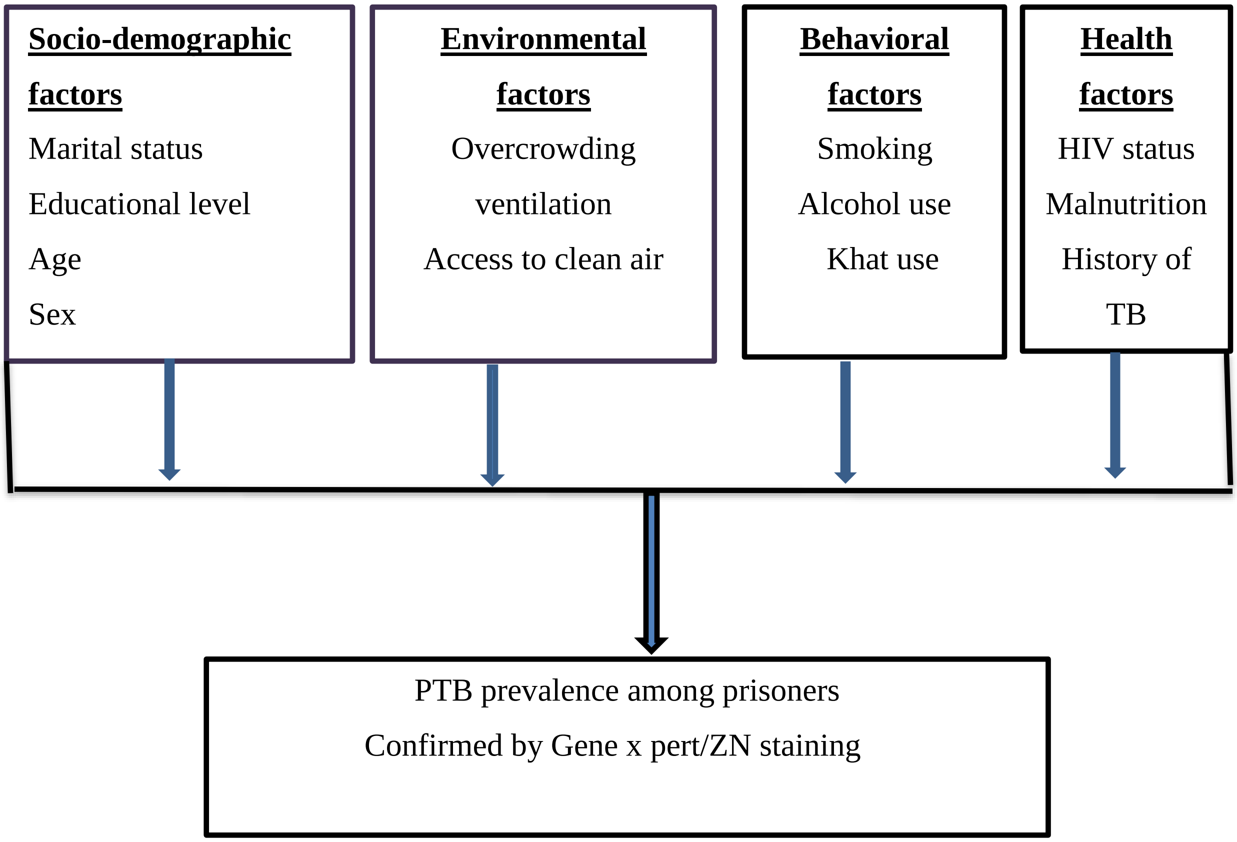
Pulmonary Tuberculosis continues to be a significant global health concern, especially in confined environment like prisons, where the conditions often facilitate the rapid spread and poor management of the disease. Over-crowding, poor ventilation, insufficient nutrition, and limited access health care services create an ideal environment for TB transmission and progression. These factors contribute to TB rates in prisons being many times higher than in the general population, with consequences that extend far beyond prison walls.

The control of Pulmonary Tuberculosis within correctional facilities is not only a matter of intimate health but also a critical aspect of public health. Since prisons are not isolated systems due to the constant movement of intimates, staff, and visitors any infectious disease that is poorly managed inside these institutions can easily spread to the surrounding community. As such, improving TB prevention, detection and strengthens national disease control programs.

This study was able to assess the prevalence of Pulmonary Tuberculosis, identify associated risk factors, and evaluate the antibiotic resistance patterns of Pulmonary Tuberculosis among prisoners in Shire, Axum and Adwa prison centers in Tigray region of Ethiopia. The information generated from this research is vital for multiple stake holders, including government health planners, correctional facility administrators, public health officials, and health care provider.it will support evidence -based decision-making, the development of targeted intervention programs, and the allocation of resource s to the most affected areas.

This research endeavors to generate contemporary, context-specific evidence on the determinants of Pulmonary Tuberculosis transmission within prison environments. The outcomes are intended to support policymakers and public health authorities in formulating evidence-based, targeted interventions that respond to the distinct health risks faced by incarcerated individuals, thereby enhancing national Tuberculosis control strategies and curbing transmission within and beyond correctional institutions (World Health Organization (WHO) [17].

1.4 Conceptual frame work



2. LITERATURE REVIEW

2.1. Overview of Pulmonary Tuberculosis

Pulmonary Tuberculosis continues to pose a major public health burden, particularly in low- and middle-income countries. According to the World Health Organization (WHO), an estimated one-quarter of the global population is latently infected with pulmonary tuberculosis, and approximately 5% to 10% of these individuals are at risk of progressing to active disease during their lifetime. In 2022 alone, there were an estimated 10.6 million new PTB cases and 1.3 million deaths globally, including over 167,000 deaths among people living with HIV [9].

Although Pulmonary Tuberculosis affects populations worldwide, the highest burden is concentrated in low- and middle-income countries, particularly in regions such as Sub-Saharan Africa and Southeast Asia. Over the past decade, notable progress has been achieved in TB control efforts, with many of the targets outlined under the Millennium Development Goals (MDGs) successfully met. However, despite these advancements, TB remains a leading cause of morbidity and mortality in high-burden regions, necessitating sustained and targeted public health interventions [9].

2.2. Epidemiology of PTB in Prisons

Globally, individuals in prison settings bear a disproportionately high burden of tuberculosis Pulmonary Tuberculosis compared to the general population. Contributing factors include overcrowded living conditions, inadequate access to healthcare services, poor ventilation, and a high prevalence of HIV. Research indicates that Pulmonary Tuberculosis incidence in correctional facilities can be up to 83 times higher than in the surrounding communities, highlighting the urgent need for targeted interventions in these high-risk environments [9].

Globally, an estimated 8 to 10 million people are incarcerated in correctional facilities at any given time, many of which present conditions that facilitate the transmission of tuberculosis (TB), such as overcrowding, poor ventilation, and limited healthcare access. In Ethiopia—a country classified among the high TB burden nations TB in prison settings remains a pressing public health concern. Evidence from a systematic review reveals that the prevalence of TB among incarcerated individuals ranges between 349 and 1,913 cases per 100,000 population, significantly surpassing the rates observed in the general community [20]. Similarly, research has identified elevated rates of tuberculosis among incarcerated individuals in the Tigray region of Ethiopia. The region has experienced significant disruptions in health service delivery due to recent armed conflict and mass displacement, which have further exacerbated TB vulnerability among prison populations [21].

Multi-drug-resistant tuberculosis (MDR-TB) poses a growing threat in correctional facilities, largely driven by incomplete treatment regimens, poor adherence, and inadequate follow-up systems. Global evidence from countries such as Russia and Pakistan has revealed disproportionately high levels of drug resistance among incarcerated populations [22] Although Ethiopia lacks extensive national surveillance data on MDR-TB within prisons, the limited available research suggests that the burden of drug resistance in these settings may surpass the national average. In the Tigray region specifically, constrained diagnostic capacity hampers the early identification of resistant TB strains, thereby heightening the risk of ongoing transmission within overcrowded prison environments [23].

Studies across Africa have consistently shown that the prevalence of PTB in prison populations is significantly higher than in the general public. A review conducted in sub-Saharan Africa ranges between 500 and 5,000 cases per 100,000 inmates, far exceeding community rates [11]. In South Africa, a prevalence of 3,300 per 100,000 in a single correctional facility, emphasizing the need for widespread screening and rapid diagnostic interventions in these high-risk settings [8].

Ethiopia, recognized by the World Health Organization as one of the high TB burden countries, reports alarmingly high TB rates in its prison systems. A study, the PTB prevalence in southern Ethiopian prisons was estimated at 1,611 per 100,000, significantly exceeding the national average found PTB rates of 1,600 to 1,800 per 100,000 in Sidama Zone prisons, mainly due to gaps in early screening and diagnosis [27, 28].

In eastern Ethiopia, PTB prevalence of 1,500 per 100,000, with key associated factors such as HIV co-infection, poor nutrition, and close proximity to active TB cases. These findings highlight the importance of enhancing TB control programs in prisons, including regular screening, timely diagnosis, and continuous treatment support [29].

The Tigray region in northern Ethiopia has faced growing challenges in TB prevention among prisoners, especially due to the recent armed conflict. Before the war began in 2020, reported a PTB prevalence of 1,724 per 100,000 in regional prisons, particularly in Shire and Axum [30].

Another study identified significant barriers to TB control in Tigray's prisons, including poor screening coverage, limited diagnostic resources, and malnutrition. Since the start of the conflict, these problems have worsened due to damage to healthcare infrastructure. According to a 2022 report by OCHA, TB detection and treatment services were critically disrupted in prisons located in Shire, Axum, and Adwa, raising serious concerns about undiagnosed TB cases and the potential for widespread transmission within those facilities [31, 32].

2.3. Clinical Features and Diagnosis of TB

Tuberculosis (TB) commonly manifests with persistent cough, night sweats, chest pain, fever, and unintended weight loss. In correctional facilities, the diagnosis of TB is frequently delayed due to limited diagnostic capacity and inadequate healthcare infrastructure [9]. The primary diagnostic tools used include Ziehl–Neelsen (ZN) smear microscopy and the Gen-expert MTB/RIF assay. While ZN microscopy is widely available and inexpensive, it has limited sensitivity, particularly in HIV co-infected individuals.

