MYCOBACTERIUM TUBERCULOSIS INFECTION AMONG HOMELESS INDIVIDUALS IN ADDIS ABABA, ETHIOPIA: DISEASE BURDEN, DRUG RESISTANCE PATTERNS AND MOLECULAR EPIDEMIOLOGY

A Dissertation Submitted to the Department of Microbial, Cellular, and Molecular Biology in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Microbial, Cellular and Molecular Biology, Infection Biology Stream.

Addis Ababa University

Addis Ababa, Ethiopia

May 2024

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I hereby notify that I have reviewed the revised version of this Ph.D. dissertation prepared under my supervision by Tsegaye Shamebo Arficho entitled: "*Mycobacterium tuberculosis* infection among Homeless Individuals in Addis Ababa, Ethiopia: Disease Burden, Drug Resistance Patterns and Molecular Epidemiology" and recommended to be accepted for fulfilling the Dissertation requirement for the Degree of Doctor of Philosophy in Microbial, Cellular and Molecular Biology, Infection Biology Stream.

Approval and signature

Name of the supervisors who approved the dissertation:

Beyene Petros (Ph.D., Prof.): ____________________ _______________

Name Signature Date

DECLARATION

I, Tsegaye Shamebo, hereby declare that the dissertation entitled "*Mycobacterium tuberculosis* infection among homeless individuals in Addis Ababa, Ethiopia: Disease Burden, Drug Resistance Patterns and Molecular Epidemiology" in fulfillment of the Requirements for the Degree of Doctor of Philosophy (Ph.D.) at the University of Addis Ababa is my independent work and has not previously been submitted for a degree at any other University. In addition, I declare that all sources that I have used or quoted have been indicated and acknowledged.

Tsegaye Shamebo	______________________	____________________
Name	Signature	Date

ABSTRACT

In high tuberculosis (TB) burden countries like Ethiopia, rapid screening and prompt treatment initiation among vulnerable groups, such as the homeless, are essential for TB control efforts. During the last three decades, Ethiopia has experienced a rise in homelessness, which is attributed to internal conflicts and economic stress. In spite of the fact that TB disproportionately affects homeless individuals, the majority of research conducted on it in Ethiopia has not adequately addressed the disease burden on this vulnerable group. This study aimed to determine the disease burden, molecular epidemiology, and drug resistance patterns of *Mycobacterium tuberculosis* (*M. tuberculosis*) among homeless individuals in Addis Ababa, Ethiopia. A cross-sectional study was conducted in Addis Ababa between February 2019 and December 2020. Homeless individuals underwent pulmonary tuberculosis (PTB) clinical screening according to WHO guidelines. Suspected cases provided sputum samples for acid-fast bacillus (AFB), Xpert MTB/RIF assay, TB culture, and drug sensitivity test (DST). The *M. tuberculosis* isolates were typed using Polymerase-Chain-Reaction (PCR) based Region of Difference-9 (RD9), spoligotyping, and 24-loci *M. tuberculosis* Interspersed Repetitive Unit-Variable Number Tandem Repeat (MIRU-VNTR) typing. DST was performed using the BD Bactec Mycobacterial Growth Indicator Tube (MGIT) 960. Data analyses were performed using SPSS software version 26 and the *M. tuberculosis* complex (MTBC) online database. Out of 5,600 homeless individuals enrolled in the study and clinically screened for PTB symptoms, 641 suspected cases were identified. Thus, the clinical prevalence of PTB was 1054 per 100,000 homeless individuals. Being homeless for more than 5 years, a body mass index (BMI) < 18.5, smoking cigarettes, living in a group of more than 5 persons,

close contact with chronic coughers, imprisonment, and HIV infections were significantly associated with the prevalence of PTB in the homeless ($P < 0.05$). Out of 59 isolates, 58 were confirmed as *M. tuberculosis* by the RD9 PCR test. Genotyping revealed three MTBC lineages and eight sub-lineages, with Euro-American lineage predominating. Furthermore, Spoligo International Types (SIT), SIT53, SIT37, and SIT149 were highly prevalent strains detected in this study. Ethiopia_3, Delhi/CAS and Ethiopia_2 were determined to be the most prevalent sub-lineages in the study population. Strain clustering rates were 77.6% using spoligotyping, 39.7% using 24-loci MIRU-VNTR typing, and 10.3% using a combination approach. Living in a group was significantly associated with strain clustering ($P < 0.05$). Three homeless individuals with PTB harbored mixed *M. tuberculosis* strains. DST revealed 6.8% (4/59) of isolates resistant to at least one first-line anti-TB drug. Overall, the prevalence of PTB in homeless individuals was higher than that in the general population of Addis Ababa. Therefore, governmental and non-governmental organizations working on TB prevention and control must consider homeless settings as hotspots for TB control. Regular PTB screening, directly observed treatment short course (DOTS) centers, and mobile clinics must be established to control TB among homeless individuals and its spread to the general population.

Keywords: Tuberculosis, Pulmonary Tuberculosis, *M. tuberculosis*, Homeless individuals, Drug sensitivity, Genetic diversity, Genotyping, Addis Ababa

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DEDICATION

I dedicate this Ph.D. dissertation to my father, Shamebo Arficho, who passed away suddenly.

TABLE OF CONTENT

CONTENTS **PAGE**

TABLE OF CONTENTS i

LIST OF TABLES v

LIST OF FIGURES vi

LIST OF APPENDICES viii

LIST OF ABBREVIATIONS/ACRONYMS ix

1. INTRODUCTION 1

1.1. BACKGROUND 1

1. 2. OBJECTIVES OF THE STUDY 9

1.2.1. General objective of the study 9

1. 2.2. Specific objectives of the study 9

1. 3. Hypothesis of the study 9

2. Genome and the Phylogeny of *Mycobacterium tuberculosis* complex (MTBC) 10

2.1. Genome of MTBC 10

2. 2. Global distribution of major lineages of MTBC 12

2. 3. Sub-lineages of MTBC 15

2.4. Epidemiology of TB 20

2. 5. Diagnosis of TB 23

2.5.1. Sputum smear microscopy 23

2. 5.2. TB Culture 23

2. 5. 3. Molecular diagnosis of TB 24

2. 6. Treatment of TB 25

2. 7. Epidemiology of drug-resistant TB 26

2. 8. Mechanisms of drug resistance in *M. tuberculosis* 29

2.9. Drug susceptibility test (DST) 31

2. 10. Genotyping of MTBC 32

2.10.1. Spoligotyping 34

2. 10.2. MIRU-VNTR 38

2.11. Genotyping method's discriminatory power for MTBC 42

2.12. TB control in Ethiopia 43

2.13. Homelessness and associated factors 45

3. MATERIALS AND METHODS 49

3.1. Study setting 49

3.2. Recruitment of study participants 51

3.3. Data collection 52

3.4. Laboratory procedures 53

3.4.1. Sputum smear microscopy..54

3.4. 2. Gene Xpert MTB/RIF assay..54

3.4.3. *M. tuberculosis* isolation..55

3.5. Drug sensitivity test..56

3.6. DNA Extraction and typing of M. tuberculosis isolates..57

3.6.1. DNA Extraction..57

3.6.2. Region of difference-9 (RD9) based polymerase chain reaction 57

3.6.3. Spoligotyping..58

3.6.4. Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat Typing..60

3.7. Anthropometric measurements..62

3.8. Rapid HIV test..62

3.9. Quality control 63

3.10. Data analysis 63

3.11. Ethical consideration..65

4. RESULTS 67

4.1. Socio-demographic characteristics of the study participants..................................67

4.2. Prevalence of PTB and associated risk factors in the homeless individuals...........70

4.3. Molecular epidemiology of MTBC strain circulating in homeless individuals in Addis Ababa, Ethiopia 74

4.3.1. Study participants 74

4.3.2. Molecular characterization of MTBC isolates 74

4.3.2.1. Speciation of the *M. tuberculosis* isolates by PCR-based-RD 9 74

4.3.2.2. Identification of lineages and sub-lineages based on spoligotyping 75

4.3.2.3. Identification of lineages and sub-lineages based on MIRU-VNTR typing 78

4. 3.2.3.1. MIRU-VNTR based allelic diversity (h) **........ 80**

4.3.2.3.2. Minimum spanning tree (MST) and dendrogram analysis 82

4.3.2.4. Discriminatory power of spoligotyping and 24-loci Mycobacterial Interspersed Repetitive Unit-variable Number Tandem Repeat typing 85

4.3.2.5. Factors associated with clustering of M. tuberculosis strains 86

4.4. Drug susceptibility test 86

4.5. Prevalence of mixed *M. tuberculosis* strain infection 89

5. DISCUSSION 90

5.1. CONCLUSIONS 101

5.2. RECOMMENDATIONS 102

6. REFERENCES 103

7. ANNEXES 129

LIST OF TABLES

LIST **PAGE**

Table 1: List of drugs used to treat TB along with genes linked to antibiotic resistance.. 26

Table 2: Drug resistance mechanisms in *M. tuberculosis* (Zhang et al. 2004)..................30

Table 3: Socio-demographic characteristics of homeless individuals (n = 641) suspected of PTB, Addis Ababa, Ethiopia, 2023..69

Table 4:Univariate analysis of PTB prevalence in homeless individuals (n = 641), Addis Ababa, Ethiopia, 2023..71

Table 5:Multivariate analysis of PTB prevalence in homeless individuals (n = 641), Addis Ababa, Ethiopia, 2023..73

Table 6:Major Lineage and lineages of MTBC isolates from homeless individuals, Addis Ababa, Ethiopia, 2023..76

Table 7:Major lineages and orphan strains of MTBC isolates from homeless individuals, Addis Ababa, Ethiopia, 2023 77

Table 8:Allelic diversity and the occurrence of MIRU-VNTR alleles among 58 MTBC isolates from homeless individuals, Addis Ababa, Ethiopia, 2023..................................81

Table 9:The ability of genotyping techniques to discriminate 58 MTBC isolates from homeless individuals, Addis Ababa, Ethiopia, 2023 85

Table 10: Factors associated with strain clustering using 24-loci MIRU-VNTR and spoligotyping for 58 MTBC isolates from homeless individuals, Addis Ababa, Ethiopia, 2023..87

Table 11: Patterns of drug resistance to first-line anti-TB drugs for 58 MTBC isolates from homeless individuals, Addis Ababa, Ethiopia, 2023. 88

Table 12: Multiple strains of *M. tuberculosis* identified from homeless individuals, Addis Ababa, Ethiopia, 2023..89

LIST OF FIGURES

LIST **PAGE**

Figure 1: The genome of *M. tuberculosis* H37Rv represented diagrammatically (Cole et al. 1998). 10

Figure 2: Based on deletions and sequence polymorphisms in five chosen genes—katG, gyrA, oxyR, pncA, mmpl6, and TbD1—the evolutionary pathway of tubercle bacilli is suggested (Barbier and Wirth, 2016). 15

Figure 3: A neighbor-joining tree displaying the phylogenetic structure of the *M. tuberculosis* complex (MTBC) lineages and sub-lineages (Niemann et al. 2016). 19

Figure 4: Eight countries with a high percentage of tuberculosis cases in 2021 (WHO, 2022) 21

Figure 5: The MTBC genome is used to represent the IS6110, MIRU, and DR regions (Barnes & Cave 2003). 33

Figure 6: A portion of the DR locus. The assay for spoligotyping uses 43 spacers. (B). amplified mycobacterial DNAs from 35 *M. tuberculosis* and 5 *M. bovis* strains showed hybridization patterns (spoligotype). The spacers on the filter are arranged in the same order as they are in the genome. As previously mentioned, strains from the Beijing family are represented by the spoligotype 6, 12, and 37 (Karmerbeek et al. 1997). 36

Figure 7:Conversion of raw spoligotyping findings into binary and octal code (Driscoll, 2009). 37

Figure 8: Tandem repeat variability....................40

Figure 9: MIRU-VNTR genotyping principle.. 40

Figure 10: Loci MIRU amplicons with a 1000 bp ladder and Ethidium bromide staining on an agarose gel...41

Figure 11: The various techniques for molecular typing *M. tuberculosis* exhibit varying characteristics regarding technical difficulties and the ability to resolve genetic distances between the strains under study (Cannans et al. 2016)..43

Figure 12: Map of the Addis Ababa study locations, or sub-cities, where study participants were recruited by setting up temporary shelters for the homeless....................................50

Figure 13: The study population, study participants, smear, Xpert, culture, molecular and DST categories in homeless individuals, Addis Ababa, Ethiopia, 2023 68

Figure 14: Picture of gel electrophoresis for RD9 deletion typing of *M. tuberculosis* isolates...75

Figure 15: Picture of gel electrophoresis for MIRU-VNTR typing of *M. tuberculosis* isolates...79

Figure 16: Minimum spanning tree based on 24-loci MIRU-VNTR profiles of 58 MTBC isolates from homeless individuals, Addis Ababa, Ethiopia, 2023. Sub-lineages are colored differently and the individual pattern is represented by the circle. The length..................82

Figure 17:Depicts the phylogenetic tree of 58 MTBC isolates from homeless individuals in Addis Ababa, Ethiopia,2023...84

LIST OF APPENDICES

LIST **PAGE**

Annex I: Consent form to study …………………………………………………..……103

Annex II. Questionnaire for TB among homeless individuals in Addis Ababa, Ethiopia. Participant's ……………………………………………………………………..….108

Annex III. Locus designations and PCR primer sequences used in this study for the 24-locus MIRU-VNTR set………………………………………………………….….114

Annex IV: Manuscript published………………..………………………………...115

Annex V: Conference presentations….…………………………………………….116

LIST OF ABBREVIATIONS/ACRONYMS

AACAHB Addis Ababa City Administration Health Bureau

AAU Addis Ababa University

AFB Acid fast bacilli

ALIPB Aklilu Lemma Institute of Pathobiology

CRISPR Clustered regularly interspersed short palindromic repeat

DOTS Directly Observed Treatment, Short Course

DR direct-repeat

EMB Ethambutol

EPHI Ethiopian Public Health Institute

FDRE Federal Democratic Republic of Ethiopia

HEW Health Extension Workers

HIV Human Immunodeficiency Virus

INH Isoniazid

LJ Löwenstein –Jensen medium

LPA Line prob assay

MDGs Millennium Development Goals

MDR Multidrug-resistant

MGIT Mycobacterium Growth Indicator Tube

MIC Minimum Inhibitory Concentration

MIRU Mycobacterial Interspersed Repetitive Units

MTB *Mycobacterium tuberculosis*

MTBC *Mycobacterium tuberculosis* complex

NAAT	Nucleic acid amplification techniques
NJ	Neighbor joining
NTCP	National Tuberculosis Control Program
NTM	Non-tuberculosis mycobacteria
PCR	Polymerase chain reaction
PLWHA	People Living with HIV
PTB	Pulmonary Tuberculosis
PZA	Pyrazinamide
RFLP	Restriction Fragment Length Polymorphism
RIF	Rifampicin
RR	Rifampin Resistance
RTI	Recent Transmission Index
SDGs	Sustainable Development Goals
SM	streptomycin
SNP	Single nucleotide polymorphism
SpolDB	Spoligotyping database
Spoligotyping	Spacer Oligo Nucleotide typing
TB	Tuberculosis
TDR-TB	Totally drug resistant tuberculosis-TB
VNTR	Variable Number Tandem Repeat
WGS	whole genome sequencing
WHO	World Health Organization
XDR	Extensively Drug Resistant
ZN	Ziehl-Neelsen

1. INTRODUCTION

1.1. BACKGROUND

Tuberculosis (TB) is an infectious disease mainly caused by the bacterial pathogen *Mycobacterium tuberculosis* (*M. tuberculosis*) worldwide. It can also be caused by *Mycobacterium bovis* (*M. bovis*) and *Mycobacterium africanum* (*M. africanum*) in certain geographical regions (Banuls et al. 2015). The disease usually affects the lungs, causing pulmonary tuberculosis (PTB), but can also affect other parts of the body as extra-pulmonary tuberculosis (EPTB) (WHO, 2022). Around 85% of TB cases occur in the lungs (Ravimohan et al. 2018).

The first known progenitor of TB may have originated in East Africa 3 million years ago (Daniel et al. 1994). More compelling evidence came from Egyptian mummies, which date back 5400 years (Zink et al. 2001). Before Johann Lukas Schönlein coined the name "tuberculosis" in 1834, the terms "consumption" "scrofula" "Pott's disease" "white plaque" and "phthisis" were used to describe the disease in the 17th and 18th centuries (Daniel et al. 1994). Hippocrates accurately recognized the symptoms of phthisis and the typical lung lesions associated with TB, as well as describing it as a fatal disease, especially in young adults (Frith, 2014).

Mycobacteria are microorganisms that are members of the Actinomycetales order, the Mycobcateriaceae family, and the genus *Mycobacterium* (Barletta et al. 2015; Brosch et al. 2002). Approximately 170 species of mycobacterium have been identified to date (Forbes, 2017). Four main families can be distinguished within the genus *Mycobacterium* based on

variations in disease, in vitro growth, and epidemiology. These include *Mycobacterium leprae*, *Mycobacterium ulcerans*, *Mycobacterium tuberculosis* complex (MTBC), and non-tuberculosis mycobacterium (NTM) (Rastogi et al. 2001). The MTBC is composed of closely related species like *M. pinnipedii* (seals), *M. microti* (rodent), *M. bovis* (bovine), and *M. caprae* (goats) (Rastogi et al. 2001). It also includes *M. tuberculosis*, *M. canettii*, and *M. africanum*.

M. tuberculosis, one of the members of the MTBC, is an intracellular pathogen characterized by its rod-shaped morphology, aerobic metabolism, lack of motility, inability to form spores, slow growth rate, and acid-fast staining properties (Delogu et al. 2013). The bacterium, *M. tuberculosis,* was first identified in 1882 by the German Scientist Robert Koch (Koch, 2010).

Transmission occurs through air droplets expelled during coughing, talking, sneezing, or spitting by individuals with laryngeal or PTB (Banuls et al. 2015; Turner and Bothamley, 2015). Due to their small size (1–5 µm in diameter), the mycobacterial aerosol droplets can easily access the lower respiratory tract and remain suspended in the air for extended periods (Banuls et al. 2015). Despite larger droplets being filtered out by the upper respiratory tract and nasopharynx (Lonnroth et al. 2010; Urdahl et al. 2011; Lienhardt et al. 2012), some bacilli in alveolar macrophages can grow intracellularly and are released upon macrophage death. This led to the development of primary active TB in 5-10% of infected individuals within weeks to decades (Emery et al. 2021; Behr et al. 2019; Urdahl et al. 2011). The remaining 90% of TB cases persist as asymptomatic or latent tuberculosis infection (LTBI). This occurs when the immune system successfully confines the invading

bacilli in granulomas (Morrison et al. 2008). Reactivation of LTBI can happen in 5–10% of those infected individuals during their lifetime (Morrison et al. 2008). However, in individuals co-infected with HIV, this risk rises to 5–15% annually (Pawlowski et al. 2012). Furthermore, factors such as diabetes, smoking, alcoholism, overcrowding, malnutrition, and drug addiction can increase the reactivation of LTBI.

The location of *M. tuberculosis* infection in the body determines the symptoms of active TB. For instance, chest pain, a cough that lasts longer than two weeks, a bloody cough, a fever, night sweats, chills, and anorexia are some of the symptoms of active TB that are most frequently diagnosed (Pfyffer, 2015). Sputum smear microscopy, utilized for detecting aid-fast bacilli (AFB) has been the gold standard for diagnosing TB for over a century and is still widely used today (MacLean et al. 2019). This technique entails the identification of bacteria in sputum samples through microscopic examination. A recent innovation in TB diagnosis, rapid molecular testing (GeneXpert MTB/RIF assay), is capable of identifying both *M. tuberculosis* and drug-resistance. In countries with more advanced laboratory facilities, culture serves as the customary reference technique for diagnosing TB cases (WHO, 2013).

More than 50% of people with active TB will die if left untreated (Andrews et al. 2012). There have been effective treatments for TB since the 1940s (Lienhardt et al. 2010). Streptomycin (SM), the first drug employed in the treatment of TB was introduced in 1944 followed by para-aminosalicylic acid (PAS) (Wainwright, 1991). However, it proved ineffective in treating TB due to the development of resistance by the bacilli. This led to the development of SM combination therapy (Crofton and Mitchison, 1948). Subsequent

discoveries of isoniazid (INH), pyrazinamide (PZA), rifampin (RIF), ethambutol (EMB), and other drugs provided crucial insights for TB control (WHO, 2009). The current recommended treatment course can cure approximately 85% of TB patients. It lasts 6-9 months for those sensitive to INH and RIF whereas 18-24 months for individuals with drug-resistant TB (WHO, 2009).

Although TB became less prevalent and gradually faded from public awareness in the 1980s due to the effectiveness of treatment, it resurged as a global emergency in 1993. This resurgence was primarily attributed to the emergence of drug-resistant strains of *M. tuberculosis* and the HIV/AIDS pandemic (Dolin et al. 1994). An estimated one-third of the global population is infected with *M. tuberculosis* which has created a large reservoir of chronic active TB (Houben and Dodd, 2016). TB now ranks as the second most common infectious killer worldwide, after coronavirus-2019 (COVID-19) (WHO, 2021). In 2022, an estimated 10.6 million people contracted TB worldwide, resulting in 1.3 million deaths, with the majority occurring in low- and middle-income countries (WHO, 2023). Eight countries account for two-thirds of the global total- Bangladesh, China, the Democratic Republic of the Congo, India, Indonesia, Nigeria, Pakistan, and the Philippines (WHO, 2022). An estimated 87% of new TB cases in 2021 occurred in the 30 high TB burden countries. Southeast Asia accounted for the largest TB cases (46%), followed by Africa (23%) and the Western Pacific (18%) (WHO, 2022).

Globally, an estimated 410 000 people developed drug-resistant forms of TB (DR-TB) in 2022 (WHO, 2023), which include multidrug-resistant/Rifampicin-resistant TB (MDR/RR-TB) and extensively drug-resistant TB (XDR-TB). In the same year, the prevalence of MDR/RR-TB was estimated to be 3.3% and 17.7% for newly and previously

treated TB cases, respectively (WHO, 2023). TB is now the primary cause of death among people living with HIV (PLWHA) (Riou et al. 2021; WHO, 2022). In 2022, it killed an estimated 167,000 people with HIV globally (WHO, 2023). HIV inhibits immune function, increases the reactivation of LTBI, and increases a patient's susceptibility to *M. tuberculosis* infection (Valdez et al. 2002; Pawlowski et al. 2012). Twenty-two of the 30 countries with the highest rates of TB-HIV co-infection are in Africa (WHO, 2019a).

TB services were one of many services disrupted in 2021 by the COVID-19 pandemic (Salina, 2023; Udoakang et al. 2023). The TB burden is increased as a result of its ongoing detrimental effects on access to diagnosis and treatment. The progress gained in the years preceding 2019 has slowed, stopped, or reversed (WHO, 2022). This suggests that there are more cases of misdiagnosed and untreated TB, which raises the risk of infections and deaths. The reported TB cases surged to 10.6 million in 2021, surpassing pre-pandemic levels (WHO, 2022).

Although Kenya, Tanzania, and Zambia have met the first milestones in their End TB Strategy, more than 33% of TB-related deaths were reported in Africa in 2021 (WHO, 2022). With a population of approximately 120 million, Ethiopia ranks 13th in the world and second in Africa in terms of population (Akwara et al.2022). It is one of the 30 high TB and TB-HIV burden countries globally. The country reported 156,000 new TB cases in 2022, with an incidence rate of 126 cases per 100,000 population (WHO, 2023). During the same period, the HIV-negative TB mortality was 21,000(17/100,000 population) while HIV-associated TB mortality was 1,700 (1.4/100,000 population). The accumulation of TB and increased burden in various population groups and geographical areas remain a challenge in Ethiopia, despite the country achieving the 2020 milestone towards ending the

TB epidemic target. This is a 20% reduction in the absolute number of TB cases between 2015 and 2020 (WHO,2021). However, this reduction must be weighed against the reported 30% miss of TB cases by the health system in Ethiopia and the estimated more than 19,000 people that lost their lives in the country, due to TB in 2021 alone (WHO, 2022). Despite Ethiopia being delisted from the global high MDR-TB burden countries, DR-TB is still a public health problem in the country. The World Health Organization (WHO) estimates that 1.1% of individuals newly diagnosed with TB and 12% of those previously treated for TB have been affected by drug resistance (WHO, 2023). According to the Addis Ababa Health Bureau's 2020 TB report, in the city, 8,525 active TB cases were reported, with 22% of them being HIV-TB co-infected (AACAHB,2020). In Addis Ababa, there is a problem with the large and congested population density of slum regions, where overcrowding affects 24.8% of all families. Overcrowding significantly impacts people's health, especially in terms of facilitating the transmission of infectious diseases (AACAHB,2020).

Although TB affects all human beings regardless of gender, age, or race, its burden is much higher in certain high-risk population groups. This includes the homeless, the poor, the elderly, prisoners, people living with HIV, refugees, immigrants, diabetics, the malnourished, and those struggling with substance addiction (Taylor, 2003; Bacha et al. 2004). Homelessness is a pressing social crisis globally, with over 100 million people homeless and 1.5 billion living in inadequate housing conditions (Donnelly et al. 2019). The majority of homeless individuals are young adults, and they are not a distinct species; rather, they are products of the society in which they live (WHO, 2000). In general, homelessness can be defined as a lack of regular, ordinary access to a traditional abode or

habitation, even though there is no single, widely accepted definition of the term (Rodriguez-Moreno et al. 2021; Lee et al. 2010). According to the European Typology of Homelessness and Housing Exclusion (ETHOS) homeless is defined as “People living in a place of habitation that is below a minimum adequacy standard, and lacking access to adequate housing" (Edgar et al. 2007; Donnelly et al. 2019).

Out of an estimated 600,000 homeless individuals residing in Ethiopia, over 100,000 are projected to be living in Addis Ababa (Shamebo et al. 2023a). The majority of young homeless individuals residing in Addis Ababa city are migrants from rural areas of the country searching for better opportunities, while some were forced into the streets due to lack of support and care from the city itself (Fekadu et al. 2014; Shamebo et al. 2023a). The primary causes of migration include internal conflict, displacement, poverty, family breaks, unemployment, and a lack of support from social networks (Shamebo et al. 2023a).

TB has been a public health problem among homeless individuals in Ethiopia and other parts of the world (Barmrah et al. 2013; Semunigus et al. 2016; Powell et al. 2017; Shamebo et al. 2023a). This is due to several risk factors including the HIV/AIDS pandemic, diabetes, smoking, alcoholism, drug abuse, delayed diagnosis, treatment non-adherence, difficulty in accessing healthcare services, and malnutrition (CDC, 2016; Semunigus et al. 2016; Aldrige et al. 2018; Rogans-Watson et al. 2020; Self et al. 2021). According to reports from developed countries, the prevalence of TB in homeless individuals is 20 times higher than its prevalence in the general population (Dolla et al.2017). Moreover, homeless individuals have an estimated 10 to 85 times higher likelihood of latent or active TB infection than the general population (Tankimovich, 2013; Ranzani et al. 2016; Cawley et al. 2022). A previous study reported 2.6% smear-positive

PTB among homeless individuals in northern Ethiopian cities (Semunigus et al. 2016). Since the study was based solely on smear microscopy, the report would be an underestimate of the true incidence. Thus, the burden of TB among homeless individuals and its spread to the rest of the community could be very high.

The strains of *M. tuberculosis* infecting the homeless are similar to those infecting the general population (Hernández Sarmiento et al. 2013). For instance, Haarlem, LAM, and T sub-lineages were more frequently isolated from both the general population and the homeless in Colombia (Hernández Sarmiento et al. 2013). However, molecular epidemiology studies conducted in Ethiopia so far have been limited to more accessible institutional populations than marginalized homeless individuals (Alelign et al. 2019; Tadese et al. 2017; Bedewi et al. 2017; Mekonnen et al.2018; Wondale et al. 2020). Therefore, this study aimed to investigate the molecular epidemiology and drug resistance patterns of *M. tuberculosis* in homeless individuals, in Addis Ababa, Ethiopia.

1. 2. OBJECTIVES OF THE STUDY

1.2.1. General objective of the study

- To investigate the characteristics of MTBC isolates circulating among homeless individuals in Addis Ababa, Ethiopia.

1. 2.2. Specific objectives of the study

1. To determine the prevalence of PTB among homeless individuals.
2. To identify possible PTB risk factors among homeless individuals.
3. To evaluate the drug sensitivity patterns of MTBC strains isolated from homeless individuals.
4. To identify the genetic diversity of MTBC strains circulating among homeless individuals.

1. 3. Hypothesis of the study

- The magnitude of TB among homeless individuals in Addis Ababa is higher than the general population and consists of diversified MTBC genotypes.

2. Genome and the Phylogeny of *Mycobacterium tuberculosis* complex (MTBC)

2.1. Genome of MTBC

M. tuberculosis H37Rv's whole genome was sequenced in 1998 (Cole et al. 1998). It turned up a circular genome with 4.4 million base pairs and about 4,000 genes (Figure 1). The genome of *M. tuberculosis* is GC-rich (65.6%), a characteristic that is more frequently found in aerobic prokaryotes (Naya et al. 2002). After four years, it was predicted that 2058 genes, or 52% of the total, would operate (Camus et al. 2002). Despite differences in host tropism, phenotypic, and virulence determinants, members of the MTBC have identical 16S rRNA sequences at the nucleotide level and exhibit 99.9% DNA similarity (Brosch et al. 2002).

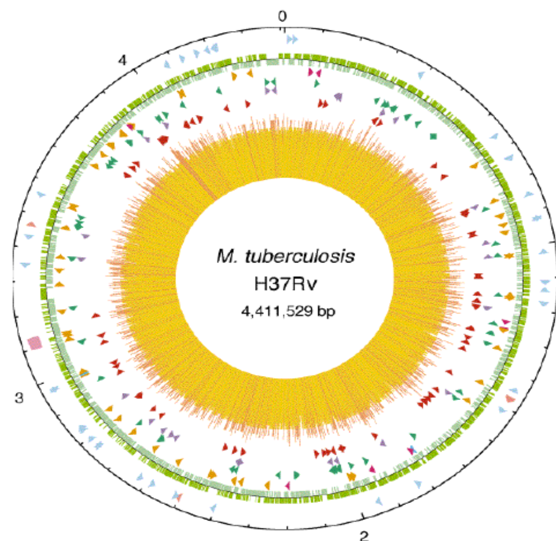


Figure 1: The genome of *M. tuberculosis* H37Rv represented diagrammatically (Cole et al. 1998).

The scale in *M. tuberculosis* is displayed in the outer circle, where 0 denotes the replication origin. First, the exterior ring indicates the positions of the direct repeat region (pink cube) and stable RNA genes (tRNA are blue, others are pink); second, the ring shows the coding sequence by strand (clockwise, dark green, anticlockwise, light green); third, repetitive DNA (insertion sequences, orange; 13E12RFP family, dark pink; prophage blue); fourth, PPE family members' positions (green); fifth, PF family members' positions (purple, excluding PGRS); and sixth, the positions of the PGRS sequences (dark red). The G+C content is shown by the histogram (middle), where < 65% G+C is shown in yellow and > 65% G+C is shown in red.

M. tuberculosis carries every gene needed for the production of essential amino acids, vitamins, and co-factors for enzymes. Enzymes involved in lipogenesis and lipolysis are encoded by most of the genes (Cole et al. 1998). Moreover, *M. tuberculosis* possesses the genes required for the catabolic Krebs cycle, the anabolic pentose phosphate pathway, which produces NADPH and pentose sugars, and the glyoxylate cycle, which converts lipids into carbon. The bacillus may thrive in a range of environments, such as the oxygen-rich lung, the center of a caseous granuloma, and macrophages. It also contains enzymes for aerobic, microaerophilic, and anoxic electron transfer (Cole et al. 1998).

Many types of repetitive DNA sequences are found in the *M. tuberculosis* genome, such as polymorphism GC-rich repetitive sequences (PGRS), major polymorphic tandem repeats (MPTR), direct repeat (DR) regions, and insertion sequences (IS) (Poulet and Cole, 1995). Better than 99.9% similarity in rDNA sequences indicates that clinical and laboratory strains of the MTBC have a high degree of sequence homogeneity (Sreevatsan et al. 1997). This similarity of sequences persists even amongst distinct species of MTBC.

The sequences of *M. bovis* and *M. tuberculosis* differ by less than 0.05% from one another (Garnier et al. 2003). This genetic similarity between MTBC and other bacteria could be considered significant. For instance, the sequence diversity between two strains of *Escherichia coli* is 1.6% (Perna et al. 2001).

2. 2. Global distribution of major lineages of MTBC

The existence of significant variation among clonal MTBC species has been verified by recent advancements in genome sequencing techniques and molecular tools (Jia et al. 2017; Tafese et al. 2021). They are all derived from the same ancestor, as evidenced by their startling similarity. The circular genomes of *M. africanum* and *M. tuberculosis*, for instance, range from 4.38 to 4.42 Mb, while the largest tubercle bacillus is *M. canettii*, whose genome is 10-115 kb larger. The main phenotypic difference between *M. canettii* and other MTBC species is that the former produces smooth colonies, while the latter produces rough colonies. While *M. africanum* is only found in Africa, and *M. canettii* seems to be limited to the horn of Africa, MTBC is found worldwide (Jia et al. 2017). Human tuberculosis is mostly caused by MTBC. MTBC has been linked to a few reported cases of bovine tuberculosis, despite the absence of an identifiable animal reservoir.

According to Comas et al. (2013), MTBC is currently split into nine evolutionary lineages. *M. canettii*, *M. bovis* (pathogen of cattle), *M. caprae* (pathogen of sheep and goat), *M. microti* (pathogen of voles), and *M. pinnipedii* (pathogen of seals and sea lions) are among the animal-adapted lineages (Firdessa et al. 2013; Coscolla and Gagneux, 2014). Additionally, there are five lineages of *M. tuberculosis* sensu stricto. With the recent

discovery of lineages 8 and 9, the MTBC now has nine lineages altogether (Nguyen et al. 2018; Ngabonziza et al. 2020).

Lineage 1 (Indo-Oceanic) (IO), Lineage 2 (East-Asian, including the Beijing family) (EAS), Lineage 3 (East-African-Indian) (EAI), Lineage 4 (Euro-American) (EA), and Lineage 7 (Ethiopia) (Tafese et al. 2021) are the five human-adapted lineages of *M. tuberculosis*. The novel Lineage 8 was originally identified in Rwanda and Uganda. Lineages 2, 3, and 4 were classified as evolutionary modern lineages, and lineage 7 appears to be intermediate between the ancient and modern lineages (Gagneux et al. 2006; Merker et al. 2018; Tafess et al. 2021). Lineages 1, 5, and 6 were classified as evolutionary ancient lineages, with *M. canetti* as the more ancestral branch. *M. tuberculosis* deletion 1 region (TbD1) is present in the ancient MTBC lineages but not in the contemporary lineages (Tafese et al. 2021) (Figure 2).

It was concluded from previous phylogenetic studies that the strain types are specifically suited to different human populations because the MTBC lineages have a high phylogeographic population structure, as their names suggest. Some lineages and sub-lineages exist globally, while others exhibit strong geographical restrictions (Brosh et al. 2002; Gagneux, 2018). More than 33–73% of all instances are traced back to Lineage 1 (Indo-Oceanic, primarily EAI family), an old lineage that has been reported from East Africa as well as from the Indian Ocean, Southeast Asia, and South Asia. This family makes up 22–25% of all instances and is also present in Oceania, Central Asia, the Middle East, and Northern Europe.

Globally expanding, lineage 2 (East Asian, mainly Beijing family) is a highly drug-resistant and virulent strain of *M. tuberculosis*. More specifically, the Beijing family makes up around 50–85% of all instances and is dominant throughout East and Southeast Asia, as well as the former Soviet Union. According to McHenry et al. (2020), this family is also rather widespread in Oceania, Africa (except from the West), and North America. Less than 10% of instances in other regions of the world, such as the Middle East, Central and South America, India, and Northern Europe, have this lineage. In the Beijing area of China, the Beijing family was found very frequently (Van Soolingen et al.1995). This family is distributed widely over Mongolia, South Korea, Hong Kong, Taiwan, Vietnam, Thailand, and Malaysia. One-third of all *M. tuberculosis* lineages were represented by this lineage.

Nine to thirty percent of all cases, mostly in the South, are found in South and West Asia, where lineage three (East-African-Indian, mainly the CAS family) is concentrated. Additionally, the Middle East and North Africa (7–12% of all cases, mostly in the East) are home to this lineage. Other locations such as Europe, Far East Asia, Oceania, and Central and North America have lower prevalence rates (0.1 - 5% of total cases). This lineage accounts for almost half of all instances and is most prevalent in Pakistan, Iran, and India.

According to Demay et al. (2012) and Coscolla et al. (2014), lineage 4 (Euro-American) is the most prevalent MTBC lineage. Europe is considered to be the lineage's origin (Mokrousov et al. 2016; Brynildsrud et al. 2018). Long-distance trade and colonialism have been credited with the lineage's growth to the Americas, Africa, Asia, and Oceania (Brynildsrud et al. 2018). This lineage is common in regions where co-infection with HIV and TB is highly prevalent. The existing evidence suggests that lineage 4 is more likely to

be associated with PTB (Taye et al. 2021). LAM, T, X, H, and S are five of the ten families represented in lineage 4 that are worldwide in distribution.

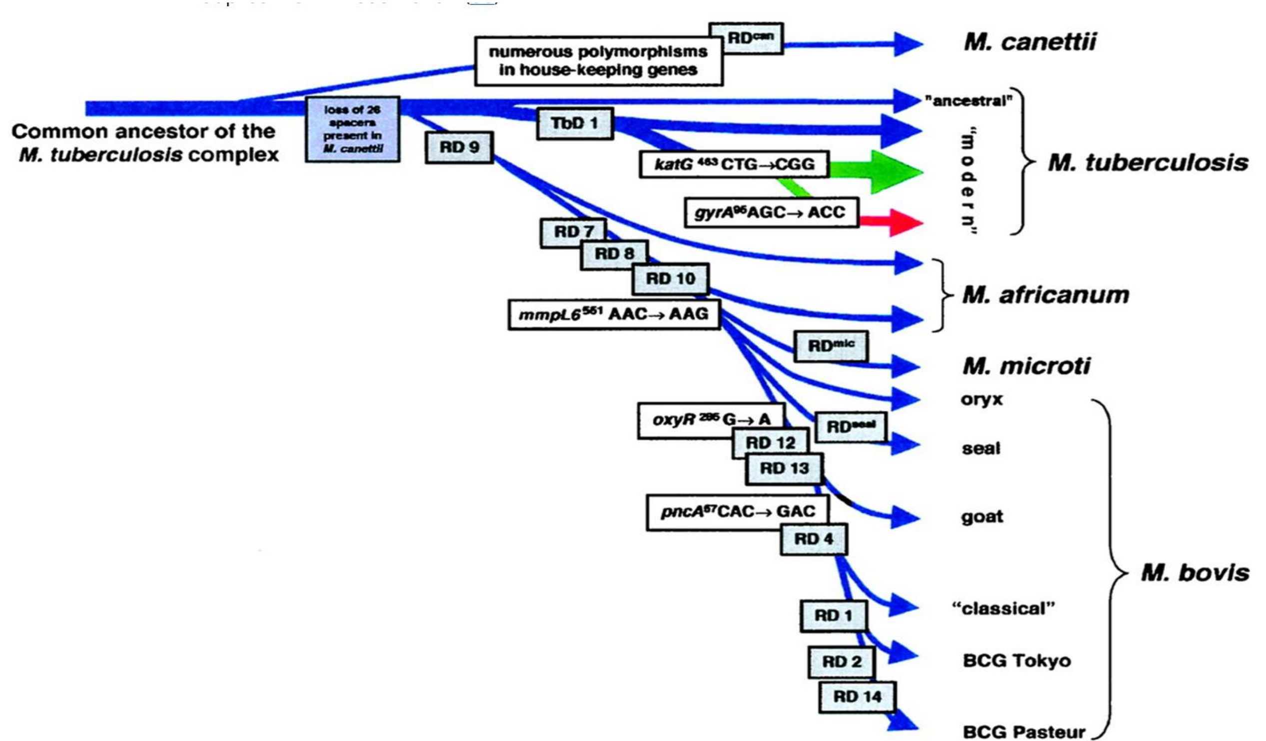


Figure 2: Based on deletions and sequence polymorphisms in five chosen genes—katG, gyrA, oxyR, pncA, mmpl6, and TbD1—the evolutionary pathway of tubercle bacilli is suggested (Barbier and Wirth, 2016).

2. 3. Sub-lineages of MTBC

The most common sub-lineages of the MTBC are EAI, MANU, Beijing, CAS, Haarlem (H), Latin-American-Mediterranean (LAM), T, X, S, Ghana, URAL, TUR, Uganda and H37Rv, AFRI, and West African (Salvato et al. 2019) (Figure 3). There are two groups of strains in the EAI sub-lineage: one group has a high number of IS6110 copies, whereas the other group has a low amount. In East Africa, India, and South East Asia, this heritage is widespread. According to Douglas et al. (2003) and Gagneux et al. (2006), the EAI lineage

may have originated in Asia, where TB may have historically found favorable spreading conditions. Based on the innovative prototypic spoligotyping-signature, Brudey et al. (2006) classified the EAI into four sub-lineages: EAI2-Nonthabuir, EAI6-Bangladesh/1, EAI7-Bangladesh/2, and EAI-Madagascar. A set of Thai strains and the EAI2 clade known as the "Manila family" were merged. It has now been shown that EAI3 and EAI4 are phylogeographically different from Vietnam and India, with the proposed designations being EAI3-IND and EAI4-VNM. Lineages from Bangladesh are unique for the eastern part of South Asia, as evidenced by the fact that they were also found in neighboring Myanmar (Chihota et al. 2018).

According to Singh et al. (2004), the "manu" family is a unique family from India that might represent an ancestral clone of strains from the basic genetic group 1. Three divisions exist within the family (Manu1, Manu2, and Manu3). Manu1 has spacer 34 deleted, Manu2 has spacers 33–34 deleted, and Manu3 has spacers 34–36 deleted, according to the spoligotyping. Medication resistance is significantly more common in people with Manu2 genotypes. It is becoming more and more obvious how important India and Asia, in general, were to the development of TB. Other African locations have also provided documentation of the lineage (Warren et al. 2002; Sola et al. 2001).

There were two sub-lineages that emerged from the previously described Central-Asia (CAS) lineage: CAS1-Delhi and CAS1-Kilimanjaro. Whereas the CAS1-kilimanjaro (SIT21) is located in Tanzania, the CAS1-Delhi type (SIT26) is mainly found in India and the Indian subcontinent (Banu et al., 2004; Filliol et al. 2002). The distinctive spoligotype hallmark is represented by the absence of spacers 4-27 and 23-34, respectively (Brudey et al. 2006). It has been demonstrated that the CAS lineage performs very well in East Africa.

The Beijing genotype is classified as belonging to Sreevatsan's genetic group 1 and has a unique spoligotyping signature that includes the insertion of IS6110 in the genomic dnaA-dnaN locus (Kremer et al. 2004), the presence of at least three spacers 35-43, and the absence of spacers 1-34 (Smith et al. 2006). The Beijing genotype was so named because it was found in over 80% of TB patients in the Beijing area of China (Van Soolingen et al. 1995). Note that the Beijing lineage is made up of extremely drug-resistant and virulent strains of MTBC.

In the Netherlands, the Haarlem (H) lineage was initially described by Kremer et al. (1999). In spoligotyping, the lineage is distinguished by the lack of spacers 29–31 and 33–36. The H lineage is divided into four sub-lineages (H1-H4). According to Brudey et al. (2006), H4 strains might be an intermediary genetic link between the H1 and H3 genotypes. The reported H strains have been detected in Armenia, Austria, Finland, Georgia, Iran, and Russia in excess of 60% of cases. The lineage is also widespread throughout the Caribbean and, to a lesser extent, Northern Europe. Due to human migration and European colonization, H has also been found in Saudi Arabia and Central Africa (Filliol et al. 2003).