The Gen-expert MTB/RIF assay, endorsed by the World Health Organization (WHO) for its ability to rapidly detect Mycobacterium tuberculosis and rifampicin resistance, offers improved accuracy and speed. However, its implementation in Ethiopian prisons remains limited, primarily due to high operational costs, restricted access, and logistical challenges [24].

2.4. PTB Transmission and Pathogenesis

Tuberculosis (TB) is predominantly transmitted via inhalation of aerosol droplets expelled by individuals with active pulmonary TB during coughing, sneezing, or even speaking [9]. These droplets, which contain *Mycobacterium tuberculosis*, can remain suspended in the air for prolonged periods, especially in confined, poorly ventilated environments. Once inhaled, the bacilli travel to the alveolar spaces of the lungs, where they are engulfed by macrophages. The host's immune response then determines the course of infection: it may eliminate the pathogen, establish latent TB infection, or permit progression to active disease. Reactivation of latent TB is particularly common in individuals with weakened immune systems, such as those with HIV, malnutrition, or other immunosuppression conditions [25].

2.5. Multidrug-Resistant Tuberculosis (MDR-TB) in Prison Settings

Multidrug-resistant tuberculosis (MDR-TB) defined as resistance to at least isoniazid and rifampicin, the two key first-line anti-TB drugs has emerged as a critical challenge within prison environments worldwide. These settings often foster conditions that promote both the transmission and evolution of drug-resistant TB, including overcrowding, delayed diagnosis, insufficient treatment monitoring, and restricted access to second-line medications. As a result, correctional facilities can act as hotspots for the propagation of MDR-TB, particularly in low-resource settings where health systems are weak.

In many African nations, prisons are densely populated and under-resourced, creating ideal conditions for the emergence and spread of MDR-TB [8]. TB incidence in South African prisons is up to 30 times higher than in the general population, with a significant proportion of cases demonstrating drug resistance. in sub-Saharan African prison systems, up to 20% of TB cases were resistant to at least one first-line drug, largely due to delayed diagnosis and lack of infection control [11].

In Zambia, a study revealed that nearly 10% of TB cases in Lusaka Central Prison involved MDR-TB, indicating serious gaps in case management [33]. In Nigeria,) documented treatment interruptions and poor adherence as major contributors to the growing challenge of drug-resistant TB in correctional settings. These findings reflect systemic issues across African prisons that hinder effective TB treatment and control [34].

Ethiopia is among the 30 countries identified by the WHO as bearing a high burden of MDR-TB. Prisons in Ethiopia are particularly vulnerable due to weak surveillance systems, diagnostic delays, and poor treatment infrastructure. A national drug resistance survey conducted by the Ethiopian Public Health Institute showed elevated MDR-TB prevalence among individuals with previous TB treatment history a group highly represented among prisoners [35]

A study shows that that 11.8% of TB cases in southern Ethiopian prisons were drug-resistant. Contributing factors included limited access to Gene X pert machines, inadequate laboratory capacity, and poor continuity of care. Furthermore, frequent prison transfers, medication stock-outs, and weak coordination between prison and public health services exacerbate the risk of treatment failure and resistance development [28].

MDR-TB was more likely among individuals with a history of interrupted treatment, a pattern common in correctional environments. A report by OCHA (2022) emphasized that prisons in Shire, Axum, and Adwa faced serious shortages of essential diagnostic tools, such as Gene X pert machines, as well as second-line drugs. These gaps, combined with overcrowded prison conditions and malnutrition, contribute to elevated risks of TB transmission and drug resistance [30, 32].

Although efforts are underway to restore TB services, the ongoing instability, resource limitations, and infrastructure damage continue to impede progress. The Tigray Health Bureau, supported by various humanitarian agencies, has made efforts to resume DOTS and expand TB screening, but coverage remains limited and inconsistent.

2.6. Risk Factors Associated with Tuberculosis in Prison Settings

The disproportionate burden of pulmonary tuberculosis in prison environments is the result of a complex interplay of environmental, biological, behavioral, and systemic factors that promote both TB transmission and disease progression. Compared to the general population, individuals in correctional facilities are more vulnerable due to conditions such as severe overcrowding, inadequate ventilation, restricted healthcare services, and a high prevalence of underlying comorbidities, including HIV/AIDS and malnutrition [8, 11].

A primary contributing factor is overcrowding, which forces inmates into prolonged close contact and increases the likelihood of inhaling infectious droplets from those with active TB. Many prisons, particularly in low- and middle-income countries, operate far beyond their intended capacity, creating optimal conditions for TB outbreaks Poor ventilation compounds this problem by allowing infectious particles to persist in the air within confined cells that are often locked for long durations [33].

HIV co-infection is another key driver of TB in prisons. Incarcerated individuals in some African countries, including Ethiopia, have higher HIV prevalence rates than the general population. This compromises immune function and accelerates the progression from latent to active TB.). Identified HIV co-infection as a strong determinant of active TB cases among inmates in the Sidama Zone [28].

Nutritional deficiency, especially under nutrition, is also common in prison populations. Limited food quality and quantity weaken immune defenses, increasing vulnerability to TB. Research from Ethiopian and Nigerian prison studies demonstrates a consistent link between low body mass index (BMI) and higher TB incidence [34].

Substance use, such as frequent alcohol consumption and khat chewing, further compromises immune responses and contributes to risky behaviors that elevate TB exposure .Compounding these risks is the lack of awareness about TB symptoms, transmission, and prevention, which delays health-seeking behaviors and perpetuates ongoing transmission in confined settings [27].

In addition, a previous history of pulmonary tuberculosis infection and close contact with active TB patients increase susceptibility among inmates. Shared spaces, bedding, and sanitation facilities make re-exposure and reinfection common in overcrowded institutions. Many prisoners originate from marginalized communities already at higher TB risk, adding to their vulnerability [30].

2.7. PTB Treatment and Control

Pulmonary Tuberculosis treatment in Ethiopia adheres to the World Health Organization's Directly Observed Treatment, Short-course (DOTS) strategy, which involves a six-month regimen of first-line anti-TB medications. While this approach has demonstrated high efficacy in the general population, treatment adherence among incarcerated individuals remains a major challenge. Factors such as intra-prison transfers, social stigma, and insufficient supervision often compromise the continuity of care. Effective TB control within prison settings requires comprehensive preventive strategies, including early case detection, improved ventilation, reduced overcrowding, and the consistent use of personal protective equipment (PPE) to mitigate transmission risks Federal Ministry of Health (FMoH) [26].

3. OBJECTIVES OF THE STUDY

3.1. General Objective

- The general objective of the study was to determine the prevalence and associated risk factors of *Mycobacterium tuberculosis* among prisoners found in Shire, Axum and Adwa, Tigray Ethiopia from October, 2024 to May, 2025 G.C.

3.2. Specific Objective

- To determine the prevalence of MTB by Zeihel - Nielseen microscopy and Gene -x pert for TB for prisoners.
- To identify possible risk factors for tuberculosis in prisoners found in Shire, Axum and Adwa.
- To determine first line drug (Rifampicin) susceptibility patterns of isolated *M. tuberculosis* using Gene-X pert.

4. MATERIALS AND METHODS

4.1. Study Area

This study was conducted in three adjacent towns of the Tigray Region in northern Ethiopia, Shire, Axum, and Adwa, located approximately 996 to 1,061 kilometers from the capital, Addis Ababa. These towns, situated in the Northwestern and Central Zones, they were selected based on the presence of established prison facilities and their strategic importance in the region's healthcare delivery, especially in tuberculosis (TB) control [36]. Geographically, these towns lie between 14°6′N to 14°10′N latitude and 38°43′E to 38°54′E longitude, with elevations ranging from 1,907 to 2,131 meters above sea level. According to the Central Statistical Agency (CSA), the estimated populations were approximately 95,491 in Shire, 94,515 in Axum, and 85,644 in Adwa, combining for a total of over 275,000 residents. There are 8 prison centers in Tigray region; Adwa 1000, Axum 980, Shire 1200, and total 5354 prisoners [Unpublished data from Tigray prison centers commission]. The region was selected due to its accessibility, concentration of incarcerated individuals, and its relevance for monitoring communicable diseases like TB in confined populations [37].

4.2. Study Design ad Period

An institution-based cross-sectional study was conducted to determine the prevalence of PTB and identify associated risk factors among incarcerated individuals. The study took place over an eight-month period, from December, 2024 to May, 2025, within prison facilities located in the towns of Shire, Axum, and Adwa in the Tigray Region of northern Ethiopia. These correctional institutions were purposively selected based on their large inmate populations and the critical importance of addressing TB control challenges in the region, particularly in the context of healthcare system disruptions following the recent conflict [26, 32]. The selection also aligned with regional health priorities and surveillance data highlighting a disproportionate TB burden in prison settings [36].

The study population comprised all incarcerated individuals residing in the selected prison centers Shire, Axum, and Adwa during the study period. Eligibility criteria included prisoners who had been present in the facilities and who exhibited a cough lasting for at least two weeks prior to data collection.

Individuals who were critically ill, mentally unstable, or unable to provide informed consent were excluded from the study to ensure both ethical compliance and data quality. A conventional sampling technique was employed to capture a representative sample of inmates from each correctional facility. To ensure proportional representation, the total sample size was distributed among the three prison centers based on the respective inmate population size at each site. This proportional allocation helped maintain balance in sampling and enhanced the generalization of the findings within the prison settings. Systematic or simple random sampling techniques may further be applied within each facility based on operational feasibility and logistical constraints [38].

A conventional sampling technique was employed to ensure representation from each prison center, with proportional allocation based on the inmate population size. This design was selected because cross-sectional studies are effective in estimating disease prevalence and identifying associated risk factors within a defined population at a specific point in time. They are particularly suitable for resource-limited settings such as prisons, where logistical constraints and health system challenges often limit longitudinal follow-up [39].

4.3. Study Populations

4.3.1 Source Population

The source populations were all incarcerated individuals residing in the correctional facilities of Shire, Axum, and Adwa at the time of the study.

4.3.2 Study Population

The actual study population was include all inmates exhibited a cough that has persisted two weeks and above during the data collection period.

4.4 Inclusion and Exclusion Criteria

4.4.1 Inclusion Criteria

Prisoners who had cough two weeks and above and those who had consent were included in the study.

4.4.2 Exclusion Criteria

Individuals those do not have a sputum sample or decline to participate through informed consent procedures were excluded from the study.

4.5. Sample size determination

To estimate the required sample size for this cross-sectional study, the single population proportion formula was utilized. This method is appropriate for determining sample sizes in prevalence studies, particularly when estimating the proportion of a health condition within a defined population. The calculation was based on a previously reported prevalence of pulmonary tuberculosis (TB) of 19.4% among prisoners in East Gojjam, Northwest Ethiopia [40]. This prevalence value was selected due to its relevance in a similar prison setting within the country, enhancing the validity of the estimate for the current study context.

Confidence level (Z) = 95% → Z-value = 1.96, Estimated prevalence (p) = 0.194, Margin of error (d) 5% or 0.05. The formula used

$$n=\frac{(Z\alpha/2)2P(1-P)}{D2}$$

$$N = \frac{(1.96)^2\, 0.194(1-0.194)}{(0.05)^2}$$

n=241

To account for non-responses, an additional 10% was added, adjusting the final sample size to:

n [final]= 241 + (241 × 0.10) = 265

The total sample size of 265 prisoners were then distributed proportionally across the three prison centers (Shire, Axum, and Adwa) based on each facility's inmate population, ensuring representative sampling from all locations.

Prison Population

Shire= 1,200, Adwa= 1,000 and Axum: =980

Total (N) population = 1,200 + 1,000 + 980 = 3,180 inmates

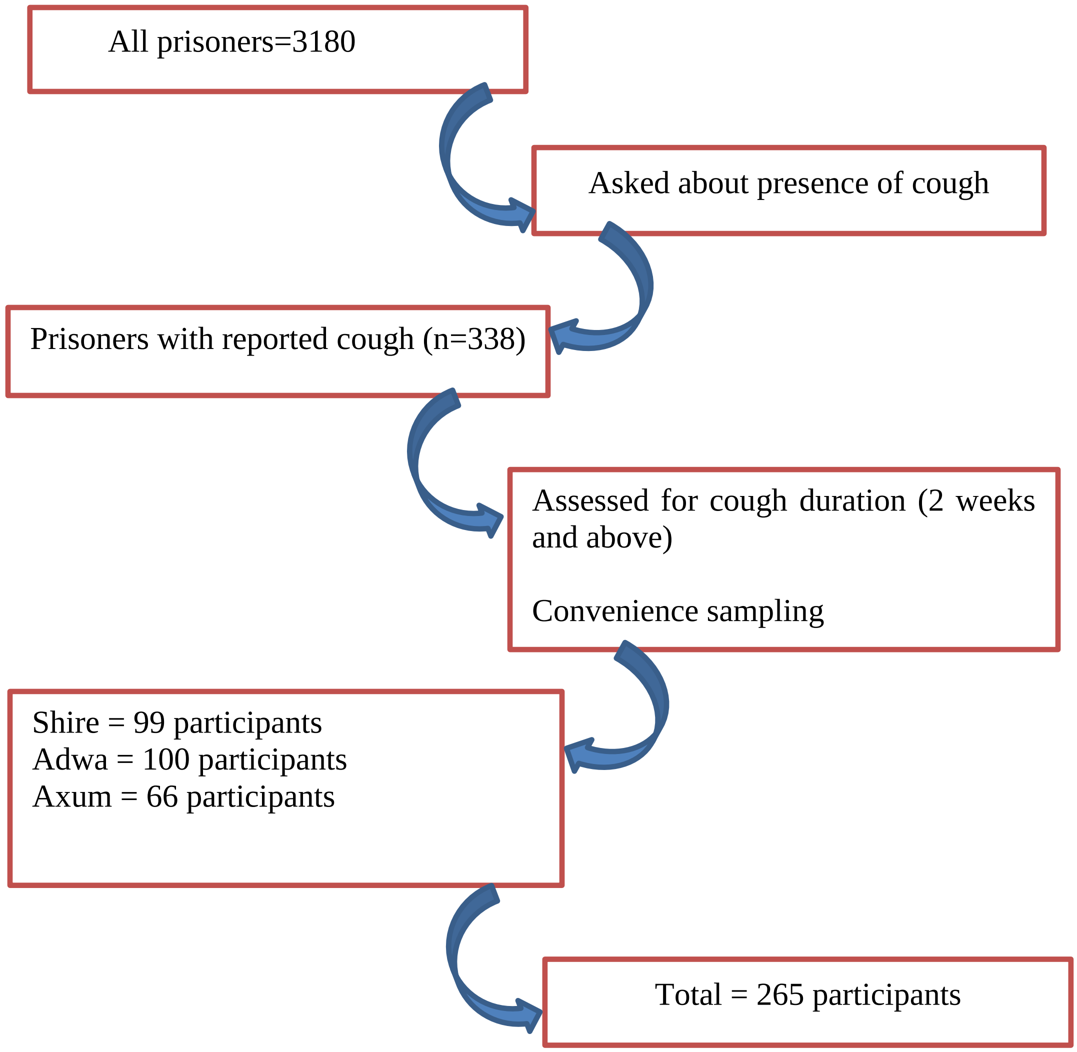
Final sample size (n) = 265

4.6. Sampling technique

The sampling process began with an initial screening of all inmates within the three selected prison centers Shire, Axum, and Adwa to identify individuals reporting symptoms of cough. Those who reported any form of cough were then undergo a follow-up interview to assess the duration of the symptom. Only inmates who reported a persistent cough two weeks and above were considered eligible for participation in the study, as this meets the inclusion criterion for presumptive tuberculosis cases.

Then we have done screening, a convenience sampling technique was employed. Eligible participants were recruited consecutively until the required sample size of 265 was obtained. This approach was considered appropriate due to the logistical and operational constraints within prison environments, where random sampling is often impractical.

4.7. Sampling and screening procedure



4.8. Study Variables

4.8.1. Dependent Variable

The primary outcome variable for this study was the prevalence of *Mycobacterium tuberculosis* among the incarcerated population.

4.8.2 Independent Variables

The explanatory (independent) variables assessed were included:

Demographic characteristics: age, sex, marital status, and educational background.

Clinical and behavioral risk factors: duration of cough, previous anti-TB treatment, HIV status, presence of diabetes mellitus and cigarette smoking,

Environmental and prison-related factors: place of residence before incarceration, occupation prior to imprisonment, number of inmates per cell, and duration of stay in the prison system.

4.9. Data Management and Statistical Analysis

The Collected data were entered into Microsoft Excel for cleaning and then imported into SPSS version 27 for statistical analysis. Descriptive statistics was summarizing the data, while logistic regression was applied to examine the associations between independent variables and TB prevalence. Variables with a p-value <0.05 were considered statistically significant, indicating strong evidence of an association between the predictor and outcome variable.

4.10. Data and Specimen Collection

4.10.1. Collection of Socio-Demographic and Clinical Risk Factors

Data on socio-demographic and clinical risk factors were collected through a structured and pre-tested questionnaire administered by trained data collectors. The questionnaire gathered information on age, sex, marital status, and educational level, history of smoking, HIV status, and previous TB treatment. This facilitated the assessment of individual-level factors potentially associated with tuberculosis (TB) infection [9, 26].

4.10.2 Sputum Sample Collection

Presumptive TB cases defined as inmates reporting a persistent cough lasting more than two weeks were asked to provide sputum samples. Two sputum specimens were collected from each participant: spot - spot samples collection. Sample collection was performed following national TB program guidelines, ensuring proper instruction on how to produce quality sputum.

4.11. Laboratory Procedures

Collected sputum samples were transported to the nearest TB diagnostic laboratories under appropriate bio-safety conditions. Laboratory analysis included:

Ziehl–Neelsen (Z-N) Staining for Microscopy

To detect acid-fast bacilli (AFB), direct smears were prepared from sputum samples on clean microscope slides and subsequently heat-fixed. The smears were then stained using carbol fuchsin as the primary dye. Following this, acid-alcohol was applied as a decolorizing agent, and the smears were counterstained with methylene blue, in strict adherence to the standard operating procedures (SOPs) of the host laboratory. The stained slides were examined under a light microscope, where acid-fast bacilli appeared.

Gene-X pert MTB/RIF Assay

For the molecular detection of Mycobacterium tuberculosis (MTB) and assessment of rifampicin resistance, sputum specimens were processed following the standard operating procedures recommended by the Gen X pert MTB/RIF assay protocol. Initially, each sputum sample was mixed with the sample reagent containing sodium hydroxide (NaOH) and isopropanol in a 2:1 ratio. This reagent serves both as a mucolytic and decontaminating agent. The mixture was vigorously shaken and then incubated at room temperature for 15 minutes to ensure adequate liquefaction and inactivation of potential pathogens. Following incubation, the treated sample was transferred into a Gene X pert cartridge, which was subsequently loaded into the Gene X pert automated system. The device performed real-time polymerase chain reaction (PCR) to simultaneously detect the presence of M. tuberculosis complex DNA and mutations in the rpoB gene, which are indicative of rifampicin resistance.

4.12. Quality Assurance

To ensure data integrity and reliability of laboratory results, the following quality assurance measures were implemented. The questionnaire was pre-tested in a similar setting and adjusted accordingly, Data collectors and laboratory staff received training prior to data collection and sample processing, Daily supervision and spot checks were conducted to ensure adherence to study protocols, Laboratory procedures followed national and WHO TB diagnostic guidelines and Positive and negative controls were used in laboratory tests to validate the accuracy of the results [26].

Microscopy Quality Assurance

All staining reagents and glass slides were inspected for expiration and physical integrity before use. Daily maintenance of microscopes were carried out to ensure consistent performance.

Gene-X pert Quality Control

Probe Check Control: Each cartridge includes an internal control to verify probe function and reaction setup before PCR begins. If the fluorescence readings were within expected thresholds, the test proceeds. Otherwise, an error is flagged and the run is aborted.