The absence of spacers 21-24 in spoligotyping and the presence of an exact tandem repeat (A) allele equal to spacer 2 originally established the Latin-American-Mediterranean (LAM) lineage (Sola et al. 2001). The group has a high level of variety, with 12 subtypes characterized by SpoIDB4 (LAM 1-LAM12) (Filliol et al. 2003). LAM7 was renamed LAM7-Turkey as new findings indicated that SIT41 is prominent in Asia Minor. The LAM10 sub-lineage was also dubbed LAM10-Cameroun. Recently, two additional lineages have been identified: LAM11-ZWE (SIT59) from Zimbabwe and LAM12-Madrid1 (SIT209) from Spain. Although the LAM lineage is most common in

Mediterranean and Latin American countries, it is also becoming more common in Africa and other regions. Based on paleopathological evidence, TB may have existed before to the arrival of the Spanish and Portuguese soldiers to America and it was endemic in either Africa or Latin America before migrating to Europe. Europeans may also have contributed to the introduction of the LAM lineages (Arriaza et al. 1995). There are members of the LAM9 sub-lineage in Africa, Belgium, Italy, and Spain. The lineage is widely known for the South African XDR-TB outbreaks, which claimed 52 out of 53 lives and garnered international attention (Mlambo et al. 2008).

The F28 clade in South Africa has been connected to the "S" lineage, which is prevalent in Sardinia and Sicily (Chihota et al. 2018). Although the origin of this genotype family is unknown, its existence in SpoIDB4 has been verified. According to Sebban et al. (2002), the "X" genotype family is identified by a low copy number of IS6110 and the absence of spacer 18 in spoligotyping. There are three sub-lineages (X1-X3) for this genotype. In the US, the UK, and the former British colonies, the ancestry is widespread. India, Mexico, South Africa, and the Caribbean are all impacted (Chihota et al. 2018).

The "T" families were still poorly described, with almost 600 spoligopatterns remaining unclassified. The lineages were split into five sub-clades (T1-T5) based on changes in single spacers (Chihota et al. 2018). Eight nested clades, excluding "Tuscany," with substantial spoligotyping-signatures were recovered; their names were derived from the nearest upper-clade designation (T1 to T5), then their purported uniqueness in the region. Examples of these are T3-Ethiopia (SIT149), T5-Russia/1 (SIT135), T1-Russia/2 (SIT280), T3-Osaka (SIT627), T5-Madrid/2 (SIT58), T4-Central Europe/1 (SIT39), T2-Uganda (SIT135), and "Tuscany" (SIT1737). In Ethiopia and Denmark, it was previously

found that the SIT 149 was common among immigrants from Ethiopia. The "URAL" family is widely distributed throughout Russia and the former Soviet Union, with isolated cases occasionally discovered outside (Mokrousov, 2012). The disease appears to have adapted to sympatric human populations based on the phylogeographic ranges of *M. tuberculosis* lineages.

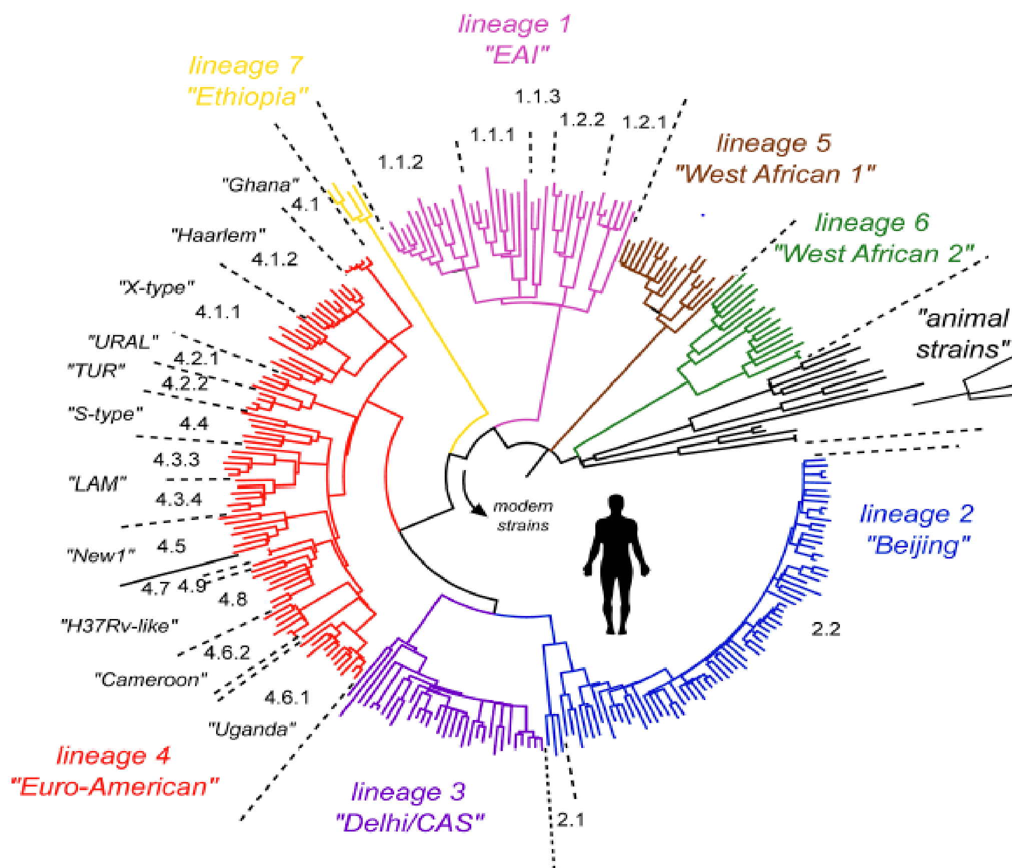


Figure 3: A neighbor-joining tree displaying the phylogenetic structure of the *M. tuberculosis* complex (MTBC) lineages and sub-lineages (Niemann et al. 2016).

2.4. Epidemiology of TB

Second only to COVID-19 and higher than HIV/AIDS, TB is a leading global cause of illness and mortality (WHO, 2022). Since TB is one of the most ancient infectious diseases that affect people, the history of the disease is entwined with human history (Daniel et al. 1994). The bacillus that causes TB, *M. tuberculosis*, has been present for 70,000 years and currently infects over 2 billion people worldwide. Each year, 10.4 million new cases of TB are detected (Raviglione and Sulis, 2016; Barberis et al. 2016). Around one-third of people on the planet are infected with *M. tuberculosis* and could develop active TB at any time (WHO, 2022). If left untreated while contagious, 10% of people infected with *M. tuberculosis* will acquire active TB. By droplet transmission, each individual with active TB infects between ten and fifteen people annually (Raviglione and Sulis, 2016).

For those with HIV, TB is also the leading cause of death. Nowadays, HIV is the primary cause of a quarter of a million TB-related fatalities, with the bulk taking place in Africa (Kwan and Ernst, 2011). In 2022, 167,000 fatalities among HIV patients are anticipated to be caused by TB (WHO, 2023). Even though TB is treatable with readily available medications, the illness claims the lives of 5000 people daily and, in 20 years, is expected to kill 35 million people worldwide if treatment is not received. In 2022, an estimated 10.6 million people worldwide (including 5.8 million men, 3.5 million women, and 1.3 million children) contracted TB, and 1.3 million of those people died from the disease, according to the World Health Organization (WHO) global TB report 2023. People living with HIV accounted for 6.3% of the total. The TB incidence rate (new cases per 100 000 population per year) rose by 3.9% between 2020 and 2022, reversing declines of about 2% per year for most of the past 2 decades. Over 80% of TB cases and deaths take place in low- and

middle-income countries. Although the disease affects the most vulnerable, such as the impoverished and malnourished, 98% of TB deaths occur in developing countries, striking young adults in their most productive years. In 2021, the WHO areas of Southeast Asia (46%), Africa (23%), and the Western Pacific (18%) had the highest number of TB cases (WHO, 2022). In the same year, approximately 87% of new TB cases occurred in the 30 high TB-burden countries, with eight countries accounting for two-thirds of the global total (WHO, 2022), including India (28%), Indonesia (9.2%), China (7.4%), the Philippines (7.0%), Pakistan (5.8%), Nigeria (4.4%), Bangladesh (3.6%), and the Democratic Republic of the Congo (2.9%) (WHO, 2022) (Figure 4). Globally, an estimated 410 000 people developed multidrug-resistant or rifampicin-resistant TB (MDR/RR-TB) in 2022 (WHO, 2023).

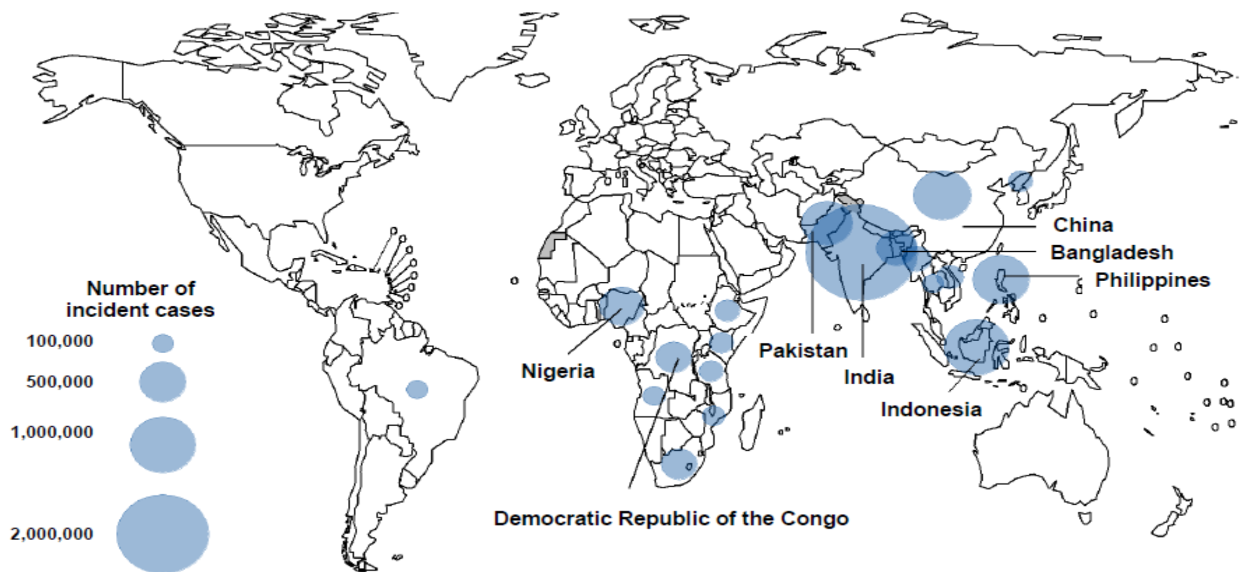


Figure 4: Eight countries with a high percentage of tuberculosis cases in 2021 (WHO, 2022)

Ethiopia is among the 30 countries in the world with the highest burden of TB, MDR-TB, and TB-HIV co-infection, with an estimated 143,000 new cases of TB infections annually

(WHO, 2021). Of the 30 countries with high TB and high MDR-TB burdens, it ranks 12th and 24th, respectively (WHO, 2021). In Ethiopia, the impact of the COVID-19 pandemic resulted in a 2.6% decrease in TB case notifications and a 10% decrease in DR-TB case notifications in 2020 compared to 2019 (WHO, 2021). This information is reported in the WHO 2021 global TB report. About 31% of TB patients in Ethiopia have HIV (Wondifraw et al. 2022). According to WHO (2022), the country's MDR-TB prevalence is currently 1.1% in newly diagnosed cases and 12% in people who have already received treatment. Nonetheless, the national TB incidence has decreased from 192 cases per 100,000 people in 2015 to 119 cases in 2021. Ethiopia is unlikely to reach the End TB objective of reducing TB death rates by 35% by 2035, despite a consistent decline in TB mortality from 26 per 100,000 persons in 2015 to 16 in 2021.

In Ethiopia, there are significant regional and population-based variations in the prevalence of TB. A recent spatial epidemiology study of TB in Ethiopia found significant geographic heterogeneity and clustering. All types of TB including those with bacteriological confirmation have concentrated in particular regions of the nation, such as the Northeast, Southwest, and South (Alene and Clements, 2019). Before this study, nothing had been done in practice to control TB among this vulnerable population, even though the National Tuberculosis Control Program (NTP) recognized homeless individuals as an important population group for TB control.

2. 5. Diagnosis of TB

2.5.1. Sputum smear microscopy

The method of choice for PTB diagnosis is sputum smear microscopy. It is the sole means of diagnosing TB in some remote areas of developing countries (Ben-Selma et al. 2009). The two staining methods that are most commonly used are fluorescent (Auramine-O/Auramine-rhodamine) and Ziehl-Neelsen (ZN) microscopy. Sputum smear microscopy is easy to use and reasonably priced, however, it has a high false negative rate (> 104 bacilli/ml of sputum to acquire a positive result) and low sensitivity, which could be misinterpreted. Notably, sputum smear microscopy is unable to distinguish between living and dead bacteria or *M. tuberculosis* from non-tuberculosis mycobacteria (Ben-Selma et al. 2009). On the other hand, ZN staining can reveal whether or not the bacilli included in patient samples are acid-fast. In some cases, this data is important for analyzing samples from people who have already undergone anti-TB therapy.

2. 5.2. TB Culture

The clinical sample can be used for mycobacterial culture. The gold standard method for diagnosing TB is still culture. Solid Lowenstein-Johnson (LJ) medium is frequently used to cultivate *M. tuberculosis*, allowing for the identification of the isolate and evaluation of its treatment sensitivity (Kenaope et al. 2020). Liquid culture techniques such as the BACTECT MGIT 960 allow for the detection of *M. tuberculosis* in a few days when compared to the LJ method (Hasan et al. 2013). Once the mycobacteria begin to grow in the tube, the automated culture system BACTECT MGIT 960 recognizes the signal of oxygen quenching fluorescence. Because the MTBC grows slowly, most cultures that test

positive for it do so in less than a week, whereas those that test negative for it take eight weeks (Kenaope et al. 2020).

2. 5. 3. Molecular diagnosis of TB

The GeneXpert MTB/RIF (Cepheid) assay is the most widely used detection method in molecular diagnosis of TB. This is a real-time, semi-nested fluorescence PCR that simultaneously detects rifampin resistance and *M. tuberculosis*. According to Boehme et al. (2010), the sensitivity of a single direct MTB/RIF test is 90.2% for smear-negative but culture-positive TB cases and 99.8% for smear-positive patients. 99.2% is the estimated specificity. The Xpert MTB/RIF ultra-developed expands upon the original Xpert MTB/RIF by including a bigger DNA reaction chamber and two distinct multi-copy amplification targets (WHO, 2017). As to Chakravorty et al. (2017), the detection limit of Xpert ultra has been increased to 15.6 CFU/ml, while that of Xpert MTB/RIF was previously 112.6 CFU/ml. Within two hours, this assay may identify rifampin resistance and MTBC DNA in sputum or concentrated sputum deposits (Bodmer and Ströhle, 2012). In December 2010, the WHO endorsed Xpert MTB/RIF for the identification of TB and treatment resistance, specifically in patients with HIV and those suspected of having multidrug-resistant TB. However, primary medical institutions are finding it difficult to meet the aforementioned Xpert MTB/RIF requirements and guarantee the quality of test results due to an increase in demand for skilled testing personnel and accompanying infrastructure (Gidado et al. 2019).

2. 6. Treatment of TB

Anti-TB drugs are used as first- and second-line therapies for TB depending on their effectiveness, side effects, and accessibility. Isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and either ethambutol (EMB) or streptomycin (SM) are the four regimens used as first-line therapy (Carr, 2022) whereas amikacin, kanamycin, thioamides (ethionamide and prothionamide), polypeptides (capreomycin, viomycin, and enviomycin), fluoroquinol (ciprofloxacin, levofloxacin, ofloxacin, and moxifloxacin), cycloserine, and parminosalcylic acid (Lienhardt et al. 2012) are the second-line anti-TB drugs.

Currently, there are two approaches used to cure TB: treatment-based approaches like chemotherapy or prevention-based approaches like vaccinations. The sole approved TB vaccine, Bacillus Calmette-Guerin (BCG), has been in use for more than a century, but it only works against TB in infants and offers minimal protection against TB in adults (McShane, 2011). Adult TB infection rates are still high even in countries where BCG is administered to children (McShane, 2011). The WHO has identified the development of a new vaccine as a major objective, and identifying potential vaccine targets requires a thorough investigation into the genetic foundations of TB. For six to nine months, taking different anti-TB drugs (Table 1) is the most effective strategy to control active TB infection. Each country has its specific regimen, but generally speaking, treatment consists of two months of isoniazid, rifampicin, pyrazinamide, and ethambutol, followed by two months of isoniazid and rifampicin alone (Kerantzas et al. 2017).

Table 1: List of drugs used to treat TB along with genes linked to antibiotic resistance. There is also information on the kind of medication and whether it is first-line (given first) or second-line (given after first line treatments have failed).

Drug	**Known genes with mutations that confer resistance**	**Drug type**	**Level**
Rifampicin (RIF)	RpoB	Rifampicin	First-line
Isoniazid (INH)	katG, inhA*, FabG*, kasA	Analogous to eth	First-line
Pyrazinamide (PZA)	pncA, clpC1, panD	Pyrazine	First-line
Ethambutol (EMB)	embB, embAB, embC	Bacteriostatic	First-line
Streptomycin (SM)	rpsL, gidB, murA	Aminoglycide	First-line
Ofloxacin (OFL)	gyrA, gyrB	Fluoroquilone	Second-line
Kanamycin (KMC)	eis, murA	Aminoglycide	Second-line
Ethionamide (ETH)	ethA, inhA*, fabG*	Analogous to INH	Second-line
Bedaquiline (BDQ)	pepQ, Rvo678, mmpL5, mmpS5, atpE	Diarylquinolines	Second-line
Linezolid (LZD)	RplC, rrl	Linezolid	Second-line

2. 7. Epidemiology of drug-resistant TB

Drug-resistant TB (DR-TB) is characterized by treatment failure of MTB by at least one of the four first-line anti-TB medications (INH, RIF, EMB, and PZA) (WHO, 2019b). When *M. tuberculosis strains* become resistant to one first-line anti-TB drug, it is referred to as mono-resistant TB. All patients with TB disease are treated with INH and RIF, the two most effective first-line TB medications (MoH, 2017; CDC, 2012). Multidrug-resistant TB (MDR-TB) is resistant to multiple anti-TB treatments (INH and RIF). The current guidelines from the WHO classify *M. tuberculosis* strains that are resistant to RIF (RR-TB) as multidrug-resistant TB (MDR-TB) (WHO, 2019b). Pre-XDR-TB, or pre-

extensively drug-resistant TB, is characterized by resistance to INH, RIF, and at least one second-line injectable medication (amikacin, capreomycin, or kanamycin). It is also resistant to RIF and any fluoroquinolone (such as Levofloxacin or Moxifloxacin). Even more hazardous is XDR-TB, which is defined as resistance to INH, RIF, a fluoroquinolone (levofloxacin or moxifloxacin), and either bedaquiline or linezolid or at least one second-line injectable medication (amikacin, capreomycin, kanamycin) (CDC, 2022). Drug resistance that develops during or after anti-TB medication in individuals who have previously had drug-susceptible TB is known as secondary drug resistance, whereas drug resistance in a patient who has never received anti-TB therapy is known as primary drug resistance.

The rise of drug-resistant TB (DR-TB) poses a significant obstacle to ending the disease. It is the primary cause of death due to antimicrobial resistance (AMR) and a danger to the security of international health systems (WHO, 2019b). The burden of DR-TB is expected to rise globally between 2020 and 2021 (WHO, 2022). The COVID-19 pandemic's impacts on TB detection are believed to be the main reason for the increase, which is ascribed to an overall rise in TB incidence between 2020 and 2021. According to WHO estimates, the number of Multi-Drug Resistant/Rifampicin Resistant TB (MDR/RR-TB) incident cases grew by 3.1% to 450,000 globally in 2021 from 437, 000 in 2020. A third of those in need started MDR/RR-TB therapy in 2021, with 161,746 patients starting treatment, and the treatment success rate was 60%, according to reports (WHO, 2022). MDR/RR-TB was anticipated to affect 3.6% and 18% of newly and previously treated cases of TB, respectively in 2021. Nineteen thousand deaths worldwide are estimated to have resulted from MDR/RR-TB in the same year. India (26%), the Russian Federation (8.5%), and

Pakistan (7.9%) had the greatest percentage of incident cases of MDR/RR-TB in 2021 (WHO, 2022). Ethiopia, Kenya, and Thailand have moved off the list of the 30 countries with a high MDR/RR-TB burden, whereas Zambia, Mongolia, and Nepal have joined it (WHO, 2022).

One of the areas impacted by the growth of drug-resistant TB is Africa, especially Sub-Saharan Africa (Migliori et al. 2010). Only in Africa were 26,845 MDR/RR-TB patients and 867 XDR-TB cases reported in 2017. This is because of a lack of resources, the COVID-19 epidemic, a staffing deficit, inconsistent medicine supplies, and the stigma attached to HIV illness (WHO, 2021). Drug-resistant TB is becoming more common in the region due to treatment dropout rates that have increased as a result of this stigma, which is linked to HIV/TB co-infection (WHO, 2020).

Ethiopia is among the 30 countries with the highest burdens of TB, TB-HIV, and MDR-TB (WHO, 2020). While the prevalence of MDR/RR-TB increased in two rounds of DR surveys conducted in Ethiopia, the third survey found that the prevalence of MDR/RR-TB was 1.1% among newly diagnosed cases and 7.5% among those who had already received treatment. As per the WHO's 2022 global TB report, Ethiopia was predicted to have 1,800 MDR/RR-TB incident cases in 2021. According to WHO (2022), the country's current MDR/RR-TB prevalence rates for newly diagnosed and previously treated cases are 1.1% and 12%, respectively. Previous research has revealed some risk factors for MDR-TB, such as inadequate patient DOTS, past TB treatment, and poor adherence to anti-TB medications (MoH 2017). Furthermore, MDR-TB is more common in those with poor socioeconomic status and other vulnerable populations, including the homeless, prisoners, migrants, and refugees (WHO, 2014a).

2. 8. Mechanisms of drug resistance in *M. tuberculosis*

According to Zhang and Yew (2015), there are four main ways that anti-TB medications work: (a) blocking RNA synthesis; (b) blocking protein synthesis; (c) blocking cell wall synthesis; and (d) interfering with the formation of cell membranes. Due to the extensive use of current anti-TB drugs and prolonged treatment, resistant *M. tuberculosis* strains have emerged and are spreading quickly. The general mechanisms of drug resistance to anti-TB medications are listed in Table 2.