Sample Processing Control (SPC): This control ensures proper sample lysis, DNA extraction, and reaction conditions. A failed SPC in analyse negative sample were lead to an INVALID result. These internal controls are essential for the reliability of Gen-expert outputs [1].

4.13. Ethical clearance

This study was conducted following the receipt of ethical approval from the Institutional Review Board (MU-IRB 2389/2024) of the College of Health Sciences, Mekelle University. The research adhered to both national and international ethical guidelines governing research involving human participants, including the Declaration of Helsinki and relevant Ethiopian national research ethics frameworks. All eligible participants were provided with clear and comprehensive information regarding the study's objectives, procedures, potential benefits, and any foreseeable risks. Written informed consent was obtained from each participant prior to inclusion in the study.

Participation was entirely voluntary, and individuals retained the right to decline or withdraw at any stage without facing any consequences or loss of benefits. To ensure confidentiality, each participant was assigned a unique identification code. Intimates positive for PTB were isolated from other intimates and they linked to the TB clinic by communication with health personnel of the prison centers. No names or personal identifiers were recorded during data collection or in any reports. All data were securely stored physically in locked cabinets and digitally on password-protected computers accessible only to the principal investigator and authorized research personnel.

4.14. Dissemination and Utilization of Results

Upon completion, the research findings will be submitted to the Department of Medical Microbiology and Immunology within the College of Health Sciences at Mekelle University to fulfill academic requirements. Additionally, key insights and conclusions will be communicated to the health authorities and administrative bodies of the Shire, Axum, and Adwa prison institutions. This aims to support evidence-based decision-making regarding tuberculosis prevention, control, and management within these facilities. Furthermore, the study outcomes will be disseminated through local and national workshops, academic symposiums, and stakeholder meetings. Efforts will also be made to publish the results in peer-reviewed scientific journals, ensuring the findings contribute to the broader scientific community and ongoing tuberculosis control initiatives, particularly in correctional settings.

5. RESULTS

5.1. Socio-demographic and economic characteristics of prisoners

The 265 prisoners from three prison centers in northern Ethiopia, specifically Shire (N=99), Axum (N=66), and Adwa (N=100), in 2025. The age distribution of the prisoners shows that the largest group fell between 18 and 30 years old (38.5%), followed by those aged 31-43 (25.3%) and 44-56 (27.5%), with the smallest group being 56-80 years old (8.7%). The prison population was overwhelmingly male (95.8%), with only a small fraction being female (4.2%). In terms of marital status, single individuals made up the majority (50.2%), closely followed by married individuals (46.4%), with smaller percentages of divorced (3.0%) and widowed (0.4%) prisoners.

The educational levels of the prisoners indicate that a significant portion were illiterate (44.9%) or had only primary education (42.6%), while a smaller percentage had secondary education (10.6%) or college and above (1.9%). The prisoners' residences were almost evenly split between urban (49.8%) and rural (50.2%) areas. Regarding monthly income before imprisonment, the largest group earned between 3001-6000 ETB (43.0%), followed by those earning 1501-3000 ETB (21.9%) and <=1500 ETB (18.5%), with smaller percentages earning >=9000 ETB (1.5%). Occupations before imprisonment were primarily daily laborers (30.9%) and government employees (29.1%), followed by self-employment (22.3%) and the unemployed (17.7%). The incarceration duration showed that half of the prisoners had been imprisoned for 2-3 years (50.2%), with 39.2% imprisoned for >=4 years and 10.6% for <=1 year. Finally, a large majority (90.2%) reported no previous incarceration, while 9.8% had been incarcerated previously (**Table 1**).

Table 1***: Socio-demographic and economic characteristics of prisoners from three prison centers (Shire, Axum, and Adwa), northern Ethiopia, 2024 (N=265)***

Variable	Category	Number	Percent (%)
Age (years)	18-30	102	38.5
	31-43	67	25.3
	44-56	73	27.5
	56-80	23	8.7
Sex	Male	254	95.8
	Female	11	4.2
Marital Status	Single	133	50.2
	Married	123	46.4
	Divorced	8	3.0
	Widowed	1	0.4
Educational Level	Illiterate	**119**	**44.9**
	Primary	113	42.6
	Secondary	28	10.6
	College and above	5	1.9
Residence	Urban	132	49.8
	Rural	133	50.2
Monthly Income (in ETB)	<=1500	49	18.5
	1501-3000	58	21.9
	3001-6000	114	43.0
	6001-8999	40	15.1
	>=9000	4	1.5
Occupation before imprisoned	Daily laborer	82	30.9
	Government employee	77	29.1
	Self-employee	59	22.3
	Unemployed	49	17.7
Incarceration duration	<= 1 year	28	10.6
	2-3 years	133	50.2
	>= 4 years	104	39.2
Previous Incarceration	No	239	90.2
	Yes	26	9.8

Behavior, prison conditions, and TB/HIV history characteristics

The data reveals that the vast majority of prisoners had no previous history of TB (97.4%), with only a small fraction reporting a prior TB diagnosis (2.6%). However, a notable proportion (39.8%) reported experiencing at least one TB symptom, all prisoners had TB symptoms. Among those tested, 12.5% were HIV-positive, 92.1% were HIV-negative, and 1.9% had unknown HIV status.

Lifestyle-related risk factors were also examined. A small percentage of the prisoners reported a history of smoking (6.0%), while the majority (94.0%) did not smoke. Alcohol consumption was reported by 15.5% of the prisoners, with 84.5% reporting no alcohol consumption history. Similarly, only 5.3% reported a history of khat chewing, compared to 94.7% who did not. Dietary habits were relatively split, with 47.9% reporting poor diet and 52.1% reporting fair diet.

It also provides insights into prison conditions and TB awareness. The number of cellmates varied, with the largest group (51.3%) sharing cells with 21-34 people, followed by 11-20 cellmates (23.4%), <=10 cellmates (19.2%), and >=35 cellmates (6.0%). All prisoners reported that their cells had ventilation/fresh air and that they were screened for TB. Despite universal TB screening, a small percentage (7.9%) reported having no knowledge of TB, while the majorities (92.1%) were knowledgeable about TB. Finally, 12.1% of the prisoners reported having a chronic basic condition, compared to 87.9% who did not, and 2.3% had contact with a TB patient, compared to 97.7% who did not.**(table 2)**

Table 2: ***Behavior, prison conditions, and TB/HIV history characteristics of prisoners from three prison centers (Shire, Axum, and Adwa), northern Ethiopia, 2025 (N=265)***

Variable	Category	Number	Percent (%)
Previous history of TB	No	258	97.4
	Yes	7	2.6
Any TB symptom/s (N=264)	No	0	0
	Yes	265	100
Tested for HIV	No	0	0.0
	Yes	265	100.0
HIV testing result	Positive	16	6.0
	Negative	249	92.1
Smoking history	No	249	94.0
	Yes	16	6.0
Alcohol consumption history	No	224	84.5
	Yes	41	15.5
Khat chewing history (N=264)	No	250	94.7
	Yes	14	5.3
Diet	BMI >18K.g/m^2	127	47.9
	BMI<18K.g/m^2	138	52.1
Cellmates (number of people per prison cell)	<=10	51	19.2
	11-20	62	23.4
	21-34	136	51.3
	>=35	16	6.0
Ventilation/fresh air	No	0	0.0
	Yes	265	100.0
Screened for TB	No	0	0.0
	Yes	265	100.0
Knowledge of TB	No	21	7.9
	Yes	244	92.1
Chronic disease Condition	No	233	87.9
	Yes	32	12.1
Contact with TB patient	No	259	97.7
	Yes	6	2.3

5.2. Prevalence of tuberculosis among prisoners

Among 265 prisoners in three prison centers (Shire, Axum, and Adwa) in Northern Ethiopia, based on data from October,2024 to May,2025,revealing that the overwhelming majority of prisoners tested negative for tuberculosis (98.1%) using Zeihel - Neelsen microscopic examination, while a small proportion tested positive (1.9%, 95% CI: 0.6% - 4.3%). Furthermore, all positive cases were confirmed positive using Gene -Xpert, and none of the TB cases demonstrated resistance to the first-line anti-TB drug, Rifampicin. **(Figure 1)**

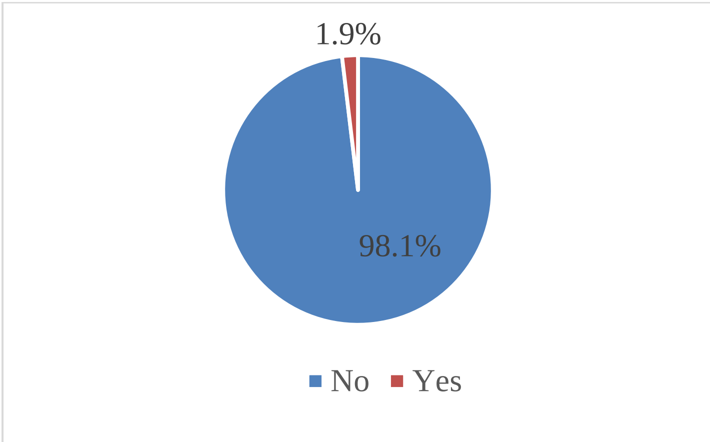


Figure 1: A pie chart depicting the prevalence of tuberculosis among prisons in three prison centers (Shire, Axum, and Adwa), Northern Ethiopia, 2025 (N=265)

5.2.1. Distribution of TB cases across prison centers

An assessment of tuberculosis (TB) distribution across the three prison centers—Shire, Axum, and Adwa revealed a total of five confirmed cases. Specifically, two cases were identified in Shire, one in Axum, and two in Adwa. This corresponds to a TB prevalence of 2.0% in Shire, 1.5% in Axum, and 2.0% in Adwa. The distribution of TB cases among the prison centers appears relatively uniform, with no significant disparity observed between the prison sites. Statistical analysis indicated no meaningful association between TB status and prison location, as reflected by an exact p-value substantially greater than 0.05. This suggests that the occurrence of TB is not significantly influenced by the specific prison center (**Fig.2**).