Drug resistance in *M. tuberculosis* is mostly caused by mutations in particular genes linked to targets of antibiotics. For example, mutations in the katG gene, such as the Ser315Thr substitution, are commonly associated with resistance to isoniazid (Zhang et al. 1992). Mutations in the rpoB gene are frequently the cause of rifampicin resistance, with the Ser531Leu mutation being particularly common (Telenti et al.1993). Furthermore, resistance to ethambutol is associated with mutations in the embB gene, namely at codon 306 (Billington et al. 199). These genetic changes make first-line TB drugs less effective, which presents serious treatment issues.

Table 2: Drug resistance mechanisms in *M. tuberculosis* (Zhang et al. 2004)

Drug	**MIC (µg/ml)**	**Gene(s) involved in resistance**	**Gene function**	**Role**	**Mechanism of action**	**Mutation Frequency %**
Isoniazid	0.02–0.2	*Kat*G	Catalase-peroxidase	Pro-drug Conversion	Inhibition of mycolic acid	50–95
		*Inh*A	Enoyl ACP reductase	Drug target	biosynthesis and other multiple effects	8–43
Rifampicin	0.05–1	*Rpo*B	β subunit of RNA polymerase	Drug target	Inhibition of RNA synthesis	95
Pyrazinamide	16–50 (pH 5.5)	*Pnc*A	Nicotinamidase/pyrazinamida se	Pro-drug Conversion	Depletion of membrane energy	72–97
Ethambutol	1–5	*Emb*B	Arabinosyl transferase	Drug target	Inhibition of arabinogalactan Synthesis	47–65
Streptomycin	2–8	*rps*L	S12 ribosomal protein	Drug target	Inhibition of protein synthesis	52–59
		Rrs	16S Rrna	Drug target		8–21
		*gid*B	rRNA methyltransferase (G527 in 530 loops)	Drug target		?
Amikacin/kanam	2–4	*Rrs*	16S Rrna	Drug target	Inhibition of protein synthesis	76
Capreomycin		*tly*A	2'-O-methyltransferase			
Quinolones	0.5–2.5	*gyr*A	DNA gyrase subunit A	Drug target	Inhibition of DNA gyrase	75–94
		*gyr*B	DNA gyrase subunit B			
Ethionamide	2.5–10	*eta*A/*eth*A	Flavin monooxygenase	Prodrug Conversion	Inhibition of mycolic acid synthesis	37
		*inh*A		Drug target		
PAS	1–8	*thy*A	Thymidylate synthase	Drug activation	Inhibition of folic acid andiron metabolism?	36

2.9. Drug susceptibility test (DST)

Several phenotypic and genotypic drug resistance detection methods are available; the resistance ratio (RR) approach is the most accurate in evaluating *M. tuberculosis* drug susceptibilities (Heifets and Cangelosi, 1999). When tested in the same experiment, this approach determines the strain's Minimum Inhibitory Concentration (MIC - the lowest concentration of antimicrobial capable of inhibiting growth). Prompt diagnosis and reporting of drug resistance is critical for disease control. The reference strain, *M. tuberculosis* H37Rv, is used as the reference strain. The strain is sensitive if the RR is less than or equal to 2; otherwise, the strain is resistant.

The modified proportion methodology works with automated liquid culture equipment like the BACTECTM MGIT 960 and requires less work than the resistance ratio method. First-line drugs are used to cultivate the strain under investigation, while a growth control strain (1:100 dilution) is grown without them. At least 1% of the bacterial population is resistant to that particular treatment when growth is found in any of the drug-containing tubes either before or at the same time as the growth control (Yusoof et al. 2022). Phenotypic detection is considered the gold standard for identifying antimicrobial susceptibilities. But when the organism has been isolated in culture, this process needs to be carried out over a number of days (Yusoof et al. 2022).

On the other hand, treatment-resistant TB can be promptly identified due to genotypic drug sensitivity techniques. Nucleic acid amplification methods (NAAT) are used in the Line Probe Assay (LPA), MTBDRplus, and GeneXpert MTB/RIF assays to directly identify the most common RIF resistance mutations from patient samples (Rufai et al. 2014).

Conversely, nucleic acid amplification techniques are more coasty and necessitate a high degree of technical proficiency in laboratory operations (Steingart, 2013).

When results from direct sputum smear microscopy are obtained in less than 24 hours, *M. tuberculosis* identification is completed in 10–14 days, and drug sensitivity testing is completed in 15–30 days following specimen collection, a laboratory is generally prepared to perform excellent quality mycobacteriology laboratory techniques (Tenover et al. 1993).

2. 10. Genotyping of MTBC

Traditional sequence-based methods like multilocus sequence typing (MLST) can only offer a limited phylogenetic resolution due to the low DNA sequence diversity of MTBC. Thus, the emphasis of early MTBC genotyping methods was on markers like transposable and repetitive genomic elements. In insertion sequence (S) 6110-restriction fragment length polymorphism (RFLP) (McEvoy et al. 2007), the first fingerprinting technique used to define MTBC was based on the variability in copy number and positioning of IS6110 throughout the genome (Van Embden et al. 1993). Subsequently, techniques based on the polymerase chain reaction (PCR) were developed, including MIRU-VNTR (Supply et al. 2000), spacer oligonucleotide typing (spoligotyping) (Karmerbeek et al. 1997), and region of differences (RD) typing.

Based on repeated genetic components such the VNTRs distributed at multiple loci throughout the MTBC genome and the clustered regularly interspersed short palindromic repeat (CRISPR) at the direct repeat (DR) locus, spoligotyping and MIRU-VNTR typing distinguish MTBC (Figure 5). Repetitive and transposable elements have been employed in molecular epidemiology as highly selective markers due to their rapid alteration (Kato-

Maeda et al. 2011). Nonetheless, convergent evolution is the consequence of the markers' quick evolution, as different MTBC strains independently produce similar fingerprinting patterns (Comas et al. 2009). According to Comas et al. (2009), homoplasy is a limitation on strong phylogenetic findings. Single nucleotide polymorphisms (SNPs) have been found due to exponential increase in whole genome sequences (WGS) of MTBC strains in recent years. These SNPs have allowed for the creation of SNP typing assays for lineage and sub-lineage typing (Stucki et al. 2012).

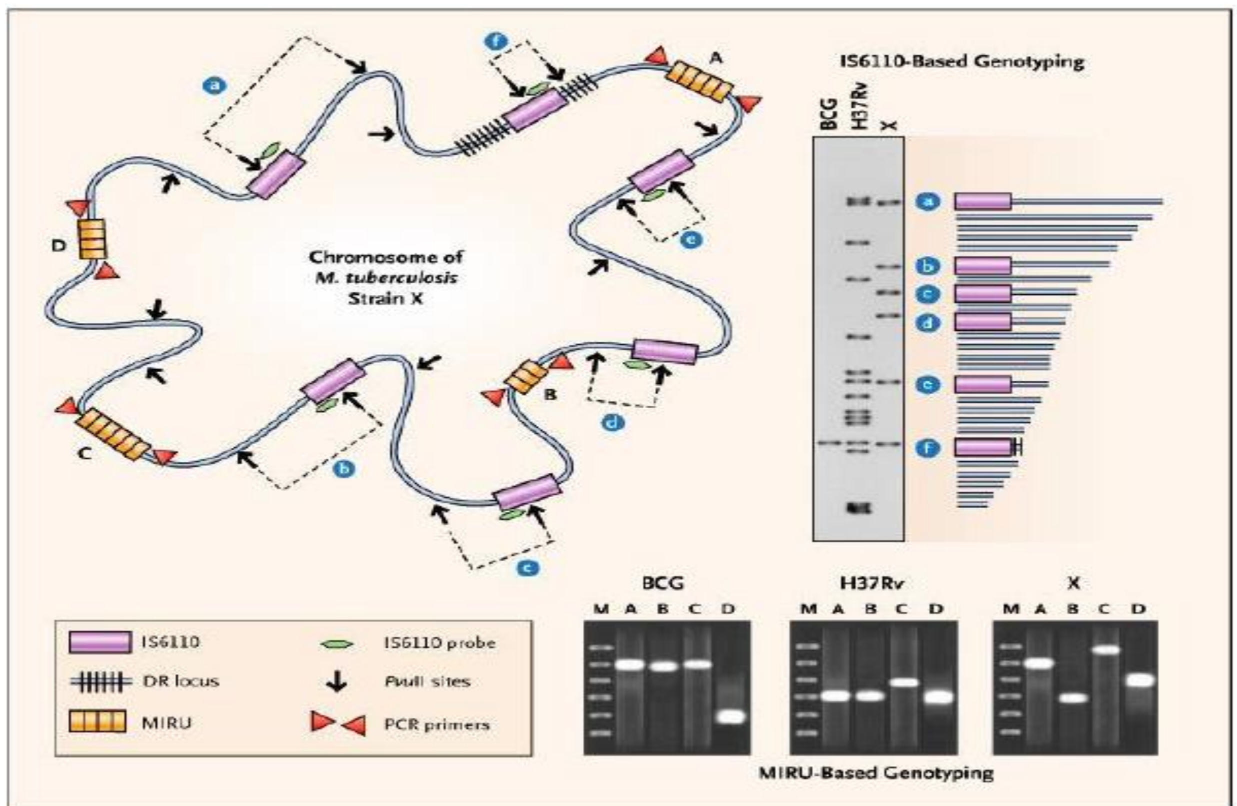


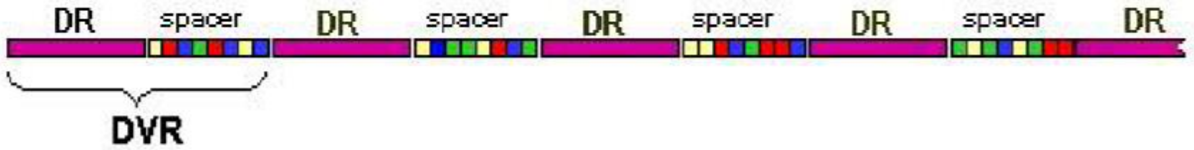
Figure 5: The MTBC genome is used to represent the IS6110, MIRU, and DR regions (Barnes & Cave 2003).

2.10.1. Spoligotyping

Karmerbeek et al. (1997) published the first description of spacer-oligonucleotide typing, or Spoligotyping. This is the first PCR-based genotyping method to detect variation in the direct repeat (DR) sections of the MTBC genome's spacer units. The MTBC genome contains a CRISPR locus called the DR locus, which is made up of multiple nearly identical 36 bp long regions interspersed with non-repetitive "spacer DNA sequences" that vary in length from 35 to 41 bp between each repeat (Hermans et al. 1991). The combination of the DR and the surrounding spacer is known as a direct variable repeat (DVR) (Goguet de la Salmoniere et al. 1997; Figure 6a). The DR locus is exclusive to this complex and exhibits good conservation among MTBC species; it is not present in any other mycobacteria. Although 43 distinct DVRs were first identified, it has now been shown that the MTBC genome may include up to 68 copies, and that the locus's order is substantially conserved (Karmerbeek et al. 1997). Among the 43 distinct spacers, different MTBC strains differ in whether or not they contain a given spacer (Karmerbeek et al. 1997, Vitol et al. 2006). The genotyping of MTBC strains is facilitated by the lack of specific spacers. Variation in the DR regions was thought to result from homologous recombination between adjacent or distinct DRs as well as transformation brought on by the insertion of IS6110, which is almost always present in the DR area (Goguet de la Salmoniere et al. 1997).

The entire DR locus is amplified, and the results are reverse hybridized to identify whether or not individual DVRs exist (Karmerbeek et al. 1997). The genomes of *M. bovis* BCG and *M. tuberculosis* H37Rv were used to choose the 43 spacers that were analyzed in the standardized spoligotyping assay (Figure 6b). In tandem with primers (DRa and DRb) that

target the DRs, the spacers are amplified. To make the PRC products detectable, one of the primers applies a biotin label. The amplicons are then hybridized to an array of capture oligonucleotides immobilized on a nylon membrane (Goguet de la Salmoniere et al. 1997). Following the hybridization process, the membrane is treated with a streptavidin-peroxidase enzyme conjugate. As Goguet de la Salmoniere et al. (1997), streptavidin attaches to the biotin label on the hybridized PCR products, and peroxidase reacts with the ECLTN (Enhanced Chemo-Luminescence Detection Kit) detection reagent to produce light. In order to detect signal, a photographic film is then treated with the membrane. A pattern of white (clear) and black (black) dots appears when the film is developed, with the black dots signifying the presence of a certain spacer and the clear dots indicating its absence (Goguet de la Salmoniere et al. 1997).



(A)

(B)

Figure 6: A portion of the DR locus. The assay for spoligotyping uses 43 spacers. (B). amplified mycobacterial DNAs from 35 *M. tuberculosis* and 5 *M. bovis* strains showed hybridization patterns (spoligotype). The spacers on the filter are arranged in the same order as they are in the genome. As previously mentioned, strains from the Beijing family are represented by the spoligotype 6, 12, and 37 (Karmerbeek et al. 1997).

Binary code is created from the raw data, where "0" denotes clear and "1" represents black. Another popular format is the octal code. The process involves breaking down the binary code into groups of three digits, then replacing each group with a single cipher that ranges

from "0" for binary "000" to "7" for octal "111" (Figure 7). Strains are grouped into clades and positioned in phylogenetic trees according to their genetic diversity.

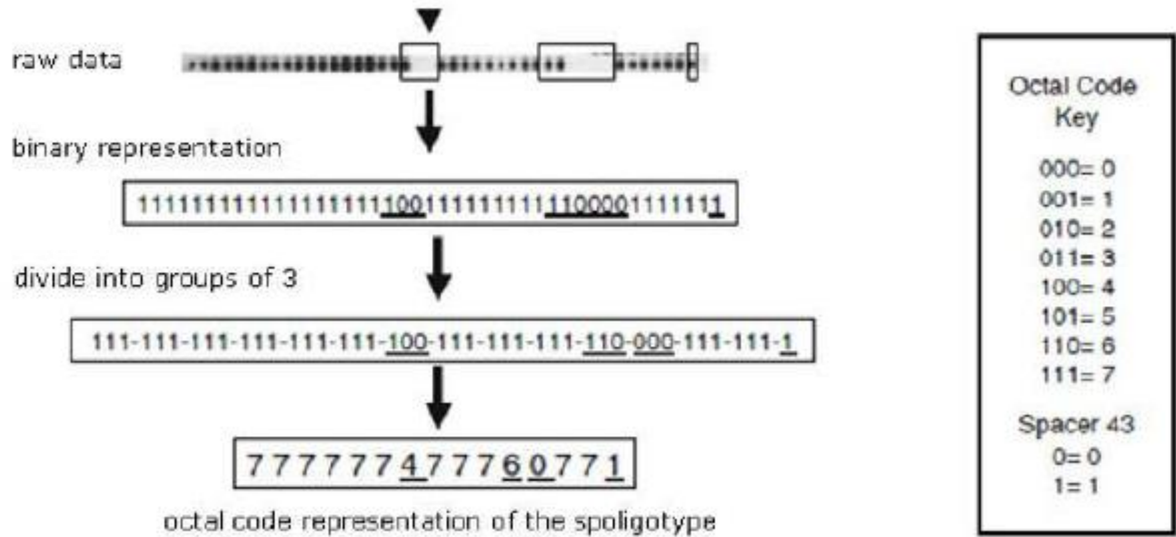


Figure 7:Conversion of raw spoligotyping findings into binary and octal code (Driscoll, 2009).

The spoligotyping results are easy to comprehend and contrast between different labs. A global spoligotyping database (SpolDB4) was made available in 2006. Online at http://www.pasteurguadeloupe.fr:8081/SITVITDemo/, the database lists 3,370 orphan types and 1,939 STs (shared types, or Spoligotype patterns shared by two or more isolates) from a total of 39,295 MTBC isolates from 122 countries that were temporarily classified into 62 clades/lineages (Brudey et al. 2006).

Because of its sensitivity, spoligotyping can be used on clinical samples right away without the need for prior culture. Its minimal genomic DNA requirement is estimated to be 10 fg of chromosomal DNA, which is comparable to the DNA from 2-3 bacterial cells. While the accuracy of the approach for detecting MTBC from clinical specimens alone is 98%

and 96%, it is 97% and 95% for identifying and genotyping *M. tuberculosis* strains using AFB-positive slides directly and from clinical specimens or mycobacterial cultures, respectively. Additionally, it has been demonstrated that Spoligotyping works well for typing on tissue slices embedded in paraffin, Ziehl-Neelsen smear slides, and nonviable cultures (Kulkarni et al. 2005). When applied to non-tuberculous mycobacteria, spoligotyping is ineffective, indicating that the method is only specific to MTBC (Goguet de la Salmoniere et al. 1997). The process takes less time, and it's being used extensively in labs all around the world. Nevertheless, the technique's primary drawback is that unrelated strains may display similar patterns, making it occasionally insufficient to identify epidemics in communities (Goguet de la Salmoniere et al. 1997). Because it only targets one genetic locus the "Direct Repeat" (DR) region, which makes up less than 0.1% of the MTBC genome it is less discriminating than IS6110 RFLP typing (Cronin et al. 2001). Spoligotyping alone is inadequate for epidemiological linkage studies due to its limitations (Goguet de la Salmoniere et al. 1997). Nonetheless, in light of the global TB pandemic, spoligotyping in conjunction with MIRU-VNTR offers a potent molecular epidemiological tool (Cannas et al. 2016).

2. 10.2. MIRU-VNTR

A novel minisatellite-like structure known as MIRU was identified by Supply et al. (1997) in the MTBC genome between 40 and 100 bp repetitive sequences. After more investigation, it was discovered that these structures are spread out over 41 locations in the *M. tuberculosis* H37Rv genome, 12 of which have polymorphisms in the copy number of unrelated MTBC isolates (Supply et al. 2000). Supply and his colleague (2000) developed

a fast and repeatable 12-locus MIRU-VNTR-based genotyping method. However, its discriminatory power was not as high as that of IS6110-RFLP. A 24-loci MIRU-VNTR typing approach was proposed as the new gold standard for molecular epidemiological investigation of *M. tuberculosis*, with a high discriminatory subset of 15-loci MIRU-VNTR (Figure 8). This showed higher discriminating power when combined with spoligotyping than the first 12-loci MIRU-VNTR and even higher than RFLP (Alonso-Rodrguez et al. 2008). When evaluating MTBC isolates and the 24-loci typing method as a high-resolution tool for phylogenetic investigations, it was shown that a specific subset of 15 loci had 96% of the total resolution power of the entire 24-loci methodology with identical predictive value (Figure 9). Hunter-Gaston discriminating index (HGD) increased from 0.895 (12-loci) to 0.920 (24-loci) in a study comparing the discriminatory strength of the 12 and 24-loci MIRU-VNTR typing approaches (Li et al. 2009).

With 78.5% concordance between RFLP and MIRU-VNTR typing, RFLP was found to have marginally higher discriminating power than MIRU-VNTR typing. Nevertheless, additional polymorphic G-C rich sequence (PGRS) RFLP typing was used for strains carrying five copies of IS6110 (de Beer et al. 2013). Automated 24-loci MIRU-VNTR typing can be performed on cell lysate; it requires less DNA and yields easily interpreted findings, making data exchange for inter-laboratory comparisons easier. High-throughput methods like spoligotyping and IS6110-RFLP typing are simpler to use when 24-loci are used (Oudrghiri et al. 2018). Therefore, it is better to use a combination of spoligotyping and 24-loci MIRU-VNTR typing techniques rather than just one to genotype MTBC.