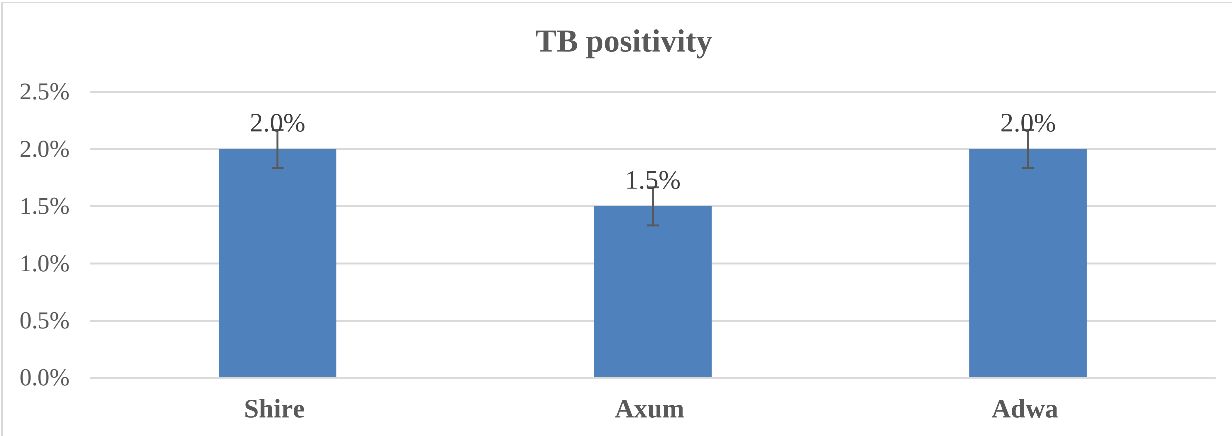


Figure 2: Bar graph depicting the prevalence of tuberculosis among prisoners in three prison centers (Shire, Axum, and Adwa) in Northern Ethiopia, 2025 (N=265).

5.2.2 Distribution of TB cases by various characteristics

The comparison of proportion of individuals with negative and positive TB status across different categories within each variable, providing a clear snapshot of the potential links between these factors and TB prevalence. Furthermore, it reports the exact p-values derived from statistical analysis, indicating the significance of the observed crude associations. These p-values are crucial in determining which variables are most likely to be independently associated with TB and should be considered for further investigation in a final exact logistic regression model.

Examining the demographic and socio-economic variables, it reveals the distribution of TB status across age groups, sex, marital status, educational level, incarceration duration, residence, and monthly income. For instance, within the age category, 2.9% of prisoners aged 18-30 years were TB positive compared to 1.2% of those older than 30 years. Similarly, the data illustrates the TB prevalence among different educational levels, with 3.4% of illiterate individuals testing positive for TB.
These descriptive statistics offer initial insights into potential risk factors, although the corresponding p-values suggest that many of these associations may not be statistically significant in this specific analysis.

It also explores into variables related to the incarcerated population, such as occupation before imprisonment, previous incarceration history, and contact with TB patients. Notably, individuals who were daily laborers or unemployed before imprisonment showed a higher proportion of TB positivity (3.9%) compared to those in government or self-employment (0.0%). While the p-value for previous incarceration was not statistically significant, a higher percentage of individuals with a history of TB contact were TB positive (16.7%) compared to those without such contact (1.5%). These findings highlight the importance of considering occupational and exposure-related factors in understanding TB prevalence within this population.

Finally, it explores health-related behaviors and conditions, including previous history of TB, presence of TB symptoms, HIV testing result, smoking history, alcohol consumption, khat chewing history, diet, cellmate numbers, knowledge of TB, and chronic chest conditions. Several variables in this section show potentially significant associations with TB prevalence, indicated by the 'C' notation for potential candidate variables. For example, individuals with positive HIV test results (6.5%) and those with a history of smoking (12.5%) exhibited a higher proportion of TB positivity. Similarly, the presence of any TB symptoms was associated with a higher TB prevalence (3.8%). These findings underscore the complex interplay of various health-related factors in the epidemiology of TB within this study population **(table 3).**

5.3. Drug Resistance Result

All five TB-positive cases identified through Ziehl–Neelsen staining and confirmed by Gene testing were further analyzed for rifampicin resistance using the Gene X pert MTB/RIF assay. The results revealed that none of the confirmed TB cases exhibited resistance to rifampicin, indicating the absence of rifampicin-resistant tuberculosis (RR-TB) among the study participants. This suggests that first-line TB treatment regimens remain effective for the cases identified in the prison centers of Shire, Axum, and Adwa during the study period **(Table 3).**

Table 3: ***Rifampicin Resistance among TB-Positive Prisoners from Three Prison Centers (Shire, Axum, and Adwa), Northern Ethiopia, 2025 (N=5)***

Drug Resistance Status	**Number of TB Cases Percent (%)**
Rifampicin-sensitive 5	100.0
Rifampicin-resistant 0	0.0

Table 4: ***Distribution and exact association of TB with candidate variables of final exact logistic regression model on TB prevalence, 2025***

Variable	Category	TB Status		Exact P-value
		Negative (%)	Positive (%)	
Age (years)	18-30 Years	99 (97.1)	3 (2.9)	0.376
	>30 years	161 (98.8)	2 (1.2)	
Sex	No	249 (98.0)	5 (2.0)	1.000
	Yes	11 (100.0)	0 (0.0)	
Marital Status	Single	130 (97.7)	3 (2.3)	1.000
	Married/Divorced/Widowed	130 (98.5)	2 (1.5)	
Educational Level	Illiterate	115 (96.6)	4 (3.4)	0.353
	Primary	112 (99.1)	1 (0.9)	
	Secondary/Secondary	33 (100.0)	0 (0.0)	
Incarceration duration	No	158 (98.1)	3 (1.9)	1.000
	Yes	102 (98.1)	2 91.9)	
Residence	Urban	131 (98.2)	1 (0.8)	0.370
	Rural	129 (97.0)	4 (3.0)	
Monthly Income (in ETB)	<=3000	103 (97.3)	4 (3.7)	0.161
	>3000	157 (99.4)	1 (0.6)	
Occupation before imprisoned	Daily laborer/Unemployed	124 (96.1)	5 (3.9)	**0.026**[C]
	Government/self-employed	136 (100.0)	0 (0.0)	
Incarceration duration	No	158 (98.1)	3 (1.9)	1.000
	Yes	102 (98.1)	2 91.9)	
Previous Incarceration	No	235 (98.3)	4 (1.7)	0.406
	Yes	25 (96.1)	1 (3.9)	

Previous history of TB	No	253 (98.1)	5 (1.9)	1.000
	Yes	7 (100.0)	0 (0.0)	
Any TB symptom/s (N=264)	No	158 (99.4)	1 (0.6)	**0.083[C]**
	Yes	101 (96.2)	4 (3.8)	
HIV testing result	Positive	14 (87.5)	2 (12.5)	**0.031[C]**
	Negative/Unknown	246 (98.8)	3 (1.2)	
Smoking history	No	246 (98.8)	3 (1.2)	**0.031[C]**
	Yes	14 (87.5)	2 (12.5)	
Alcohol consumption history	No	220 (98.2)	4 (1.8)	0.571
	Yes	40 (97.6)	1 (2.4)	
Khat chewing history (N=264)	No	245 (98.0)	5 (2.0)	1.000
	Yes	14 9100.0)	0 (0.0)	
Diet	BMI>18K.Kg/m^2	123 (96.8)	4 (3.2)	0.197
	BMI<18Kg/m^2	137 (99.3)	1 (0.7)	
Cellmates	<=20	111 (98.2)	2 (1.8)	1.000
	>20	149 (98.0)	3 ()2.0	
Knowledge of TB	No	21 (100.0)	0 (0.0)	1.000
	Yes	239 (97.9)	5 (2.1)	
Chronic BC Condition	No	228 (97.8)	5 (2.2	1.000
	Yes	32 (100.0)	0 (0.0)	
Contact with TB patient	No	255 (98.5)	4 (1.5)	0.109
	Yes	5 (83.3)	1 (16.7)	

[C]: Potential candidate variables for the final exact logistic regression analysis

5.4. Factors associated with TB prevalence

The results of an exact logistic regression analysis examining factors associated with tuberculosis (TB) prevalence among 265 prisoners from three prison centers in Northern Ethiopia: Shire, Axum, and Adwa. The details the number and percentage of TB-positive cases within each category of the variables under study. Crucially, it provides both the Crude Odds Ratio (COR) and the exact Adjusted Odds Ratio (AOR) with their corresponding 95% exact Confidence Intervals (CI), allowing for an assessment of the unadjusted and adjusted relationships between these factors and TB positivity. The statistical significance of these associations is denoted by an asterisk (*) where the p-value is less than 0.05.

The analysis reveals that the prisoners' occupation before imprisonment significantly influences TB prevalence. Specifically, prisoners who were government or self-employed had a significantly lower odds of TB (exact AOR = 0.119, 95% CI: 0.000, 0.946, p=0.0432) compared to those who were daily workers or unemployed. Similarly, a strong association was observed with HIV status. Prisoners with negative or unknown HIV results demonstrated significantly lower odds of TB (exact AOR = 0.033, 95% CI: 0.001, 0.722, p=0.0281) in comparison to prisoners who were HIV-positive.