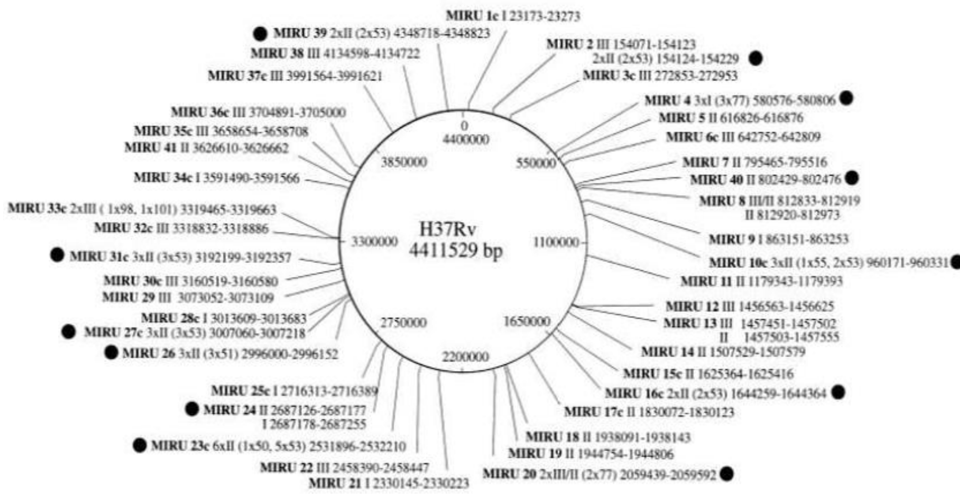


Figure 8: Tandem repeat variability

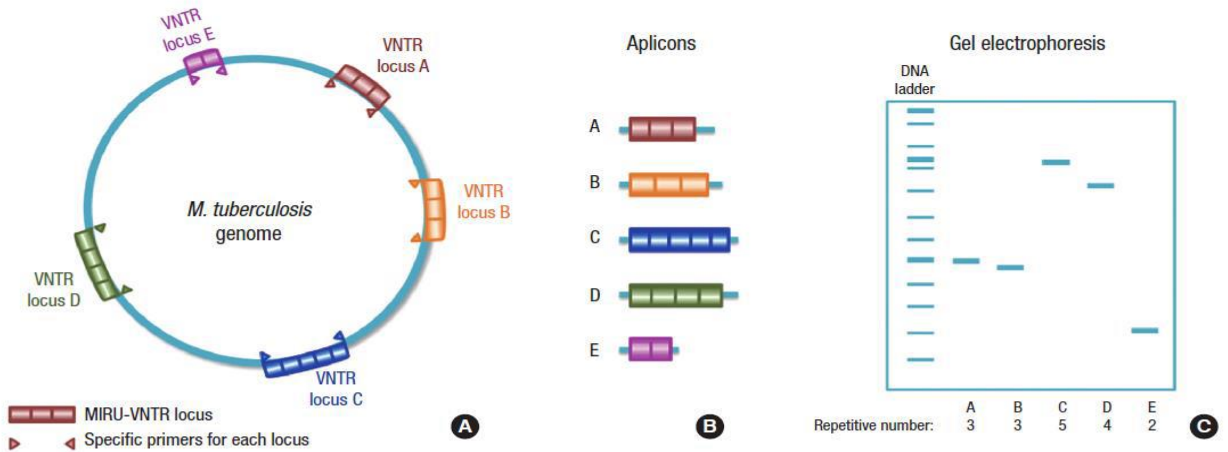
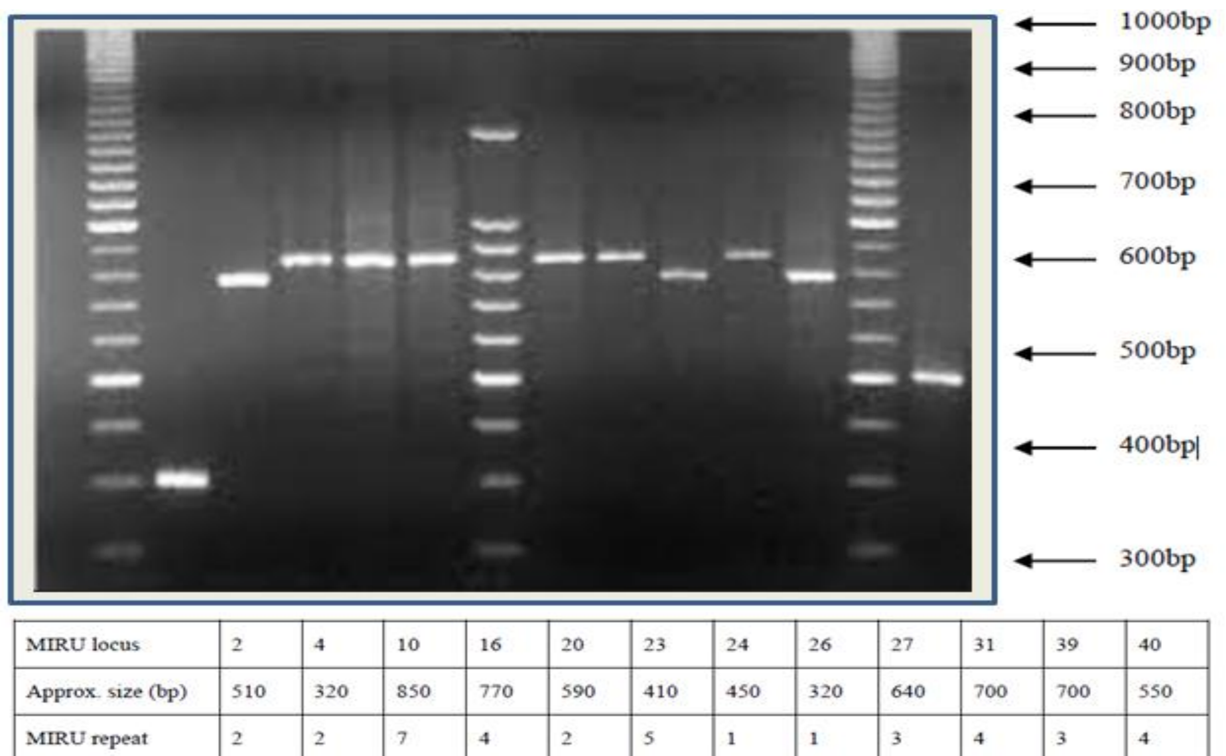


Figure 9: MIRU-VNTR genotyping principle. (A) Specific primers are used for each MIRU-VNTR site, which are dispersed throughout the *M. tuberculosis* genome and have varying repeat numbers. (B) Variations in amplicon sizes following PCR. (C) Following gel electrophoresis, amplicons with varying sizes that represent the repeated number of each VNTR locus can be seen (Ei et al. 2016).

The *M. tuberculosis* 24-loci genome were selected for MTBC genotyping because sequence analyses of 12, 15, and 24-loci revealed that they have greater differences in

tandem repeat copy numbers (Ei et al. 2016; Jagielski et al. 2014). Using specific MIRU primers, each MIRU-locus is amplified by PCR in the MIRU-VNTR procedure. The amplicon sizes are then ascertained by gel electrophoresis (Supply, 2005). As a result, each locus under study has a repetition number that is represented by a multi-digit numerical code known as the MIRU-VNTR code (Figure 10). According to Allix-B'eguec et al. (2008), this coding technique facilitates data deposit in international databases over the Internet for extensive epidemiological and population genetic research. It also makes it simple to compare results across laboratories globally. Moreover, handling a large number of strains is made simpler by typing by comparison of these numerical codes (Han et al. 2007).



MIRU locus	2	4	10	16	20	23	24	26	27	31	39	40
Approx. size (bp)	510	320	850	770	590	410	450	320	640	700	700	550
MIRU repeat	2	2	7	4	2	5	1	1	3	4	3	4

Figure 10: Loci MIRU amplicons with a 1000 bp ladder and Ethidium bromide staining on an agarose gel (Han et al. 2007).

2.11. Genotyping method's discriminatory power for MTBC

The ability of molecular markers to discriminate is closely correlated with each marker's genetic stability. It has been discovered that there are slight variations in the DNA fingerprint patterns of *M. tuberculosis* strains that have been obtained from patients on different dates as well as from epidemiologically linked TB cases (Yeh et al. 1998). Whole Genome Sequencing (WGS) appears to offer the best resolution of *M. tuberculosis* isolates in molecular epidemiological investigations when comparing the discriminatory power of genotyping approaches, but it also comes with a higher technical difficulty (Cannas et al. 2016) (Figure 11).

The developed world, where TB is less clinically significant, has access to the most sophisticated and advanced procedures for identifying *M. tuberculosis*, while developing nations, where TB is more common, have extremely limited or no access to these techniques. Thus, research facilities in poor countries with high disease burdens, like Ethiopia, should have access to cutting-edge molecular tools like Whole Genome Sequencing and MIRU-VNTR, which are standard procedural components in developed countries.

Figure 11: The various techniques for molecular typing *M. tuberculosis* exhibit varying characteristics regarding technical difficulties and the ability to resolve genetic distances between the strains under study (Cannans et al. 2016).

2.12. TB control in Ethiopia

Early in the 1960s, Ethiopia built sanatoriums and TB hospitals in three of its cities. The national TB control program (NTCP) central office (CO) was founded in 1976. In 1992, the government used the WHO's direct observation short course therapy (DOTS) technique following a fruitful pilot study in the South East Ethiopian zones of Arsi and Bale (MoH, 2017).

The prevention and control of TB was given top priority in Ethiopia's Health Sector Transformation Plan (HSTP) for the years 2016–2020. The country's National TB Strategic Plan fully incorporates the "Milestones" and "Targets" of the WHO End TB Strategy. Improving individual service access through the more than 40,000 Health Extension

Workers (HEWs) spread throughout the state was a key goal. The HEWs received training to help patients with a range of ailments, including those exhibiting indications of TB, obtain access to diagnostic and treatment facilities (WHO, 2014a).

Over the past few decades, Ethiopia has made significant strides toward TB control and has met the MDG target. However, the yearly projected TB cases were over 143,000, showing that attaining the MDGs aim alone is insufficient for TB control in Ethiopia, given the prevalence of 119/100,000 population in 2021 (MoH, 2021). The successful implementation of the DOTS and Stop TB strategies through the massive expansion of the DOTS center, the inclusion of TB prevention and control in the Health Extension Program (HEP), the effective participation of private health care providers in TB care and control activities, the effective collaborative implementation of the HIV/AIDS and TB/HIV program, and the strong commitment by all partners and stakeholders in response to the TB epidemic were some of the factors that helped Ethiopia meet its MDG targets.

The five-year TB National Strategic Plan (TB-NSP), which runs from 2021 to 2026, is now being revised by the NTP. By concentrating on the following initiatives, the TB-NSP hopes to lower TB incidence and mortality from 151 cases per 100,000 and 22 deaths per 100,000 in 2018 to 91 cases per 100,000 and 7 deaths per 100,000 by 2026:

- ❖ Scaling-up the use of rapid molecular diagnostics for routine screening;
- ❖ Engaging all care providers in TB diagnosis and care;
- ❖ Prioritizing reaching vulnerable populations (like refugees, prisoners, miners, slums etc.);
- ❖ Decentralization of TB care and treatment;

- Mitigating the catastrophic cost of TB care on patients and their households;
- Increasing contact screening coverage and integrating TB preventive treatment (TPT) in
- community-based and other types of health services;
- Mitigating TB-related stigma in the community and healthcare settings and monitoring
- progress through regular assessment; and
- Proactively finding ways to manage and mitigate potential COVID-19 impacts (MoH, 2021).

2.13. Homelessness and associated factors

The European Federation of National Organizations working with the homeless (FEANTSA) defines homelessness as "people living in a place of habitation that is below a minimum adequacy standard, and lacking access to adequate housing" (Donnelly et al. 2019), despite the fact that there is no widely accepted definition of homelessness (Rodriguez-Moreno et al. 2021). ETHOS identified many forms of homelessness, including living on the streets, insecure housing, and inadequate housing (FEANTSA, 2007; Echenberg and Jensen, 2012; Dworsky et al. 2013). The ETHOS conceptual frameworks consider a person's deviance, imperfections, and housing situation. According to ETHOS, homelessness is a form of social exclusion (FEANTSA, 2007). Being shut out of social, political, economic, and religious spheres of society is referred to as social exclusion, and it is necessary for social integration on an individual and family level. According to Echenberg and Jensen (2012), those who are socially excluded are oftentimes

separated from other members of their community in terms of social, economic, and physical aspects.

Individual and socio-structural elements come together to generate homelessness (Anderson and Christian, 2003; Piat et al. 2015). Among the structural causes are a lack of affordable housing options, social isolation, unemployment, poverty, and unsuitable housing (Shinn, 2010; Crane et al. 2014). According to Nooe and Patterson (2010), individual factors include traumatic events, familial conflict, poor physical and mental health, and substance addiction. According to Crane et al. (2014), homelessness is therefore seen to result from both structural and personal factors that make a person incapable of overcoming hardship.

Globally, it is anticipated that over 100 million people will be homeless and that 1.5 billion will live in substandard housing (Donnelly et al. 2019). According to several studies (Ayano et al. 2017; Groenewald *et al*., 2018; Ayenew et al. 2020; Shamebo et al. 2023a), homelessness is becoming a more significant socioeconomic issue in Africa. Inadequate housing solutions have led to a rise in homelessness and poorly maintained informal settlements on the periphery of the metropolitan area. 75% to 99% of people live in subpar housing in several African cities (Marutlulle, 2021). Approximately 20% of all homeless people worldwide reside in this region (Marutlulle, 2021). It is estimated that about 500,000 individuals in Ethiopia are involved in street life. Between 60,000 and 100,000 homeless people live on the streets of Addis Ababa, the capital of Ethiopia, according to Shamebo et al. (2023a).

Several risk factors, such as delayed diagnosis, healthcare barriers, HIV/AIDS, malnutrition, alcoholism, smoking, diabetes, and drug abuse, increase the likelihood that homeless people will get *M. tuberculosis* and develop active TB (Semunigus et al. 2016; Aldrige et al. 2017; Rogans-watson et al. 2020; Crosby et al. 2023). Moreover, homeless people regularly congregate in places where there is a significantly higher risk of tuberculosis transmission and use homeless shelters (Tibbetts et al. 2020).

Since the early 20th century, TB has been acknowledged as a significant problem for the homeless population. According to international studies, the death rate for homeless people is four times higher than that of the general population. It is widely known that secure housing is linked to excellent health (Nordentoft and Wandall-Holm, 2003). The prevalence of tuberculosis (TB) among the homeless can be up to 20 times greater in wealthy nations than in the general population (Dolla et al. 2017). Moreover, homeless people are predicted to be 10 to 85 times more likely than the general population to have a latent or active TB infection. TB is the third most common cause of illness among homeless people (Tankimovich, 2013; Ranzani et al. 2016; Scholze et al. 2022). A thorough analysis involving data from multiple nations revealed a higher incidence of tuberculosis among the homeless (Beijer et al. 2012). 2.6% of homeless people in northern Ethiopian cities had smear-positive PTB (Semunigus et al. 2016). Resistance to anti-drugs TB is a serious problem for those who are homeless. For instance, research among homeless people in the United States, Korea, and London revealed higher prevalence of drug-resistant tuberculosis (2.7%, 11.5%, and 6.5%, respectively) (Haddad et al. 2005; Heo et al. 2012; Hernández Sarmiento et al. 2013). A higher percentage of previous anti-TB treatment defaulters may be the cause of this.

On the other hand, Haarlem, LAM, and T sub-lineages have been found in earlier genetic epidemiology investigations among homeless people (Hernández Sarmiento et al. 2013). This implies that different strains of MTBC could infect homeless individuals worldwide. In addition, higher rates of strain clustering have been reported in Hungary (Lukács et al. 2004), Colombia (Hernández Sarmiento et al. 2013), Los Angeles (Barnes et al. 1996), France (Bernard et al. 2012), and San Francisco (Moss et al. 2000), suggesting recent transmission of TB among homeless people. According to Bamrah et al. (2013), there was an overrepresentation of homeless people in genotype clusters that suggested local transmission. Ethiopia lacks baseline data despite the prevalence of TB among homeless people. Therefore, the goal of this study was to determine the incidence of PTB and related risk factors in addition to evaluating the treatment susceptibility profiles of MTBC strains that were isolated from Addis Ababa, Ethiopia, homeless people.

3. MATERIALS AND METHODS

3.1. Study setting

A cross-sectional study was conducted in Addis Ababa city, the capital of Ethiopia, between February 2019 and December 2020. The city is located on a plateau in the geographic center of the country at an elevation of 2408 meters above sea level. It has a total area of 530.14 square kilometers. Administratively, the city is divided into 10 sub-cities and 100 kebeles (districts) (Figure 12). The current estimated population of the City is 4,851,253, with an annual growth rate of 3.8% (CSA, 2019; Haile et al. 2020). About 52.4% of the population is female, and 70% fall within the age range of 15-59 years old (CSA, 2019). The city is inhabited by diverse Ethiopian ethnic groups and only 1% of the population is foreign. It is the fastest-growing city in Africa (Ozlu et al. 2015). Addis Ababa city has the highest rural-urban migration accounting for 40% of its population growth. The migration has contributed to the increased number of homeless individuals in the city. To support homeless individuals, the Addis Ababa city Administration has established temporary shelters in the city during the study period. The temporary shelters were established in six sub-cities such as Arada, Yeka, Bole, Akaki-Kality, Chirkos, and Addis Ketema. It provided meals, bedding, clothing, health care services, and counseling. The shelters served as a center for the recruitment of the study participants.

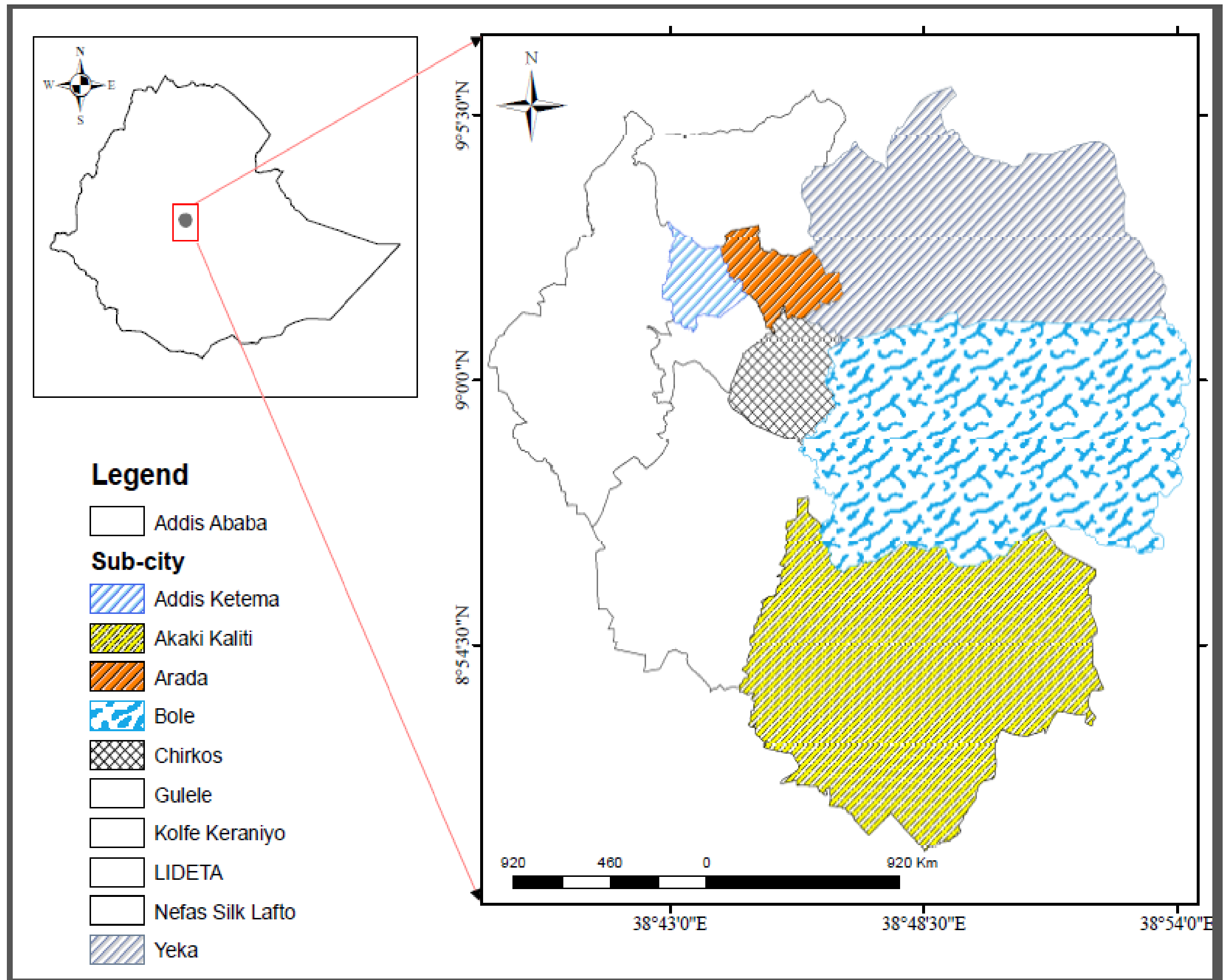


Figure 12: Map of the Addis Ababa study locations, or sub-cities, where study participants were recruited by setting up temporary shelters for the homeless (Mapped by Challa Adugna).

3.2. Recruitment of study participants

This study defined homeless individuals as those living on the streets. Voluntary homeless individuals were enrolled from the streets of Addis Ababa through the outreach program to get temporary shelter services established by the city's government. The outreach program was carried out by an outreach team that encompasses health professionals, social workers, and voluntary community workers. A snowball sampling technique was used to enroll homeless individuals. Enrollment was conducted in the early morning and afternoon when homeless individuals were expected to be visible on the streets. Then, homeless individuals were brought to the established temporary shelters, sensitized, and screened for eligibility and symptoms of PTB before admission to the shelters. Age greater than 18 years, voluntary to participate in the study, being homeless for at least 6 months, and not on anti-TB treatment were included in the study and screened for PTB based on the standard TB symptom screening documents of the WHO guidelines upon admission to the shelters. The study excluded individuals below 18 years to focus on the adult homeless population, which is more likely to have prolonged exposure to TB risk factors and present with active disease. The ethical and logistical challenges of obtaining consent and working with below 18 were significant considerations. Despite the crucial importance of sample size determination for the generalization of findings, this study did not calculate the sample size. Instead, the intention was to enroll as many eligible homeless individuals as possible to ensure sufficient power to detect significant findings.

3.3. Data collection

Pre-structured questionnaires were administered by trained health professionals to collect pertinent data. The questionnaire included sociodemographic data (age, gender, total duration of homelessness, previous residence, living in groups, educational background, and marital status) and TB risk behaviors (cigarette smoking, alcohol consumption, chewing khat, drug abuse, HIV infection, and malnutrition) and TB specific information (history and symptoms of TB, history of imprisonment, history of contact with chronically coughing individuals, and history of contact with TB patients) (WHO, 2013). Trained laboratory technologists conducted laboratory investigations using AFB, Xpert MTB/RIF, TB culture, and DST. The principal investigator of the study has closely supervised overall screening procedures, sample collection, and laboratory investigations. Sensitization was carried out before the admission of temporary shelters in cooperation with the labor and social affairs department and the health bureau of the Addis Ababa City administration. Additionally, before being screened for PTB symptoms upon admission to the temporary shelters, homeless individuals were informed of the study's objective. The following symptoms were used to identify presumed PTB cases: cough for more than two weeks (score = 2), sputum production (score = 2), loss of weight in the previous three months (score = 1), appetite loss (score = 1), and chest pain (score = 1). These criteria were based on the WHO PTB symptom screening guidelines (Maher et al. 1998). Those who were homeless and had a score of at least five or had received anti-TB treatment within the previous five years were considered to have a positive screen.

Every homeless individual whose PTB symptom screened positive was invited to participate in the study and asked to answer a set of standard questions about

sociodemographic characteristics and risk factors related to TB. Next, the participants were requested to provide two sputum samples in sterile falcon tubes for laboratory investigation of *M. tuberculosis*. The first sample of sputum was collected on the spot and sent to Ras-Desta Memorial Hospital, Tirunesh Beijing Hospital, Zewuditu Memorial Hospital, and St. Peter Hospital for ZN sputum smear microscopy and Xpert MTB/RIF investigation (Gu et al. 2015; MoH, 2016) in a cold box. The second sputum sample was then collected early the next day (morning sputum), transported in a cold box to the TB Laboratory of Aklilu Lemma Institute of Pathobiology (ALIPB), Addis Ababa University on the day of collection, and stored at 2-8°C until mycobacteria isolation was performed as previously described (Parija, 2013). Both sputum samples were cultured at ALIPB to isolate *M. tuberculosis*. The participants were given guidelines on how to collect appropriate samples of sputum. At the shelter clinics, sputum samples were taken and entered into the registration log book. All methods were performed in accordance with the relevant guidelines and regulations. Every data-collecting form was verified for legitimacy by the lead investigator. Homeless individuals who had tested positive for TB were also tested for HIV.

3.4. Laboratory procedures

Laboratory testing procedures and sputum specimen handling were carried out in accordance with the Standard Operating procedures (SOPs) of ZN sputum smear microscopy, Xpert MTB/RIF assay, and LJ sputum culture (WHO, 2014b; Tadesse et al. 2017; Kent and Kubica, 1985).

3.4.1. Sputum smear microscopy

Sputum smear microscopy was performed in the hospital's laboratory with the ZN staining procedure, as previously reported by (Ben-Selma et al. 2009). In a nutshell, a microscopic smear was created by placing a drop of the re-suspended sputum specimen on a microscope slide. After the smear was heat-fixed to kill the microorganisms, it was placed on a staining rack over a sink. Approximately 1 milliliter of carbon fuchsin was applied to the slide, and it was heated for 5 minutes until a smear developed. The slide was then labeled to empty the solution and give it good forceps wash in water. A 3% acid-alcohol solution was applied to the slides completely, and it was allowed to work for five minutes. After flooding the slides with the methylene blue solution for a minute, water was used to wash them. The slides were air-dried and examined under a light microscope to see if AFB was present after one last rinsing. Bacillus with a blue background and a pink or red tint were thought to be positive for AFB. If a second reader saw and confirmed that there was one or more AFB for every 100 immersion fields, the slide was considered positive.

3.4. 2. Gene Xpert MTB/RIF assay

AFB-negative sputum specimens were all subjected to further testing with the GeneXpert MTB/RIF assay. Briefly, the Ethiopian Federal Ministry of Health (FMoH) recommended that the sputum specimens be processed with a sample reagent that contained isopropanol and NaOH (MoH, 2016). After that, the treated samples were put into the cartridge, which was then inserted into the GeneXpert device under the manufacturer's instructions (Davis et al. 2013). Within two hours, the process generates mutations in the RNA polymerase

beta (repoB) gene in the *M. tuberculosis* genome by identifying *M. tuberculosis* DNA using fluorescent markers known as molecular beacons.

3.4.3. *M. tuberculosis* isolation

A sputum culture was performed in the ALIPB TB laboratory. Briefly, the sputum sample was decontaminated as previously described (Stinson et al. 2014) by mixing it with an equal volume of 4% N-acetyl- L-cysteine-NaOH (NaLC-NaOH). The remaining space was filled with phosphate buffer saline (PBS) which is composed of disodium/potassium hydrogen phosphate and sodium/potassium chloride at PH~ 7.4. The mixture was centrifuged for 15 minutes at 3000 rpm at room temperature (25ºC). Using phenol red as an indicator, 2N HCl was used to neutralize the sediment after the supernatant was decanted. It was thought that a change in color from purple to yellow indicated that the neutralization had been achieved. After that, the suspension was inoculated onto two sterile LJ medium slopes that were either supplemented in 0.75% pyruvate or 0.6% glycerol. After that, the inoculated medium was incubated for one week at 37°C when slanted, and for eight weeks while standing up. The incubated media were examined for possible contamination at 24, 48, and 72 hours. Every week until the eighth week of culture, the bacteria's growth was observed. A culture that showed no growth or colony after eight weeks of incubation was considered negative.

After a week of incubation, any visible growth or colony was confirmed using ZN microscopy and sub-culturing on blood agar. When these strains were ZN microscopically positive and showed no signs of growth on blood agar, they were classified as members of the *Mycobacterium* genus. Following their removal from LJ media, AFB-positive isolates were inoculated into two different cryotubes, one holding one milliliter of freezing media

and the other 0.3 milliliters of distilled water (dH2O). For one hour, isolates in deionized water were subjected to heat killing in a water bath set at 80°C. Following that, both isolates were kept at -80°C until MIRU-VNTR typing, RD9, DST, and spoligotyping could be completed.

3.5. Drug sensitivity test

Drug sensitivity test for *M. tuberculosis* isolates was performed by standard phenotypic method at the National TB Reference Laboratory, Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia. According to its handbook (Becton Dickinson, USA), a BD BACTECTM MGITTM 960 system protocol was used to perform the DST (Rüsch-Gerdes et al. 2006). Briefly, following the liquefaction of the frozen MTBC isolates, 100µl of aliquots were added to 2 milliliters of phosphate buffer saline (PBS). The samples were then ready for decontamination using equal volumes of a 2.9% sodium citrate and a 4% sodium hydroxide (Na-citrate-NaOH) solution. A decontaminated suspension was cultivated using the MGIT 960 technique. The antibiotic medications from the BD BACTECTM MGITTM 960 SIRE kit and PZA kit were lyophilized and reconstituted in dH2O. The final concentrations utilized were ethambutol (EMB) 5 µg/ml, isoniazid (INH) 0.1 µg/ml, rifampicin (RIF) 1 µg/ml, PZA 100 µg/ml, and streptomycin (STM) 1 µg/ml. Each isolate had a drug-free growth control (GC) tube, and the software algorithm of the BD BACTECTM MGITTM 960 system determined the relative growth ratio between the drug-containing tube and the GC tube. The DST results were given qualitatively. Quality control was maintained by testing the batch of MGIT medium, SIRE kit, and PZA kit using the *M. tuberculosis* H37Rv strain.

3.6. DNA Extraction and typing of M. tuberculosis isolates

3.6.1. DNA Extraction

Extraction of DNA of the *M. tuberculosis* isolates was performed in ALIPB, TB Laboratory, Addis Ababa University, Ethiopia. Briefly, a loop full of freshly grown bacterial colonies was boiled in 100 µL of distilled water at 80°C for 60 minutes to extract the DNA of *M. tuberculosis* isolates. The extracted DNA samples were kept at -80ºC until molecular characterization was performed by using Region of difference (RD)-9, spoligotyping, and MIRU-VNTR typing.

3.6.2. Region of difference-9 (RD9) based polymerase chain reaction

As previously mentioned (Grange and Zumla, 2009), heat-killed cells were exposed to Region of Difference (RD) 9-based PCR to differentiate between MTBC strains and other mycobacteria, such as non-tuberculosis mycobacteria (NTM). Briefly, in the PCR reaction, three primers RD9 flankF, RD9intR, and RD9 flankR were used. The mixtures were amplified using Thermal Cycler PCR equipment (VWR thermal cycler, UK). Briefly, the 20µL PCR mixture contained 2µL of heat-killed DNA template, 7.1µL of distilled water, 0.3µL of each of the three primers, and 10µL of HotStar Taq Master Mix (Qiagen, UK). The PCR reaction was heated to 95°C and 72°C for one minute each. Following a 10-minute holding period at 72°C, the product was removed from the thermal cycler and put through agarose gel electrophoresis. To perform gel electrophoresis, 8 µL of PCR products and 2 µL of loading dye were mixed, applied to a 1.5% agarose gel, and electrophoresed at 110 volts and 400 mA for 35 minutes. After that, the gel was seen using a UVP photodoc

imaging system. The band sizes were compared to the standard 100 bp ladder using a photo camera. Distilled water was used as a negative control, and DNA from *M. bovis* BCG and *M. tuberculosis* H37Rv was used as a positive control. Results were interpreted as *M. tuberculosis* when a band size of 396 bp was detected (RD-9 positive), but the detection of a band size of 575 bp was interpreted as positive for the other MTBC species (Parsons et al. 2002), and the absence of any band interpreted as invalid.

3.6.3. Spoligotyping

Using the procedure described by Kamerbeek et al. (Kamerbeek et al. 1997), *M. tuberculosis* isolates that tested positive by RD9 PCR typing were then further typed by spoligotyping by the instructions provided by the manufacturer of the spoligotype kit (Ocimum Bio solution Company, Ijsselstein and the Netherlands). Known strains of *M. tuberculosis* H37Rv and *M. bovis* were employed as positive control while Qiagen distilled water was used as negative control. Using oligonucleotide primers (DRa and DRb) generated from the DR sequence, the DR region of the isolates was amplified by PCR. Briefly, PCR involved using 25µl of the subsequent reactions: To produce the mixture, 10µl Hot Star Taq, 2µl Master Mix from each primer, 5µl heat-killed cell suspension, and 8µl distilled water were used. After being heated to 96°C for 15 minutes, the mixture was run through 30 cycles of 96°C for 1 minute, 55°C for 1 minute, and 72°C for 35 seconds each. The Standard thermo cycler (VWR Thermo cycler, UK) was used to amplify the PCR. The membrane containing 43 covalently bound synthetic immobilized oligonucleotides representing the various spacers selected from *M. bovis* BCG (spacers 20-21 and 33-36) and *M. tuberculosis* H37Rv (spacers 1-19, 22-32, and 37-43) was hybridized with the amplified biotinylated PCR products (Barnes and Cave, 2003). After that, the

membrane was twice cleaned for ten minutes at $60^{O}C$ in 2× SSPE/0.5% sodium dodecyl sulfate (SDS). Subsequently, 4 µl of the streptavidin-peroxidase conjugate was added, and it was incubated at 42°C for an hour in a rolling bottle. The membrane was rinsed with 2× SSPE for 5 minutes at room temperature after being twice washed for 10 minutes at 42°C in 2× SSPE/0.5% SDS. By subjecting the membrane, which was coated in a transparent plastic wrap, to X-ray film for five minutes (Hyperfilm ECL, Amersham), as directed by the manufacturer, hybridized DNA was found using the enhanced chemiluminescence technique. On the film, black and white squares represented the presence and absence of DNA spacers, respectively.

Binary notation was used to write the spacers, with "1" standing for black squares and "0" for white squares. In order to identify the Spoligotype International Type (SIT) and family, the binary formats were converted to octal code and entered into a query box. This allowed the strain in question to be compared with those previously recorded in the SpolDB4 database (http://www.pasteur-gaudeloupe.fr:8081/SITVIT_ONLINE/) (Demay et al. 2012). Orphan strains were defined as strains that were not included in the SpolDB4 database, while unknown strains were defined as strains with spoliogpatterns that matched the SITs but could not be connected to any family. The *M. tuberculosis* family similarity was searched for using the SPOTCLUST tool, which was developed from the SpolDB3 database (http://tb insight.cs.rpi.edu/run_spotclust.html) (Vitol et al. 2006). Additionally, the primary lineages and sub-lineages were predicted using this online tool employing a knowledge-based Bayesian network (KBBN) and a conformal Bayesian network (CBN), respectively. According to Kamerbeek et al. (1997), strains with a single spoligopattern

were classified as single/unique strains, whereas two or more isolates that shared identical spoligotype patterns were categorized as clusters.

3.6.4. Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat Typing

In this work, 58 *M. tuberculosis* isolates were subjected to 24-loci MIRU-VNTR typing following a standardized methodology by Supply et al. (2000). Briefly, each isolate's 24 MIRU-VNTR alleles were amplified by PCR using 24 forward and reverse primers. 5µl of 5×buffer (HF), 2µl of 10mM dNTPs, 0.5µl of Phusion DNA polymerase, 0.8µl of heat-killed cell DNA template, and 15.7µl of dH2O were combined to create the PCR Master Mix. After multiplying each of these by 26 (i.e., 24 loci and 2 contingency), the total volume is 624µl. Next, each of the 24 PCR tubes was filled with 24 µl of the Master Mix. After that, one microliter primer was added into 24 µl of each Master Mix and formed a total volume of 25 µl that was amplified. Using PCR in 96-well plates, each MIRU-VNTR region was amplified individually. This is because the study's primers were chosen based on those found in Supply et al. (2006) (Annex III). The PCR program was performed under cycling conditions that included a 15-minute enzyme activation stage at 95 °C, 40 cycles of denaturation at 94 °C, annealing at 59 °C, and extension at 72 °C for one minute and thirty seconds. *M. tuberculosis* H37Rv and distilled water were used as a positive and negative control, respectively.

Gel-electrophoresis was used to extract the amplified Okazaki fragments (Cowan et al. 2005; Supply, 2005). Briefly, 300 ml of 1× TBE buffer was mixed with 5.4 grams of UltraPureTM Agarose powder (Sigma Life Science) and heated in a microwave until it

dissolved. When the agarose solution was dissolved and 45°C, or when a bottle could be held in one's hand, ethidium bromide was added and the mixture was gently homogenized. Subsequently, a gel tray (Thermo Scientific Owl A2 Large Gel System, 20 x 25 cm) was filled with the agarose solution and fitted with 26 lane gel combs of 1 mm width. The comb was carefully removed after 45 minutes, and 2.5 µl of loading dye and 3 µl of the PCR product were combined and added to the corresponding well. Every gel run contained standard DNA molecules measuring 50 bp and 100 bp. Ultimately, the gel tank was positioned, linked to a power supply, and operated for five hours at 120 volts and 400 milliampere. With the aid of a UV transilliminator, the product size was seen and captured in pictures. The items' band diameters were contrasted with those of the standard DNA ladder bands. To guarantee quality, *M. tuberculosis* H37Rv were loaded, and reagent contamination was managed by using sterile water.

Based on their band size, the number of tandem repeats or MIRU-VNTR alleles was determined (Supply et al. 2005). The results were displayed as the number of MIRU sequence repeats as a series of digits known as the MIRU code. The strains were categorized into primary evolutionary lineages using the MIRU-VNTR 24-loci profiles. The reference strain collection and identification tools are accessible online at *http://www.miru-vntrplus.org/MIRU/.* Based on the absence of spacer 13 and 10-19, Ethiopian strains, Ethiopia_2 and Ethiopia_3, respectively were manually assigned (Firdessa et al. 2013; Tessema et al. 2013; Biadglegne et al. 2015). Neighbor-joining (NJ) trees, and minimum spanning trees (MST) were created using a combination of spoligotyping and MIRU-VNTR results. All molecular typing studies were performed at AKLIPB, AAU, Ethiopia.

3.7. Anthropometric measurements

The weight and height of each study participant were measured by trained health professionals to estimate body mass index (BMI). A digital scale was used to measure the weight of each study participant, and weight was measured to the nearest 0.1kg, while height was measured with the vertical measuring rod to the nearest 0.1cm (Seligman et al. 2007; Saha et al. 2008). The formula for calculating BMI, which measures nutritional status, is weight (in kilograms) divided by height (in meters). According to Seligman et al. (2007), a BMI of less than 18.5 kg/m2 was considered underweight.

3.8. Rapid HIV test

The HIV status of the study participants was determined through the provision of pre-test counseling by trained health professionals. The rapid HIV diagnostic test was run on the clinically identified cases. Briefly, a whole blood sample was collected by finger prick. The presence of antibodies against HIV_1 and HIV_2 was determined by using HIV antibody colloidal gold (1+2) rapid diagnostic kits (KHB, Shanghai Kehua Bio-engineering Co Ltd, China) as a screening test, followed by HIV1/2 STAT-PAK® (Chembio Diagnostics, USA) test, when the KBH test result was reactive. When the STAT-PAK® result was discordant with KBH, a third test, Unigold TM HIV (Trinity Biotech, Ireland), was also used as a tiebreaker to determine the test result following the manufacturer's instruction. Finally, post-test counseling was provided to all participants (Constantine and Zink, 2005). HIV-positive study participants were linked to the health facilities for treatment and follow-up.

3.9. Quality control

All methods were performed in accordance with the relevant guidelines and regulations. The questionnaire was created in English and translated into the local language (Amharic) and then back into English by an expert fluent in both languages to preserve the data's quality. Moreover, informal translators were used in case when the participant was unable to speak Amharic language. The questionnaires were read by the data collector when the participant unable to read the information sheet. It was pretested among 30 randomly chosen homeless individuals in Adama town, which is roughly 100 kilometers east of Addis Ababa, prior to the actual data collection. The data collection process was closely supervised by the project supervisor and principal investigator day to day. Reagents and culture media were checked for sterility and performance characteristics in each batch of newly prepared reagent lots.

3.10. Data analysis

Clinical and demographic data were organized, checked, and cleaned before actual analysis. Then, the data were imported into Microsoft Excel version 2013 and exported to SPSS version 26 statistical software (IBMCorp, USA) for analysis. The patients' sociodemographic, behavioral, environmental, and morbidity history data were analyzed using descriptive statistics.

Genotyping data were analyzed using the online system SpolDB4 database (http://www.pasteur-gaudeloupe.fr:8081/SITVIT_ONLINE/) for spoligotyping (Demay et al. 2012) and MIRU-VNTR*plus* database (http://www.miru-vntrplus.org) (Allix-Béguec et al. 2008). Additionally, the *M. tuberculosis* family similarity was searched by using the

SPOTCLUST tool, which was developed from the SpolDB3 database (http://tbinsight.cs.rpi.edu/run_spotclust.html) (Vitol et al. 2006).

The MIRU-VNTR and spoligotyping data, which were coded in the Excel sheet, were transferred to the online tools to determine the molecular characteristics of the isolates. Lineages and sub-lineages were identified using the spoligotyping online tool employing a knowledge-based Bayesian network (KBBN) and a conformal Bayesian network (CBN), respectively, and the MIRU-VNTR*plus* database. Identification of MTBC isolates was carried out by best match analysis and the Minimum Spanning tree (MST) was determined by using the MIRU-VNTR*plus* database to ascertain strain classification (Allix-Béguec et al. 2008). Based on the copy number of the 24-loci MIRU-VNTR and spoligotyping data, a dendrogram was created using the unweighted pair group technique with neighbor joining (NJ).

The Hunter-Gaston Discrimination Index (HGDI) was used to assess the genotyping techniques' discriminatory power (Hunter and Gaston, 1988).

$$HGDI = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} n_j(n_j - 1)$$

Where nj is the number of isolates that belong to the jth pattern, s is the total number of distinct patterns, and N is the total number of isolates

The MIRU-VNTR*plus* was used to calculate the allelic diversity (h) for each of the 24 loci in the MIRU-VNTR. According to Sola et al. (2003), alleles with $h > 0.6$, $0.3 \leq h \leq 0.6$, and $h < 0.3$ were categorized as highly discriminative, moderately discriminative, and

poorly discriminative, respectively. The recent transmission index was calculated using the following formula (Small et al. 1994):

$$RTI = \left(\frac{T(c)-N(c)}{T(p)} \right)$$

where N(c) is the total number of clusters, T(p) is the total number of patients who participated in the study, and T(c) is the total number of clustered patients.

According to Meehan et al. (2018), patients were categorized as clustered if their patterns on spoligotyping and/or MIRU-VNTR were the same. Logistic regression analysis was performed to identify patient risk factors associated with PTB, *M. tuberculosis* strain clustering and drug resistance. All variables that significantly associated with drug resistance, strain clustering, and PTB at the univariate level were included in the multivariate logistic regression analysis. Odds Ratio (OR) and the 95% confidence interval (CI) were determined to evaluate the strength of association between the variables. P-value < 0.05 was considered statistically significant.

3.11. Ethical consideration

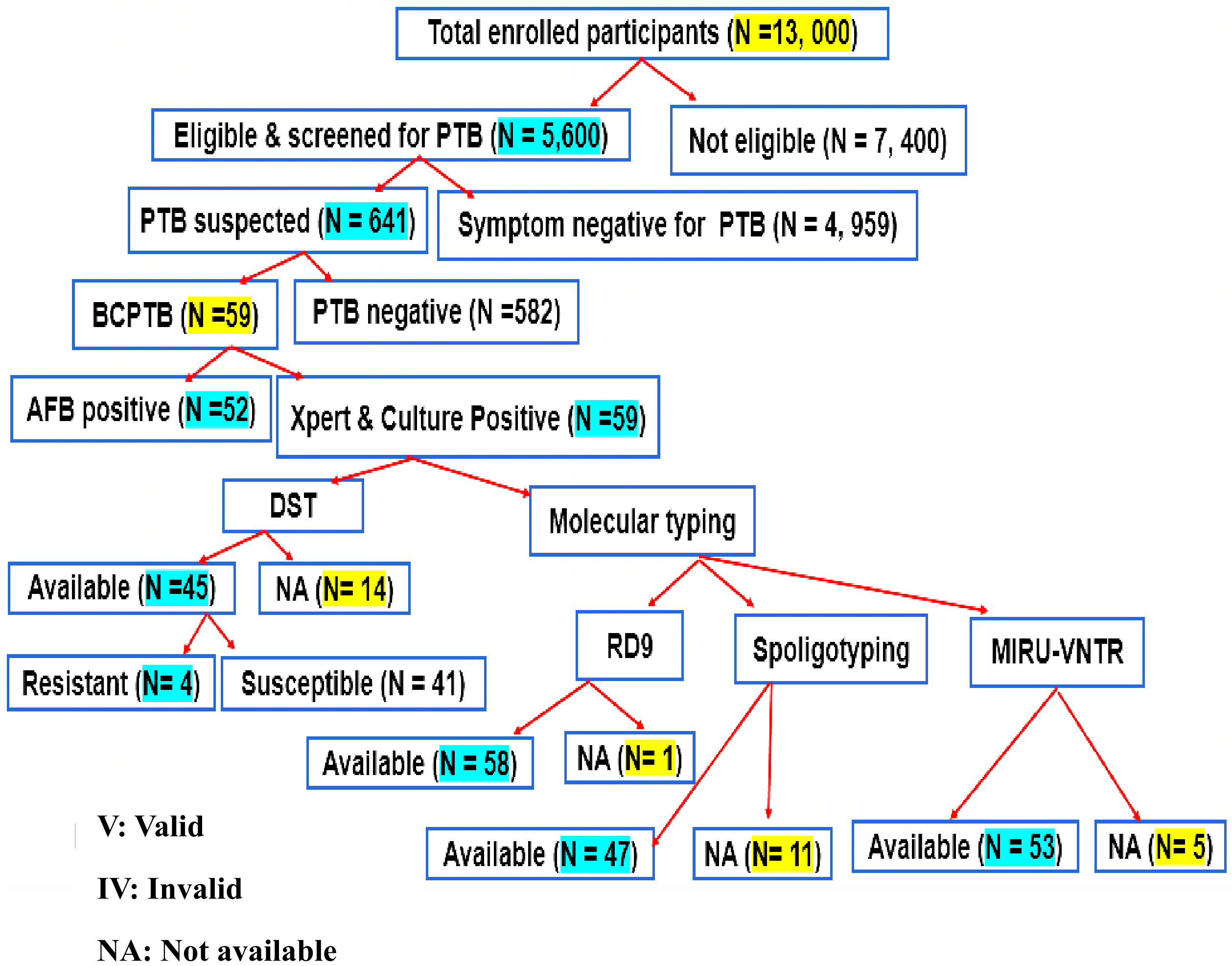
The protocol for the study was approved by the Institutional Review Board (IRB) of the College of Natural and Computational Sciences, Addis Ababa University, Ethiopia (Ref. No. IRB/036/2018). Furthermore, the Addis Ababa City Administration Health Bureau provided ethical clearance (Ref. No. A/A/H/3981/227). Then, a letter of support (Ref. No. A/A/L/S /66/116/163) was acquired from the Labor and Social Affairs Bureau of the Addis Ababa City Administration. All study participants who provided sputum samples gave their written informed consent after receiving sufficient information about the study's potential

benefits and dangers. Patients who tested positive in AFB or Xpert MTB/RIF assay were referred to the clinics in the temporary shelters for treatment under the directly observable therapy short course (DOTS) program.