While it also explores the impact of TB symptoms and smoking history on TB prevalence, the adjusted odds ratios did not show these factors to be statistically significant factors in this study population. Prisoners who reported having any TB symptom showed higher odds of TB (exact AOR = 17.404, 95% CI: 0.980, 1578.523, p=0.0526), and those with a history of smoking also had elevated odds (AOR = 11.212, 95% CI: 0.694, 193.745, p=0.0936), but these findings did not reach the conventional 0.05 significance level. **(Table 4)**

Table 5: ***Exact logistic regression to identify actors associated with TB prevalence among prisoners from three prison centers (Shire, Axum, and Adwa), Northern Ethiopia (N=265)***

variable	**Category**	**Crude OR**	**P-value**	**AOR (exact 95% CI)**	**Exact P-value**
Occupation	Daily worker/Unemployed	1.000 (Reference category)		1.000 (Reference category)	
	Government /self-employed	0.137	0.0525	0.119 (0.000, 0.946)	**0.0432***
	No	1.000 (Reference)		1.000 (Reference)	
Any TB symptom	Yes	6.216	0.1665	17.404 (0.980, 1578.53)	0.0526
HIV result	Positive	1.000 (Reference)		1.000 (Reference)	
	Negative/unknown	0.087	0.0616	0.033 (0.001, 0.722	**0.0281***
Smoking history	No	1.000 (Reference)		1.000 (Reference)	
	Yes	11.436	0.0616	11.212 (0.694, 193.745)	0.0936

1:000: Reference category, COR: Crude Odds Ratio, AOR: Adjusted Odds Ratio; CI: Confidence Interval, *: Significant at 5% level α

6. DISCUSSIONS

Tuberculosis (TB) remains a major public health challenge globally, particularly in confined settings such as prisons, where transmission risk is significantly elevated. Correctional facilities often concentrate individuals from vulnerable backgrounds in overcrowded and poorly ventilated environments, creating conditions that facilitate the spread of TB. In Ethiopia, prison populations are at heightened risk due to systemic challenges in health service delivery, limited awareness, and inadequate disease surveillance. This study sought to assess the prevalence of TB and identify associated risk factors among prisoners in the Shire, Axum, and Adwa correctional facilities in the Tigray region, thereby contributing to the growing body of evidence needed to inform targeted interventions in such high-risk environments [9].

This study examined the prevalence of tuberculosis (TB) and its associated risk factors among prisoners in three correctional facilities—Shire, Axum, and Adwa—located in the Tigray region of Northern Ethiopia. The findings revealed a TB prevalence of 1.9% (95% CI: 0.6%–4.3%), which aligns with previous research indicating that prison environments are high-risk settings for TB transmission due to factors such as overcrowding, poor ventilation, and delays in diagnosis and treatment [8, 9, 17].

A closer examination of the socio-demographic variables revealed that TB prevalence was slightly higher among younger prisoners (18–30 years), individuals with no formal education, and those from rural areas. Although these associations were not statistically significant, they underscore potential vulnerabilities that warrant further investigation. Notably, occupation prior to imprisonment emerged as a significant factor: prisoners who were previously government-employed or self-employed were significantly less likely to have TB compared to daily laborers and the unemployed. This disparity may reflect differences in socioeconomic status and access to healthcare before incarceration [11, 41].

The study also identified key health-related factors significantly associated with TB. HIV-positive prisoners had markedly higher odds of TB infection compared to those who were HIV-negative or whose status was unknown. This finding aligns with the well-established link between HIV infection and increased susceptibility to TB, underscoring the need for integrated TB/HIV screening and management programs within prison settings [42, 43].

Although not statistically significant at the 5% level, the presence of TB-related symptoms and a history of smoking were both associated with increased odds of TB infection? These associations are biologically plausible and have been supported by previous research. The lack of statistical significance in this study may be attributed to the small number of TB-positive cases, which limits statistical power [44, 45, 46].

Interestingly, factors such as alcohol use, khat chewing, previous incarceration, dietary quality, number of cellmates, and knowledge of TB were not significantly associated with TB status in this sample. However, the high prevalence of poor dietary practices (47.9%) and overcrowding (51.3% of prisoners sharing cells with 21–34 individuals) remain significant public health concerns. These conditions can facilitate TB transmission and contribute to adverse health outcomes over time, even if not statistically significant in this particular analysis [47].

Although the TB prevalence found in this study was relatively low compared to some earlier studies in Ethiopia—such as a 3.4% prevalence among prisoners in Gondar [48].it remains significantly higher than in the general population. According to the Ethiopian Public Health Institute the national TB prevalence is estimated at 132 per 100,000 populations, which is considerably lower than rates observed in prison settings. This disparity underscores the urgent need for intensified TB surveillance and targeted intervention strategies in closed and high-risk environments [35].

The uniform distribution of TB cases across the three prison centers—Shire (2.0%), Axum (1.5%), and Adwa (2.0%)—and the absence of statistically significant differences by location suggest that institutional-level factors may be similarly influencing TB transmission. Overcrowding, as evidenced by over half of the prisoners sharing cells with 21 to 34 other inmates, along with limited separation of suspected TB cases, likely creates an environment conducive to TB spread, irrespective of geographic setting [8, 9, 11].

Significantly, this study identified key factors independently associated with TB prevalence. Prisoners who had been daily laborers or unemployed prior to incarceration were more likely to develop TB compared to those who had been government or self-employed (adjusted odds ratio [AOR] = 0.119, 95% CI: 0.000–0.946, p = 0.043). This finding aligns with previous research indicating that socioeconomic disadvantage and occupational instability are major contributors to TB risk. Precarious employment often correlates with poor nutritional status, limited access to healthcare, and increased exposure to environmental stressors—all of which elevate the risk of developing TB [46].

HIV infection was another significant predictor of TB. The odds of having TB were substantially lower among individuals who were HIV-negative or had unknown HIV status compared to those who were HIV-positive (AOR = 0.033, 95% CI: 0.001–0.722, p = 0.028). This finding is consistent with a large body of evidence demonstrating that HIV-induced immunosuppression significantly increases susceptibility to TB, particularly in high-burden settings (24). As such, integrated TB/HIV programs within prison environments are critical to managing co-infection and curbing TB transmission [49].

While not statistically significant, several other factors exhibited elevated odds ratios and warrant consideration for their potential relevance. Prisoners presenting with any TB-related symptoms had over 17 times the odds of testing positive for TB (AOR = 17.404), and those with a history of smoking had over 11 times the odds (AOR = 11.212), although both associations narrowly missed conventional levels of statistical significance (p = 0.0526 and p = 0.0936, respectively).

The association between smoking and TB is well established, as tobacco smoke compromises pulmonary immune defenses and increases susceptibility to respiratory infections, including TB [45]. The presence of TB symptoms remains a clinically meaningful indicator and highlights the need for consistent symptom-based screening, even in the absence of laboratory confirmation.

Interestingly, variables such as number of cellmates, dietary status, and previous incarceration were not statistically associated with TB in this analysis. Nevertheless, their potential roles in TB transmission should not be overlooked, as prior studies have demonstrated significant associations under different conditions [10]. The relatively small number of TB-positive cases in this study (n = 5) may have limited the statistical power needed to detect meaningful associations with these factors.

Despite the implementation of universal TB screening and the presence of ventilation in all prison cells, the study revealed that 39.8% of inmates reported experiencing at least one TB-related symptom. This finding points to potential gaps in early case detection efforts or the possible progression of latent tuberculosis infections to active disease. Furthermore, 7.9% of the incarcerated population reported having no knowledge of TB, highlighting a critical shortfall in health education within the correctional system. These insights emphasize the importance of integrating targeted educational interventions to enhance inmates' awareness of TB, encourage timely health-seeking behaviors, and ultimately support early diagnosis and treatment within prison settings.

Overall, this study underscores the persistent risk of tuberculosis among incarcerated populations and reinforces the importance of implementing routine screening, early case detection, and the integration of TB/HIV services within correctional facilities. Special consideration should be given to individuals from socioeconomically disadvantaged backgrounds and those with known co-morbidities, such as HIV infection or a history of smoking, as these factors may compound vulnerability to TB. However, given the relatively small number of confirmed TB cases in this study, further research involving larger and more diverse samples is warranted to validate these findings and to more comprehensively assess the impact of additional risk factors

7. LIMITATIONS OF THE STUDY

The limitations of the study were:

- Done on only in three Study Sites including three distinct prison centers.
- It used ZN staining were used, rather than Gene-x pert for most samples.

8. CONCLUSIONS AND RECOMMENDATIONS

8.1. Conclusions

This study aimed to determine the prevalence of tuberculosis and its associated risk factors among inmates in three prison centers of Shire, Axum, and Adwa located in Northern Ethiopia. The results revealed a TB prevalence of 1.9%, which is substantially higher than that observed in the general population, thereby reinforcing the notion that incarceration is a major risk factor for tuberculosis transmission.

Multivariate analysis was identified occupation prior to imprisonment and HIV status as statistically significant risk factors. Inmates who were unemployed or working as daily laborers before incarceration, as well as those who were HIV-positive, had significantly higher odds of testing positive for TB.

8.2. Recommendations

In light of the findings of this study, the following recommendations were proposed:

- Implement intensified TB screening among high-risk inmates, particularly those with a history of unemployment.
- Integrate TB and HIV Services: Strengthen the integration of TB and HIV services within prison health systems to facilitate early detection and treatment.

9. ACKNOWLEGEMENT

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11. ANNEXES

Annex 1: Participant Information Sheet (Template)

Title of the project: "Prevalence and associated risk factors of TB *Mycobacterium tuberculosis* among prisoners in Shire, Axum and Adwa prison centers"

Principal investigator: Girmaslasie fiseha

Advisors: Major advisor- Ephrem Tsegay,

Co-advisor-Dawit Gebrezgabher

Department: Department of Microbiology division of Immunology. College of Health sciences, Mekelle University

Introduction:

You are invited to take part in a research study entitled "Prevalence and Associated Risk Factors of Tuberculosis among Prisoners in Shire, Axum, and Adwa Prison Centers, Tigray Region, Ethiopia."

This study is being conducted as part of an academic requirement for master degree of microbiology department of microbiology and immunology. The purpose of the research is to assess how common tuberculosis (TB) is among prisoners and to identify the factors that may increase the risk of developing TB in prison settings.

Tuberculosis is a major public health problem, especially in overcrowded environments like prisons, where people may have limited access to health care, proper nutrition, and hygiene. This study will help us better understand the current situation in the prison centers of Shire, Axum, and Adwa, and provide valuable information that could help in planning interventions and improving health services for prisoners.

Your participation is completely voluntary, and the information you provide will be kept confidential. Please read the following sections carefully before deciding whether or not to take part.

1. Purpose of the Study: This research aims to assess the prevalence of tuberculosis (TB) and identify associated risk factors among prisoners in Shire, Axum, and Adwa prisons, located in the Tigray region of Ethiopia.

2. Significance of the Study: Understanding the burden and contributing factors of TB in prison settings can help design targeted prevention, control, and treatment strategies for this vulnerable population.

3. Procedures: If you choose to participate, you will be asked to respond to a standardized questionnaire covering socio-demographic information and potential TB risk factors. In addition, a sputum sample will be collected from each participant to test for pulmonary TB.