4. RESULTS

4.1. Socio-demographic characteristics of the study participants

A total of 5,600 homeless individuals or 93.1% of all homeless individuals enrolled for shelter accommodations in Addis Ababa City during the study period were screened for PTB using the WHO TB symptom screening guidelines and 641 had a positive symptom screen (Figure 13). With a mean ± SD age of 27.8 ± 9.5 years, the majority of PTB suspected participants were male (80.3%) and single (86.7%) (Table 4). More than 97.7% of the participants originated out of Addis Ababa City, with the largest percentage coming from the Southern Nations, Nationalities and Peoples Regional state (SNNPR) (37%), which was followed by the Oromia (29.6%) and Amhara Regional state (23.7%) (Table 3).



V: Valid

IV: Invalid

NA: Not available

Figure 13: The study population, study participants, smear, Xpert, culture, molecular and DST categories in homeless individuals, Addis Ababa, Ethiopia, 2023

Table 3: Socio-demographic characteristics of homeless individuals (n = 641) suspected of PTB, Addis Ababa, Ethiopia, 2023

Variables		**Screened n (%)**	**PTB suspected n (%)**	**BCTB n (%)**
Gender:	Female	1,100 (19.6)	126 (19.7)	7(11.9)
	Male	4,500(80.4)	515(80.3)	52 (88.1)
Age:	18-27	753 (13.4)	86 (13.4)	6(10.2)
	28-37	2,744 (49.0)	314 (49.0)	34 (57.6)
	38-47	1,540(27.5)	176(27.5)	15(25.4)
	48-57	372(6.6)	42(6.6)	3(5.1)
	58 and older	191(3.4)	23(3.7)	1(1.7)
Marital status:	Single	4,869(86.9)	557(86.9)	49(83.1)
	Married	158(2.8)	39(6.1)	6 (10.2)
	Divorced	374 (6.7)	34 (5.3)	3(5.1)
	Widowed	199(3.6)	11(1.7)	1(1.7)
Educational status:	Illiterate	2,230 (39.8)	255 (39.8)	30(50.8)
	Primary school	2,632 (47.0)	301 (47.0)	22(37.3)
	Secondary school	704(12.6)	71(11.1)	6(10.2)
	Higher education	35(0.6)	14(2.2)	1(1.7)
Former residence:	Urban	920 (16.4)	233 (36.3)	18(30.5)
	Rural	4,680(83.6)	408(63.7)	41(69.5)
Previous region	Addis Ababa	126 (2.3)	34(5.3)	2 (3.4)
	Amhara	1,329 (23.7)	152 (23.7)	14 (23.7)
	Tigray	11 (0.2)	11(1.7)	(00)
	SNNPR	2,071 (37.0)	238(37.1)	23 (39.0)
	Oromia	1,660 (29.6)	190(29.6)	19 (32.2)
	Other	403 (7.2)	16 (2.5)	1 (1.7)
Total		**5, 600**	**641**	**59**

4.2. Prevalence of PTB and associated risk factors in the homeless individuals

Six hundred forty-one sputa samples were subjected to a Ziehl-Neelsen sputum smear microscopy, Xpert MTB/RIF assay, and LJ culture. Of these, 59 sputum samples were positive for PTB in both the LJ culture and the Xpert MTB/RIF assay, while 52 sputum samples were positive by ZN. Among homeless individuals who were suspected of TB 9.2% were bacteriologically confirmed PTB cases. The clinical prevalence of TB in this study was 1,054 cases per 100,000 individuals. The majority 88.1% (52/59) of the PTB-positive participants were males and the age range of 28–37 had 57.6% (34/59) of the PTB cases. Of the BCPTB cases, 32.2% (19/59) had co-infection with HIV.

It was shown that more than 82.1% of the study participants smoked cigarettes, whilst 78.0% drank alcohol, 73.6% chewed khat, and 32.8% used drugs. Of the study participants, 82.1% had been homeless for more than five years. According to univariate analysis, alcohol consumption [COR = 2.72, 95% C: 1.14, 6.46], living or sleeping in a restricted area with more than five people [COR = 13.97, 95% C: 1.92, 101.97], BMI < 18.5 [COR = 2.17, 95% CI: 1.26, 3.75], cigarette smoking [COR = 6.27, 95% CI: 1.30, 466], history of incarcerations [COR = 1.55, 95% CI: 1.04, 2.68], drug abuse [COR = 3.44, 95% CI: 1.22, 6.87], close contact with people who cough chronically [COR = 3, 28, 95% CI: 1.16, 9.23], and HIV infection [COR = 1.91, 95% CI: 1.07, 3.42] were significantly associated with PTB ($P < 0.05$), (Table 4).

Table 4:Univariate analysis of PTB prevalence in homeless individuals (n = 641), Addis Ababa, Ethiopia, 2023

Variables		PTB Suspected n (%)	PTB + n (%)	COR (95%CI)	P-value
Time spent without a home:	Greater than or equals to 5 years	526 (82.1)	55(93.2)	**3.24(1.15, 9.13)**	**0.026**
	Less than 5 years	115(17.9)	4(6.8)	1	
Body mass index	Less than 18.5	181(28.2)	33(55.9)	**2.17 (1.26, 3.75)**	**0.005**
	Greater than or equals to 18.5	460(71.8)	26(44.1)	1	
Alcohol consumption:	Yes	498 (77.7)	53(89.8)	**2.72 (1.14, 6.46)**	**0.024**
	No	143(22.3)	6(10.2)	1	
Drug use:	Yes	210 (32.8)	11(18.6)	**3.44(1.22, 6.87)**	**0.018**
	No	431 (67.2)	48(81.4))	1	
Smoking cigarette:	Yes	534 (83.3)	57(96.6)	**6.27 (1.51, 26.10)**	**0.012**
	No	107(16.7)	2(3.4)	1	
Chewing khat:	Yes	517 (80.7)	49(83.1)	1.19 (0.59, 2.43)	0.625
	No	124 (19.3)	10(16.9)	1	
The average number of homeless individuals sharing a confined space and sleeping together was	Five or more persons	527(82.2)	58(98.3)	**13.97 (1.92, 101.97)**	**0.009**
	Less than 5 persons	114 (17.8)	1(1.7)	1	
	Yes	124 (19.3)	3(5.1)	**1.30(1.01, 4.66)**	**0.008**
Past incarcerations:	No	517(80.7)	56(94.9)	1	
Close contact with people who are known to have TB:	Yes	212 (33.1)	25(42.4)	**1.55 (1.04, 2.68)**	**0.023**
	No	429 (66.9)	34(57.6)	1	
Close proximity to those who cough persistently:	Yes	525 (81.9)	55(93.2)	**3.28 (1.16, 9.23)**	**0.025**
	No	116 (18.1)	4(6.8)	1	
Previous TB history:	Yes	92(14.4)	12((20.3)	0.81 (0.42, 1.56)	0.523
	No	549 (85.6)	47(79.7)	1	
HIV- status:	Positive	135 (21.1)	19(32.2)	**1.91(1.07, 3.42)**	**0.030**
	Negative	506 (78.9)	49 (83.1)	1	

Except for alcohol and drug misuse, all variables that were significantly associated with the prevalence of bacteriologically confirmed PTB in the univariate logistic regression analysis continued to be significant in the multivariable logistic regression analysis. As a result, the following factors were found to be significantly associated with the prevalence of PTB ($P < 0.05$): BMI < 18.5 [AOR = 3.20, 95% CI: 1.68, 6.18], smoking cigarettes [AOR = 5.71, 95% CI: 1.29, 25.28], living or sleeping in a restricted area with more than five people [AOR = 7.82, 95% CI: 1.02, 60.16], close contact with chronic coughers [AOR = 3.53, 95% CI: 1.14, 10.91], history of imprisonment [AOR = 3.21, 95% CI: 1.10, 9.37], duration of homelessness (AOR = 2.26, 95% CI: 1.08, 5.89), and HIV infection [AOR = 2.34, 95% CI: 1.17, 4.71] (Table 5).

Table 5:Multivariate analysis of PTB prevalence in homeless individuals (n = 641), Addis Ababa, Ethiopia, 2023

Variables		**PTB suspected n (%)**	**PTB + n (%)**	**COR (95%CI)**	**P-value**
Time spent without a home:	Greater than or equal to 5 years	526 (82.1)	55(93.2)	**3.99 (1.31, 11.59)**	**0.014**
	Less than 5 years	115(17.9)	4(6.8)	1	
	Less than 18.5	181(28.2)	33(55.9)	**3.20 (1.68, 6.09)**	< **0.001**
Body mass index	Greater than or equal to 18.5	460(71.8)	26(44.1)	1	
	Yes	498 (77.7)	53(89.8)	1.88 (0.76, 4.66)	0.174
Alcohol consumption:	No	143(22.3)	6(10.2)	1	
	Yes	210 (32.8)	11(18.6)	0.54 (0.26, 1.11)	0.093
Drug use:	No	431 (67.2)	48(81.4))	1	
	Yes	534 (83.3)	57(96.6)	**5.71 (1.29, 25.28)**	**0.022**
Smoking cigarette:	No	107(16.7)	2(3.4)	1	
	Five or more	527(82.2)	58(98.3)	**7.81 (1.09, 60.16)**	**0.048**
The average number of homeless individuals sharing a confined space and sleeping together was:	Less than 5 persons	114 (17.8)	1(1.7)	1	
	Yes	124 (19.3)	3(5.1)	**2.26 (1.08, 5.89)**	**0.023**
History of imprisonment:	No	517(80.7)	56(94.9)	1	
	Yes	525 (81.9)	55(93.2)	**3.21 (1.10, 9.37)**	**0.033**
Close contact with chronic coughers:	No	116 (18.1)	4(6.8)	1	
	Positive	135 (21.1)	19(32.2)	**2.34 (1.17, 4.71)**	**0.017**
	Negative	506 (78.9)	49 (83.1)	1	
HIV-status:					

4.3. Molecular epidemiology of MTBC strain circulating in homeless individuals in Addis Ababa, Ethiopia

4.3.1. Study participants

A total of 59 LJ culture-positive *M. tuberculosis* isolates were included in the molecular epidemiology investigation. Then, the 59 isolates were identified using RD 9-based PCR, spoligotyping, and MIRU-VNTR typing.

4.3.2. Molecular characterization of MTBC isolates

4.3.2.1. Speciation of the *M. tuberculosis* isolates by PCR-based-RD 9

Using PCR-based-RD 9, 58 of the 59 isolates were confirmed to be *M. tuberculosis* (Figure 14). One sample did not give a valid result. The isolates were further genotyped using spoligotyping and 24-loci MIRU-VNTR typing for the identification of lineages and strains.

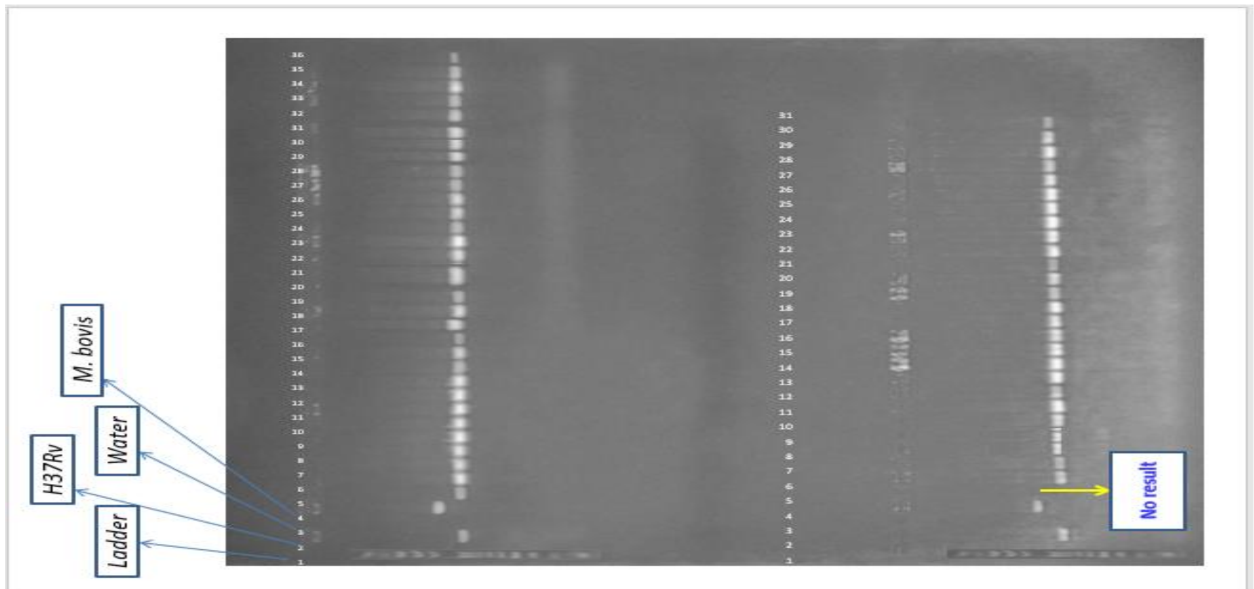


Figure 14: Picture of gel electrophoresis for RD9 deletion typing of *M. tuberculosis* isolates. Lane1, is a DNA ladder, Lane 2, *M. tuberculosis* H37Rv (Positive control), Lane 3 Distilled water (Negative control), Lane 4, *M. bovis* (control), Lane 5-67 *M. tuberculosis* isolates to be investigated.

4.3.2.2. Identification of lineages and sub-lineages based on spoligotyping

Table 6 illustrates the spoligotyping results. Based on the spoligotyping results 81% (47/58) of the isolates belonged to 16 shared Spoligotype International Type (SIT). According to the TB-insight RUN TB-Lineage, Euro-American (L4) (EA) major lineage was the most dominant 89.65% (52/58) followed by East-African Indian (L3) 8.6% (5/58) and Indo-Oceanic (L1) 1.7% (1/58) lineages. The three most commonly found spoligotype were SIT53, SIT149, and SIT37, which included 11, 9, and 6 isolates, respectively. However, 19% (11/58) of the isolates in the SpolDB database lacked SIT numbers; as a result, these isolates were classified as orphan (new) spoligotype (Table 7).

Table 6:Major Lineage and lineages of MTBC isolates from homeless individuals, Addis Ababa, Ethiopia, 2023

SIT	Octal code	Binary code	Major Lineages (TB insight)	Lineage (SITVITWEB)	Lineages (MIRU-VNR *plus*)	Isolates N (%)
25	703777740003171		EAI	CAS1_DELHI	Delhi/CAS	2(3.4)
26	703777740003771		EAI	CAS1_DELHI	Delhi/CAS	1(1.7)
37	777737777760771		EA	T3	Ethiopia_2	6(10.3)
41	777777404760771		EA	Turkey	TUR	1(1.7)
42	777777607760771		EA	LAM9	LAM	1(1.7)
47	777777774020771		EA	H1	Haarlem	2(3.4)
53	777777777760771		EA	T1	Ethiopia_3	11(19.0)
54	777777777763771		EA	Manu2	TUR	5(8.6)
118	777767777760771		EA	T1	Unidentified	2(3.4)
119	777776777760771		EA	X1	X	2(3.4)
121	777777775720771		EA	H3	Haarlem	1(1.7)
149	777000377760771		EA	T3-ETH	Ethiopia_3	9(15.5)
302	777756777760771		EA	X1	X	1(1.7)
584	777775777760731		EA	T2	Unidentified	1(1.7)
952	603777740003771		EAI	CAS1_DELHI	Delhi/CAS	1(1.7)
1312	703777740003131		EAI	CAS1_DELHI	Delhi/CAS	1(1.7)

Abbreviations: EA: Euro-American, EAI: East-African Indian, IO: Indo-Oceanic

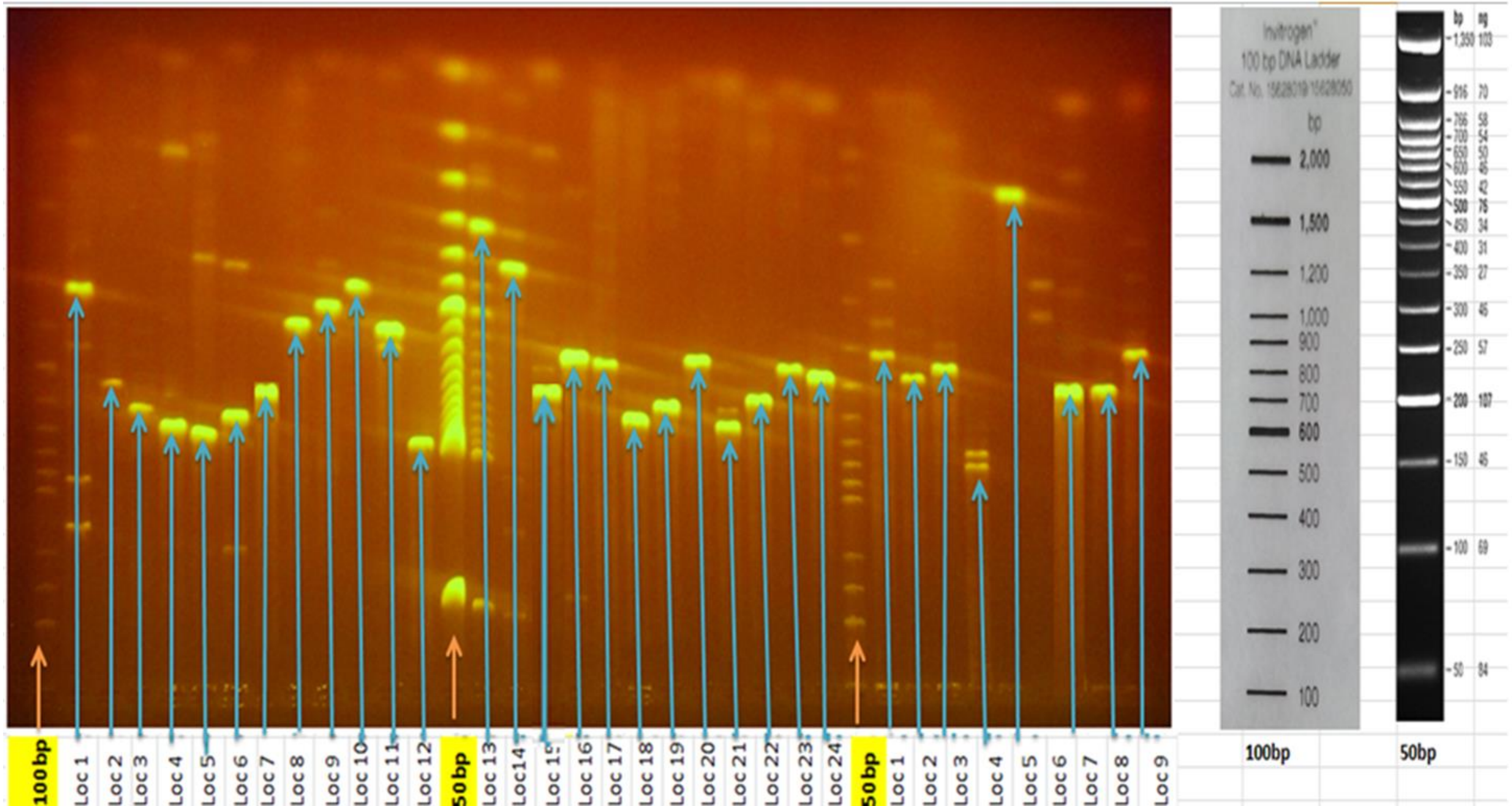
Table 7:Major lineages and orphan strains of MTBC isolates from homeless individuals, Addis Ababa, Ethiopia, 2023

SIT	Octal code	Binary code	Major Lineages (TB insight)	Lineage (SITVIT WEB)	Lineage (MIRU-VNTR *plus*)	Isolates N (%)
Orphan-1	777776777760600		EA	Unknown	Haarlem	1(1.7)
Orphan-2	777777377770771		EA	Unknown	Unidentified	1(1.7)
Orphan-3	777737757760771		EA	Unknown	Ethiopian_H_{37}RV-Like	2 (3.5)
Orphan-4	777775777760721		EA	Unknown	Ethiopian_H_{37}RV-Like	2(3.5)
Orphan-5	763777777563771		EA	Unknown	Delhi/CAS	1(1.7)
Orphan-6	776737777760771		EA	Unknown	X	2(3.5)
Orphan-7	777777774030771		IO	Unknown	Delhi/CAS	1(1.7)
Orphan-8	777777775730771		EA	Unknown	Unidentified	1(1.7)

Abbreviations: EA: Euro-American, EAI: East-African Indian, IO: Indo-Oceanic

4.3.2.3. Identification of lineages and sub-lineages based on MIRU-VNTR typing

Using MIRU-VNTR typing, sub-lineages of Ethiopia_3 (34.5%), Delhi/CAS (12.1%), Ethiopia_2 (10.3%), TUR (10.3%), X-type (8.6%), Ethiopia_H37Rv-like strain (6.9%), Haarlem (6.9%) and Latin-American Mediterranean (LAM, 1.7%) were identified. Nevertheless, the MIRU-VNTR*plus* database was unable to identify the lineages of 8.6% of the isolates. Figure 15 illustrates a typical image of the MIRU-VNTR gel run.



GLB-71 **GLB-485**

Abbreviation: GLB: Sample ID stands for global site of the shelter

Figure 15: Picture of gel electrophoresis for MIRU-VNTR typing of *M. tuberculosis* isolates.

4. 3.2.3.1. MIRU-VNTR based allelic diversity (h)

Table 8 displays the allelic diversity (*h*) for each MIRU-VNTR locus. Based on their allelic diversity and discriminative power, MIRU-VNTR loci are categorized into three groups. Eleven loci of MIRU-VNTR (424, 802, 960, 1644, 1955, 2163b, 2347, 2401, 2996, 4052, and 4156) were highly discriminative (HGI > 0.6) while 10 MIRUVNTR loci (577, 580, 2059, 2165, 2461, 2531, 3007, 3192, 3690 and 4348) were moderately discriminative (0.3 < HGI < 0.6) and the remaining three MIRU-VNTR loci (154, 2687 and 3171) were poorly discriminative (HGI< 0.3).