4. Potential Risks: There are no anticipated physical or health risks associated with your participation in this study, aside from the time required for completing the questionnaire and providing a sample.

5. Participant's Role: Your participation involves providing basic personal details (such as age, sex, and address), responding to questions related to TB risk factors, and submitting a sputum sample for analysis.

6. Voluntary Participation and Right to Withdraw: Participation in this study is completely voluntary. You have the right to refuse to participate or withdraw at any point without facing any consequences. You may also decline to answer any questions or to provide a sample. Your decision will not affect the services you receive.

7. Benefits: Although you will not receive direct payment, if your laboratory test yields any important medical findings, the results will be shared with your physician for appropriate care and management. You may request your test results at any time.

8. Incentives and Compensation: There will be no financial costs or payments for participating in this study. You are entitled to receive full information about the study procedures and related matters in the language of your choice.

9. Confidentiality: All information you provide will be kept strictly confidential. Your name will not be recorded on any forms or associated with the data. All results will be used solely for research purposes and reported anonymously.

10. Contact Information: If you have any questions about this study, or if you would like to be informed of the findings upon the study's completion, please feel free to contact:

Institutional Review Board (IRB): Mekelle University, College of Health Sciences-institute of Biomedical Sciences, IRB Office

If you want more information and check about this project you can contact the following people **MEKELLE UNIVERSITY COLLEGE OF HEALTH SCIENCES, IRB** Office Tel.+251-0344-40-66-80

Principal Investigator Name and Address: Girmaslasie fiseha

Tel: +251946472570

Advisor's Name and Address: Major advisor-Ephrem Tsegay,

Co-advisor-Dawit Gerezgabher

Department of Microbiology and Immunology, College of Health sciences, Mekelle University

Mobile: +251913422945

Annex 2: Informed Consent Form (Specimen)

Title of the project: **"Prevalence and associated risk factors of *Mycobacterium tuberculosis* among prisoners in Shire, Axum and Adwa prison centers**"

I have been well aware of that this research undertaking is for a partial fulfillment of master degree Microbiology which is fully supported and coordinated by the Department of Microbiology, College of health sciences , Department Microbiology and Immunology and the designate principal investigator is Girmaslasie Fiseha. I have been fully informed in the language I understand about the research project objectives that are to understand the purpose, procedures possible risks and benefits, and my rights as a participant.

I have been informed that all the information I shall provide to the interviewer will be kept confidential. I understood that the research has no any risk and no composition. I also knew that I have the right to withhold information, skip questions to answer or to withdraw from the study any time I have acquainted nobody will impose me to explain the reason of withdrawal. It is also enlighten there would have no effect at all in my health benefit or other administrative effect that I get from the refuge.

I have assured that the right to ask information that is not clear about the research before and or during the research work and to contact
Mekelle University, College of health
science IRB Office

Tel. +251-0344-40-66-80

Principal Investigator's Name: Girmaslasie Fiseha Tel: +251946472570

Advisor's Name and Address: Major advisor -Ephrem Tsegay.co advisor -Dawit Gerezgabher.

I have read this form, or it has been read to me in the language I comprehend and understood the condition stated above, therefore, I am willing and confirm my participation by signing the consent.

Name of the participant

____

Agreed to participate in the study: Yes /No (mark one of them for verbal
Consent) Signature ______________________ (if written consent)
Name of witness signature ______________________ (Data collector, supervisor, any third person) Signature ________________
Date _______________________

Annex 3: Questionnaire English Version and Tigrigna version

1.Questionnaire: for investigation of prevalence and associated factors of mycobacterium tuberculosis among prisoners in shire, Axum and Adwa prison centers, Tigray Ethiopia from October, 2024 to May, 2025 G.C English version.

Section 1: Socio demographic characteristics		Date / /
		ID.No
		Phone
1.	Prison center?	
2.	Age?	
3.	Gender	Male
		Female
4.	Marital status?	Single
		Married
		Divorced
		Widowed
5.	Education level?	No formal education
		Primary school
		High school
		College and above
7.	Duration of incarceration?	<6 months
		6-12 months
		1-5 years
		>5 tears
8	Do you have Previous incarceration history?	Yes
		No
9.	The work before incarceration?	Daily worker
		Government employee
		Entrepreneur
		Unemployed
10.	Residence?	Urban
		Rural
11.	Monthly household income?	<4500
		4500
		>4500

Section 2: TB Diagnosis and symptoms			
12.	Do you have previous TB history?	Yes	
		No	
13.	Have you experienced any of the following symptoms in the past 6 months?	Persistent cough >2 weeks	Yes
			No
		Fever	Yes
			No
		Night sweat	Yes
			No
		weight loss	Yes
			No
		Chest pain	Yes
			No

Section 3: Risk factor for TB		
14.	Have you been tested for HIV?	Yes
		No
15.	If yes, what was the result?	Positive
		Negative
16.	Do you have History of smoking cigarettes?	Yes
		No
17.	Do you have History of chronic alcohol consumption?	Yes
		No
		No
18.	Khat chewing?	Yes
		No
19.	How would you describe your diet in prison?	BMI>18Kg/m^2
		BMI <18Kg/m^2
20.	How many intimates share your cell?	11-20 21-33
		>35
21.	Is the ventilation in your cell adequate?	Yes
		No
22.	How often are you allowed outside for fresh air?	Daily
		Weekly
		Rarely
		Never
23.	Were you screened for TB during you incarceration?	Yes
		No
		I do not know

Section 4: Knowledge and awareness of TB		
24.	Do you know how TB is transmitted?	Yes
		No
25.	Do you know the symptoms of TB?	Yes
		No
26.	Are you aware of the importance of completing TB treatment?	Yes
		No

Section 5: Additional information		
27.	Do you have any other chronic medical conditions?	Yes
		No
28.	If the answer is yes, specify it?	Asthma
		Diabetes
		Malnutrition
		Other
		No
		I do not Know
29.	Have you been in close contact with someone diagnosed with TB?	Yes
		No

Thank you for your participation in this study.

.Annex 3:ቃለ - መሕትት ትግርኛ ቅዳሕ

ንመፅናዕቲ ፦"ሽፋን ናይ ቲቢን ቲቢ ከምፅኡ ዝኽእሉ መንገድን አብ ሽረ፡ ኣክሱምንዓድዋን ዘርከቡ ናይ ሕጊ ተሃነጽቲ ንምፍላጥ ኣብ ዝግበር ፅንዓት ዝውዕል መሕትት

ክፋል 1: መለክዒ ማሕበራዊን ዲሞገራፊን ባህርታት		ዕለት / /
		መለለይ ቁጽሪ
		ስልኪ ቁጽሪ
1.	ስም ቤት ህንጸት?	
2.	ዕድመ?	
3.	ጾታ	ተባ
		ኣን
4.	ኩነታት ሓዳር?	ዝተመርዐወ/ት
		ዘይተመርዐወ
		ዝተፋትሐ/ት
		ሰብኣያ/ሰበይቱ ዝሞታ/ ዝመተቶ
5.	ደረጃ ትምህረቲ?	ቐለም ዘይቆጸረ
		ቀዳማይ ደረጃ
		ካልኣይ ደረጃ
		ኮሌጅን ልዕሊኡን
7.	ኣብ ቤት ህንጸት ዝጸንሕሉ ግዜ?	.<6 ኣዋርሕ
		.6-12 ኣዋርሕ
		.1-5 ዓመት
		.>5 ዓመት
8	ቅድሚ ሐዚ ተኣሲሮም የፈልጡ ዶ?	እወ
		ኣየፋል
9.	ቅድሚ ምእሳሮመ ዝነበሮም ስራሕ?	ዓልታዊ ሰራሕተኛ
		መንግሰቲ ሰረሕተኛ
		ናይ ግለይ ስራሕ
		ስራሕ አይነበርኒን
10.	ቅድሚ ምእሳሮመ ኣበይ ይነብሩ ኔሮመ?	ኣብ ገጠር
		ኣብ ከተማ
11.	ቅድሚ ምእሰሮመ ወርሓዊ ኣታዊኦመ ክንደይ ኔሩ?	<4500
		4500
		>4500

ክፍሊ 2: ምርመራ ቲቢን ምልክታትን			
12.	ቅድሚ ሕዚ ብሕማም ቲቢ ተታሒዞም ይፈለጡ ዶ ?	እወ	
		ኣይፋል	
13.	ቅድሚ ክልተ ሰሙን ኣቢሉ ናይ ቲቢ ምልክታት ተራእይዎም ድዩ?	ሰዓል>2 ሰሙን	እወ
			ኣይፋል
		መቑት	እወ
			ኣየፋል
		ረሃጽ ለይቲ ለየቲ	እወ
			ኣየፋል
		ክብደት ምቕናስ	አወ
			አይፋል
		ቓንዛ ኣፍልቢ	አወ
			አየፋል

ክፈሊ 3: መንቀሊታት ቲቢ		
14.	ምርመራ ኤች ኣዪቪ ጌሮም ዶ?	አወ
		አይፋል
15.	ምርመራ እነተጌረመ ዉጽኢቶም እንታይ እዩ?	ፖሰቲብ
		ነጻ
16.	ቅረድሚ መእሳሮመሸጋራ የትክኩ ዶ ኔሮም ?	አወ
		አይፋል
17.	ቅድሚ ምእሳሮመ ኣለኮላዊ መስተ የዝወትሩ ዶ ኔሮመ?	አወ
		ኣየፋል
18.	ቅድሚ ምእሳሮም ጨት የቕሕሙ ዶ ነየሮም?	እወ
		አይፋል
19.	ኩንታት ኣመጋግበኦመ እነታይ መስል?	ትሑት
		ድሓን
		ጽቡቕ
20.	ኣብ ሐደ ገዛ ክንደየ ሰባት ትነብሩ?	<10
		11-20
		21-30
		›30
21.	ትነበሩሉ ገዛ ንፋሰ የኣ ዶ?	እወ
		ኣይፋል
22.	ንጹህ አየር ክትቕበሉ መዓዝ መዓዝ ይፍቀደሎም?	መዓልታዊ
		ሰሙናዊ
		ሓልሓሊፉ
		በፍጹም
23.	ናብ እስርቤት ክኣተዉ ከለዉ ናይ ቲቢ ምርመራ ተጌሩሎመ ዶ?	አወ
		ኣይፋል
		Iኣይፈለጥኩን

ክፍሊ 4: ግንዛበን ፍልጠትን በዛዕባ ቲቢ		
24.	ሕማም ቲቢ ክመይ ከምልመሓላለፍ የፈልጡ ዶ?	ኣወ
		ኣየፋል
25.	ምልክታት ሕማም ቲቢ የፈልጠዎም ዶ?	እወ
		ኣይፋል
26.	ጥቕሚ ብኣገባቡ መድሐኒት ቲቢምውሳድ ግንዛቤ ኣለዎም ዶ?	እወ
		ኣይፋል

ክፍሊ 5: ተወሳኽቲ ሓበሬታታት		
27.	ካልእ ሕዱር ሐማም ኣለዎም ድዩ?	ኣወ
		ኣየፋል
28.	መልሰም እወ እንተኮይኑ?እንተይ ዓይነት ሕማም?	ኣሰሚ
		ሽኮረያ
		ሕጽረት ምግቢ
		ካልእ
29.	ምስ ቲቢ ዘሓዞ ሰብ ንክክእ ኔርዎም ደዩ?	ኣወ
		ኣይፋል

ኣበዚ መጽናዕቲ ስለዝተሳተፉ ነምስግን !

Annex 4: Laboratory Procedures

Sputum smear preparation

- Sputum smear should be prepared nearer to the flame (spirit lamp/Bunsen burner).
- Label a new clean, unscratched slide at one end with the laboratory number using diamond tipped.
- Take muco purulent portion for smear preparation.
- Transfer an appropriate portion of the specimen to smear the specimen over an area of approximately 2 by 3 cm.
- Make a good smear (not too thin or too thick).
- Allow smears to air-dry for 15 minutes. Do not use heat for drying.
- Fix the smear to the slide by passing it over the flame 3 to 5 times for 3 to 4 seconds each
- After making smear, burn and dispose the broom-stick or flame wire loop thoroughly using side burner prior to re-use.
- Flood the slides with freshly filtered carbol fuschin and wait for 5 minutes.
- Wash well with running water followed by adding acid alcohol.
- Then add a counter stain methylene blue for 30 seconds.
- Wash the slide by running water and air dry the slides.

Precautions: Avoid over staining and over discoloring since both leads to false positive and false negative results.

PROCEDURE AND REPORTING OF RESULTS

First switch on the mercury vapor lamp and wait about 10 minutes to reach full intensity. Using the low power objective (1ox) first examine a known positive slide to ensure that the microscope is correctly set up. With Zihel- Nielsen staining, the bacilli appear as slender red, standing out clearly against a blue background.

Microscopy Examination: Examine the stained slide under oil immersion (100x objective).Acid-fast bacilli (AFB) will appear as bright red rods against a blue background.

Result Interpretation:

AFB Count per 100 Fields	Reporting
No AFB seen	Negative
1–9 AFB in 100 fields	Scanty
10–99 AFB in 100 fields	+
1–10 AFB per field	++
>10 AFB per field	+++

Procedure for X-pert MTB/RIF Assay

The Gene X pert MTB/RIF system is a fully automated nested real-time PCR system, which detects MTB complex DNA in smear positive and negative sputum samples and some types of non-sputum samples. It simultaneously identifies mutations in the rpo B gene, which are associated with rifampicin resistance.

The Gene X pert MTB/RIF system consists of the instrument, a computer, a barcode scanner and requires single-use disposable X pert MTB/RIF cartridges that contain assay reagents. Following a 3-step sample preparation in the laboratory, the specimen is transferred into the MTB/RIF cartridge and entered into the Gene X pert instrument. By starting the test on the system software, the Gene X pert automates all following steps, including sample work-up, nucleic acid amplification, detection of the target sequence and result interpretation.

The primers in the X pert MTB/RIF assay amplify a portion of the rpo B gene containing the 81 base pair "core" region. The probes are able to differentiate between the conserved wild-type sequence and mutations in the core region that are associated with RIF resistance. Furthermore, the assay includes a sample processing control to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction. A Probe Check Control verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

Specimens

Note: Any incoming specimen must be properly labeled, as a minimum with a unique identification number. This identification is also written on the request form and must correspond with the identification in the laboratory register.

Type of Specimen

Collect minimum 1ml of sputum. Do not accept specimens with obvious food particles or other solid particulates. The Gene X pert MTB/RIF is only validated for sputum and concentrated sputum. No other samples may be used.

Storage of Specimens: Specimens should be held at 2–8 °C prior to processing whenever possible. If immediate processing is not possible, the specimens can be stored at a maximum of 35°C for 3 days or at 4°C for 4-10 days.

Storage and handling of equipment and materials

- Store the X pert MTB/RIF cartridges and reagents at 2–28°C.
- Do not use reagents or cartridges that have passed the expiration date.
- The cartridge is stable up to 7 days after opening the package.

Note: Samples should be liquefied with no visible clumps of sputum. If there are still clumps of sputum, shake again vigorously and incubate for another 3-5 min.

Interpretation of Results

The results are interpreted by the Gene X pert DX System from measured fluorescent signals and embedded calculation algorithms and will be displayed in the "View Results" window. Lower Ct values represent a higher starting concentration of DNA template; higher Ct values represent a lower concentration of DNA template. MTB Detected MTB target DNA is detected. • MTB Detected—The MTB result will be displayed as High, Medium, Low or Very Low depending on the Ct value of the MTB target present in the sample. Table 1 lists the Ct value ranges for the displayed MTB results.

Table1.MTB result name and Ct value range

MTB Result	Ct Range
High	<16
Medium	16-22
Low	22-28
Very low	>28

Rif Resistance DETECTED, Rif Resistance NOT DETECTED, or Rif Resistance INDETERMINATE will be displayed only in MTB DETECTED results and will be on a separate line from the MTB DETECTED result. **MTB Not Detected**

10. MTB target DNA is not detected, SPC meets acceptance criteria.
11. MTB NOT DETECTED—MTB target DNA is not detected
12. SPC— Pass; SPC has a Ct valid range and endpoint above the endpoint minimum setting.

Probe Check—PASS; all probe check results pass.

RIF target DNA is not detected, SPC meets acceptance criteria.

29. RIF NOT DETECTED—RIF target DNA is not detected
30. SPC— Pass; SPC has a Ct valid range and endpoint above the endpoint minimum setting.
31. Probe Check—PASS; all probe check results pass.

INVALID

Presence or absence of MTB cannot be determined, repeat test with extra specimen. SPC does not meet acceptance criteria, the sample was not properly processed, or PCR is inhibited.

- MTB INVALID—Presence or absence of MTB DNA cannot be determined.
- SPC—FAIL; MTB target result is negative and the SPC Ct is not within valid range.
- Probe Check—PASS; all probe check results pass.

ERROR

- MTB—NO RESULT
- SPC—NO RESULT
- Probe Check—FAIL*; one or more of the probe check results fail.

*If the probe check passed, the error is caused by a system component failure.

- NO RESULT
- MTB—NO RESULT

- NO RESULT
- MTB—NO RESULT
- SPC—NO RESULT
- Probe Check—NA (not applicable)

Reasons to Repeat the Assay

Repeat the test using a new cartridge or initiate alternate procedures if one of the following test results occurs:

5. An INVALID result indicates that the SPC failed. The sample was not properly processed or PCR was inhibited.
6. An ERROR result indicates that the Probe Check control failed and the assay was aborted possibly due to the reaction tube being filled improperly, a reagent probe integrity problem was detected, or because the maximum pressure limits were exceeded or there was a Gene Xpert module failure.
7. A NO RESULT indicates that insufficient data were collected. For example, the operator stopped a test that was in progress.

Annex 5: Assurance of Principal Investigator

I, the undersigned, agree to accept all responsibilities for the scientific and ethical conduct of the research project and for the provision of required progress reports as per the terms and conditions of the requirements of the department. I will provide timely progress report to my advisors and seek the necessary advice and approval from my major advisor in the course of the research.

Name of The Master's

Student: Girmaslasie Fiseha

Approval of the Major

Advisor

Name of the primary:

Supervisor: Signature:

Date:

Annex 6: Declaration

I hereby declare that this MSc thesis is my original work and has not been presented for a degree in any other university and all sources of material used for this thesis have been duly acknowledged.

Name: _Girmaslasie Fiseha

Signature: Date:

This MSc thesis had been submitted for examination with my approval as thesis advisor.

Name: Major advisor-Ephrem Tsegay, Co-Advisor-Dawit Gerezgabher

Signature… …………..

Date……………………..

Signature………………….

Date.........................

Annex 7: Examiner's Approval Sheet

MEKELLE UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPATMENT OF MICROBIOLOGY AND IMMUNOLOGY.

Examiners' Approval Sheet

We, the undersigned, members of the Board of Examiners of the final open defense by

"Girmaslasie Fiseha" have read and evaluated his/her thesis "Prevalence and associated risk factors o *mycobacterium tuberculosis* among prisoners in Shire, Axum and Adwa prison centers" and evaluated th candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of th requirements for the Master's Degree in Microbiology.

Name of Chairperson	Signature	Date
Name of Major Advisor	Signature	Date
Name of Internal Examiner	Signature	Date
Name of External Examiner	Signature	Date

Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the thesis to the candidate's Department through the office of the Department Graduate Program Coordinator.

Thesis Approved by

Graduate Program Coordinator	Signature	Date
___________________________	_________	______/______/_____

Annex 8: Certification of the Final Thesis

I hereby certify that all the corrections and recommendations suggested by the Board of Examiners are incorporated into the final thesis entitled “Prevalence and associated frisk factors of *Mycobacterium tuberculosis* among prisoners in Shire, Axum and Adwa prison centers” by Girmaslasie Fiseha.

Department Head	Signature	Date
_______________	______________	_____/_____/_____

Stamp of the Department of

Remark

Use this form to submit the thesis accepted with minor and major modifications as suggested by the examining board.