Table 8:Allelic diversity and the occurrence of MIRU-VNTR alleles among 58 MTBC isolates from homeless individuals, Addis Ababa, Ethiopia, 2023

MIRU-VNTR				**Allele No.#**												**h**	**Discriminant status**
Loci	24	**15**	**12**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**			
MIRU02	154				3	55										0.05	Poorly
Mtub04	424				19	28	2	5	3		1					0.65	**Highly**
ETRC	577				1	7	9	35	6							0.59	Moderately
MIRU04	580					48	3	6	1							0.34	Moderately
MIRU40	802			2	3	18	16	10	8	1						0.75	**Highly**
MIRU10	960			8	6	2	10	9	17	3	1	1	1			0.81	**Highly**
MIRU16	1644			1	16	2	31	5	1		2					0.61	**Highly**
Mtub21	1955				2	11	30	5	10							0.70	**Highly**
MIRU20	2059				4	48	1	5								0.34	Moderately
QUB11b	2163b				10	20	14	9	3	2						0.77	**Highly**
ETRA	2165					15	38	4		1						0.44	Moderately
Mtub29	2347				1	2	5	34	15	1						0.61	**Highly**
Mtub30	2401				2	29		22	4	1						0.61	**Highly**
ETRA	2461				4	48	4	2								0.37	Moderately
MIRU23	2531					1	2	14	40	1						0.49	Moderately
MIRU24	2687			3	54		1									0.18	Poorly
MIRU26	2996				19	2		14	18	3		2				0.74	**Highly**
MIRU27	3007			1		6	50	1								0.34	Moderately
Mtub34	3171				1	3	49	4	1							0.24	Poorly
MIRU31	3192			1		5	35	10	3	2	1					0.53	Moderately
Mtub39	3690					2	40	11	5							0.45	Moderately
QUB26	4052				1	3	10	7	9	11	11	4		1		0.84	**Highly**
QUB4156	4156				2	24	23	9								0.60	**Highly**
MIRU39	4348				2	46	7	1			1		1			0.34	Moderately

The number of alleles in the MIRU-VNTR locus in the *M. tuberculosis* genome corresponds to the frequency at which a unique repeat unit was found in each of the 58 isolates. There are three types of discriminant: poor (h < 0.3), moderate (0.3 < = h < =0.3), and high (h > 0.6). The alleles used for the 12-loci and 15-loci MIRU-VNTR approaches are displayed in shaded boxes.

4.3.2.3.2. Minimum spanning tree (MST) and dendrogram analysis

A Minimum Spanning Tree (MST) was created using MIRU-VNTR data, and isolate genotypes were connected according to double-locus variations to analyze the genetic relatedness or evolutionary relationships between different strains of *M. tuberculosis.* Thirty-four singleton patterns were left after twenty-two genotypes (matching to twenty isolates) were clustered into seven clonal complexes. While clonal complexes 3, 4, 5, 6, and 7 each included two genotypes, clonal complexes 1 and 2 contained nine and three genotypes, respectively (Figure 16).

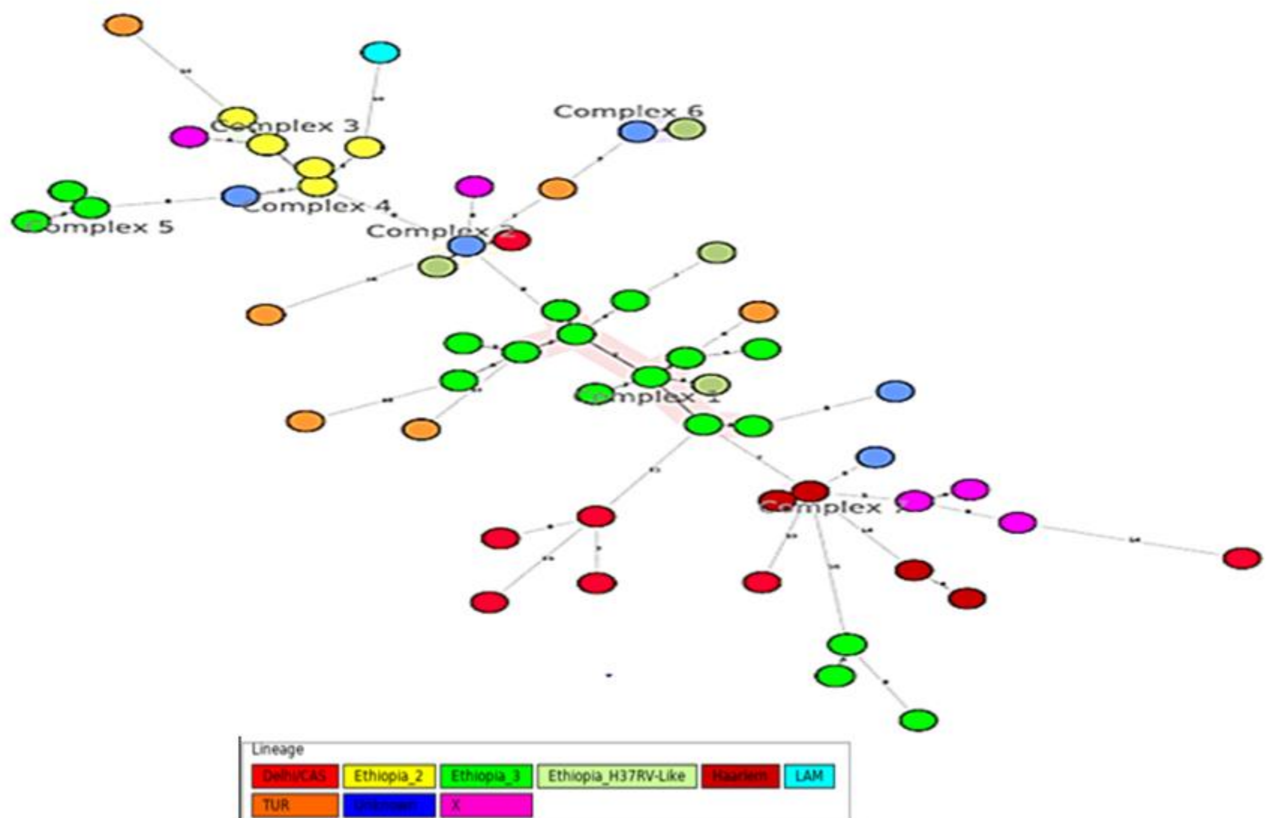


Figure 16: Minimum spanning tree based on 24-loci MIRU-VNTR profiles of 58 MTBC isolates from homeless individuals, Addis Ababa, Ethiopia, 2023. Sub-lineages are colored differently and the individual pattern is represented by the circle. The length

of the branch shows the distance between patterns. A maximum locus difference within a clonal complex of MIRU-VNTR types is double locus variation. The reference strain, *M. canetti*, was taken from the MIRU-VNTR *plus* database.

The MIRU-VNTR dendrogram was constructed using http://WWW.miruvntrplus.org/MIRU/index to determine the genetic relatedness or similarity among different strains of *M. tuberculosis*. The dendrogram divided the 58 isolates into 7 clonal groups. There was one isolate in the second group. There were 3, 9, 21, 10, 4, and 10 isolates in the second, third, fourth, fifth, sixth, and seventh groups, respectively (Figure 17).

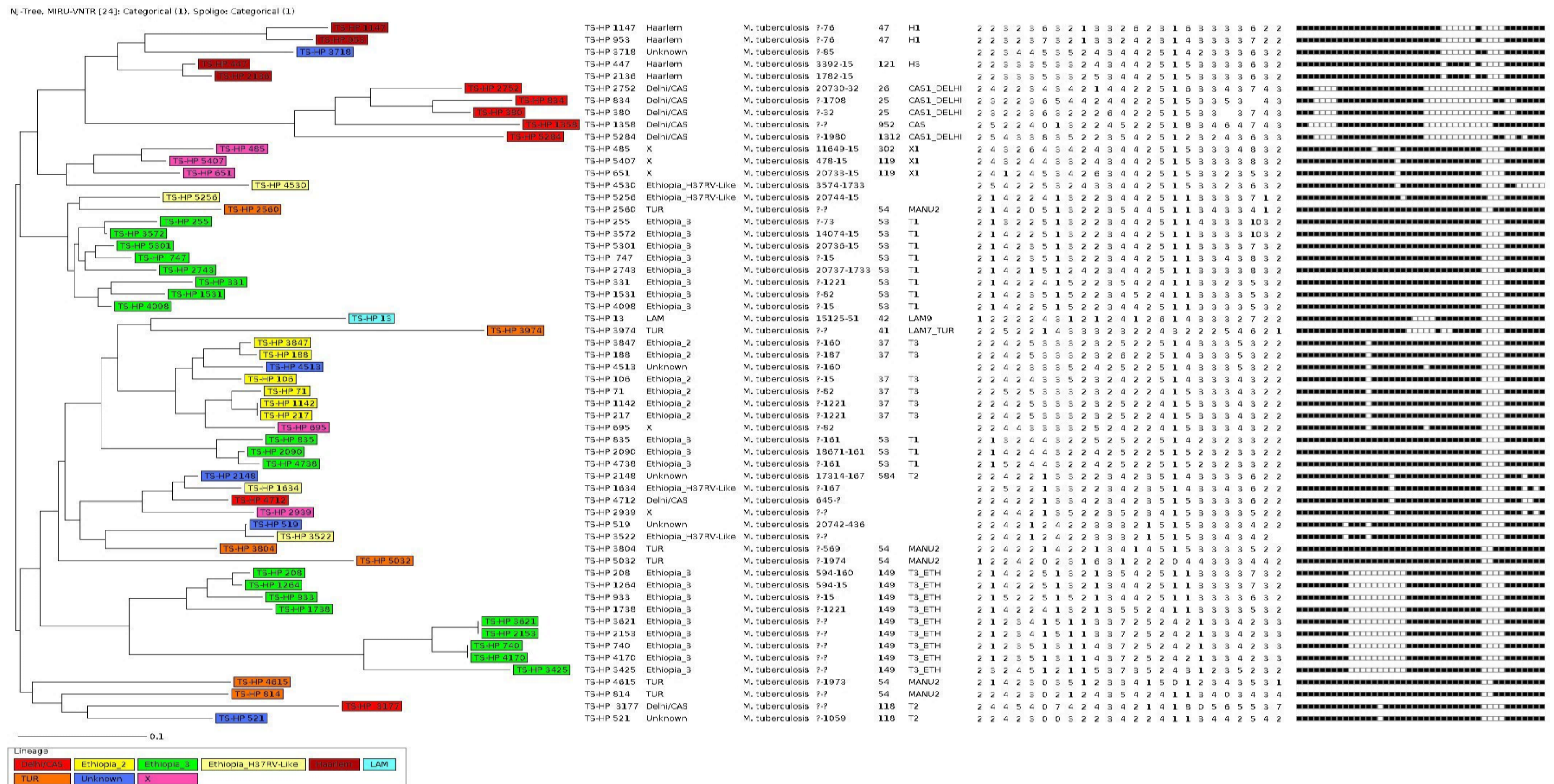


Figure 17:Depicts the phylogenetic tree of 58 MTBC isolates from homeless individuals in Addis Ababa, Ethiopia,2023. The phylogenetic connection between the MTBC strains isolated from homeless individuals in Addis Ababa city and the reference MTBC strains in the MIRU-VNTR*plus* database is displayed in a neighbor-joining (NJ) tree. Using 24-loci MIRU-VNTR data and spoligotyping, the NJ tree was built. Along with the NJ tree, spoilage patterns and MIRU-VNTR alleles of 58 isolates were also displayed. The novel MTBC isolates from Addis Ababa were predicted to belong to which lineage using this phylogenetic tree. To create the phylogenetic tree, the web tools MIRU-VNTRplus (https://www.miru-vntrlus.org) were used.

4.3.2.4. Discriminatory power of spoligotyping and 24-loci Mycobacterial Interspersed Repetitive Unit-variable Number Tandem Repeat typing

Spoligotyping and 24-loci MIRU-VNTR had discriminatory powers of 0.9864 and 0.9987, respectively. Twenty-four distinct spoligotype patterns 11 clustered and 13 unique were found through spoligotyping analysis. On the other hand, MIRU-VNTR*plus* produced 55 distinct patterns, of which 3 were grouped and 52 were unique. When spoligotyping and 24-loci MIRU-VNTR were combined, the overall clustering rate was 10.3%, and when both genotyping techniques were combined, the RTI was 5.2% (Table 9).

Table 9:The ability of genotyping techniques to discriminate 58 MTBC isolates from homeless individuals, Addis Ababa, Ethiopia, 2023

Typing techniques/methods	**Different patterns n (%)**	**Clusters (n)**	**Size of the cluster (n)**	**Isolates in clusters n (%)**	**Number (%) unique isolates**	**The percentage of clustering (%)**	**RTI %**	**HGDI**
Spoligotyping	24/58 (41.4)	11	2-16	45 (77.6)	13 (22.4)	77.6	58.6	0.9864
MIRU-VNTR-24	53/58 (91.4)	7	2-9	23(39.7)	35(60.3)	39.7	27.6	0.9987
Spoligotyping + MIRU-VNTR	55/58 (94.8)	3	2	6(10.3)	52(89.7)	10.3	5.2	0.9999

**HGDI* Hunter Gaston Discrimination Index

4.3.2.5. Factors associated with clustering of *M. tuberculosis* strains

The clustering of *M. tuberculosis* strains was significantly associated with living in groups (AOR=7.2, 95% CI: 4.64, 15.41) according to the logistic regression analysis (Table 10).

4.4. Drug susceptibility test

The drug sensitivity test (DST) data was available for 76.3% (45/59) of the total isolates tested. Of this, 91.1% (41/45) of the strains were found to be pan-susceptible, whereas 6.8 % (4/59) were found to be mono-resistant to the first-line anti-TB drugs. The rate of mono-resistance was 4.4% to either INH or SM. The resistance rates of Ethiopia_3 and LAM strains were 5.1% (3/59) and 1.7% (1/59), respectively (Table 10). When tested for resistance to second-line anti-TB drugs, all isolates that were resistant to first-line drugs were found to be non-resistant.

Table 10: Factors associated with strain clustering using 24-loci MIRU-VNTR and spoligotyping for 58 MTBC isolates from homeless individuals, Addis Ababa, Ethiopia, 2023

Variable		**Genotype**		**AOR (95% CI)**	**P value**
		Clustered n (%)	**Unique n (%)**		
Age	18-27	4(57.1)	2(28.6)	1	
	28-37	31(91.2)	3(8.8)	3.1 (0.93, 4.24)	0.21
	38-47	10(66.7)	5(33.3)	7.1(0.96,12.33)	0.068
	48-57	NA	3	NA	NA
Living with more than five individuals in one restricted place	No	5(45.5)	6 (54.5)	1	
	Yes	40(85.1)	7(14.9)	**7.2 (4.64, 15.41)**	**0.032**
History of TB	No	43(78.2)	12(21.8)	1	
	Yes	2(66.7)	1(33.3)	1.84(0.44, 6.23)	0.85
SM	Susceptible	43(76.8)	13(23.2)	1	
	Resistant	2(4.4)	NA	NA	NA
INH	Susceptible	43(76.8)	13(23.2)	1	
	Resistant	2(4.4)	NA	NA	NA
RIF	Susceptible	45(77.6)	13 (22.4)	1	
	Resistant	0(0)	NA	NA	NA
EMB	Susceptible	45(77.6)	13 (22.4)	1	
	Resistant	0(0)	NA	NA	NA
Any resistance to FLDs	No	41(91.1)	12(23.5)	1	
	Yes	3(6.7)	1(2.2)	2.81(0.92, 6.22)	0.74

FLDs: First-Line Drug resistance, NA: Not available

The three types of sub-lineages identified in this were lineage-1(EAI), lineage-3(Delhi/CAS), lineage-4 (Ethiopia_3, Ethiopia_2, TUR, X-type, Ethiopia_H37Rv-like strain, Haarlem, LAM and unknown) and their patterns of drug resistance shown in table 12. Two strains each consisting of three Ethiopia_3 and one LAM demonstrated the highest drug resistance (Table 11).

Table 11: Patterns of drug resistance to first-line anti-TB drugs for 58 MTBC isolates from homeless individuals, Addis Ababa, Ethiopia, 2023.

Lineage (N= 58)	**INH**		**RIF**		**EMB**		**SM**		**ANY resistance**		**MDR**
	R	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	
Ethiopia_3 (20)	**1 (5%)**	**3(15%)**	**0(0)**	**3 (15%)**	**0(0)**	**7(35%)**	**2(10%)**	**4(20%)**	**3(15%)**	**17(85%)**	**0(0)**
Ethiopia_2 (6)	**0(0)**	**1(16.7)**	**0(0)**	**4 (20%)**	**0(0)**	**1(16.7%)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**
Delhi/CAS (7)	**0(0)**	**3(42.9)**	**0(0)**	**4 (57.1%)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**
TUR (6)	**0(0)**	**2 (33.3%)**	**0(0)**	**3(50%)**	**0(0)**	**1 (16.7%)**	**0(0)**	**0(0**	**0(0)**	**0(0**	**0(0)**
X-type (5)	**0(0)**	**1(20%)**	**0(0)**	**3(60%)**	**0(0)**	**1(20%)**	**0(0)**	**1(20%)**	**0(0)**	**0(0**	**0(0)**
Ethiopia_H37Rv-like (4)	**0(0)**	**2 (50%)**	**0(0)**	**2 (50%)**	**0(0)**	**0(0**	**0(0)**	**0(0**	**0(0)**	**0(0**	**0(0)**
Haarlem (4)	**0(0)**	**1(25%)**	**0(0)**	**2(50%)**	**0(0)**	**1(25%)**	**0(0)**	**0(0**	**0(0)**	**0(0**	**0(0)**
LAM (1)	**1**	**0(0**	**0(0)**	**0(0**	**0(0)**	**0(0**	**0(0)**	**0(0**	**0(0)**	**0(0**	**0(0)**
Unknown (5)	**0(0)**	**2 (40%)**	**0(0)**	**2 (40%)**	**0(0)**	**1(20%)**	**0(0)**		**0(0)**		**0(0)**
Total 58	**2 (3.4%)**	**15 (25.9)**	**0(0)**	**23(39.6%)**	**0(0)**	**12(20.7%)**	**2(3.4%)**	**4(8.6%)**	**3(5.2%)**	**(29.3%)**	**0(0)**

R: Resistant S: Sensitive

4.5. Prevalence of mixed *M. tuberculosis* strain infection

It was discovered that three of the 58 patients had multiple strains of *M. tuberculosis*. All individuals who had multiple strains of the infection were newly diagnosed and one of them was positive for HIV. One patient (patient # 3804) had three alleles in two loci, whereas the other two patients (patient # 2560, # 933) each revealed double alleles in two loci. The allelic diversity was observed at loci 1644, 3192, and 3690 (Table 12).

Table 12: Multiple strains of *M. tuberculosis* identified from homeless individuals, Addis Ababa, Ethiopia, 2023

Patient ID	**MIRU-VNTR loci**																							
N	**154**	**424**	**577**	**580**	**802**	**960**	**1644**	**1955**	**2059**	**2163b**	**2165**	**2347**	**2401**	**2461**	**2531**	**2687**	**2996**	**3007**	**3171**	**3192**	**3690**	**4052**	**4156**	**4348**
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
2560	2	1	4	2	0	5	1	3	2	2	3	5	4	4	5	1	1	3	4	3,2	3,2	4	1	2
3804	2	2	4	2	2	1	4,3,2	2	2	1	3	4	1	4	5	1	5	3	3	3	3,2,2	5	2	2
933	2	1	5	2	2	5	1,2	5	2	1	3	4	4	2	5	1	1	3	3	3,2	3	6	3	2

ID patient identification number

5. DISCUSSION

The current cross-sectional study was conducted in Addis Ababa, Ethiopia, to investigate the prevalence of PTB, associated risk factors, molecular epidemiology of *M. tuberculosis,* and its drug resistance patterns in homeless individuals. This is the first study carried out in Addis Ababa, Ethiopia, where there are a higher estimated number of homeless individuals as a result of the highest rural-urban migration.

In line with earlier studies of a similar nature conducted in Ethiopia (Semunigus et al. 2016) and Brazil (Santos et al. 2021), 80% of the homeless participants in this study were males. Similar to previous studies, TB was predominant in the young homeless individuals in the current study (Moonan et al. 2012; Bamrah et al. 2013). This could be due to the socioeconomic crisis of low-income countries, as parents in both urban and rural regions are unable to provide basic needs and demands for their children, forcing them to turn to the streets in search of support (Atnafu et al. 2014; Abeje, 2021). This leads to a significant increase in the number of young homeless individuals and a high prevalence of TB in this age group (Semunigus et al. 2016). Regarding the gender disparity, there are a variety of factors that contribute to a higher proportion of males among homeless individuals in Addis Ababa, Ethiopia. One of the causes is a socio-cultural impact in Ethiopia that makes it difficult for females to leave their families and live on the streets. As a result, even if females in Ethiopia endure substantial socioeconomic challenges in their parents' homes, leaving their families and living on the street is socially unacceptable (Shamebo et al. 2023a). Furthermore, it is customary that female youth have better employment opportunities because the majority of them work as maids in people's houses in Ethiopia

or overseas in Middle Eastern countries. Moreover, most hotels, bars, and cafeterias prefer to hire females over males.

In the present study, the prevalence of PTB in homeless individuals was nine times higher than its prevalence in the general population of Ethiopia (Kebede et al. 2014) and four times higher than the general population of Addis Ababa City (Temesgen et al. 2021; Alene et al. 2022). Similarly, several studies from other countries reported (Barmah et al. 2013; Tabuchi et al. 2011; Johnston et al. 2019; Story et al. 2007) that the prevalence of PTB in homeless individuals could be 10 times higher than its prevalence in the general population. However, other studies from London (Moss et al. 20000) and the United States (Haddad et al. 2005) reported a lower prevalence value of PTB than the prevalence recorded in this study. This could be due to methodological and socio-economic differences between countries. Differences in TB screening and diagnostic practices, as well as varying levels of access to health care services and resources, may contribute to variations in reported PTB prevalence rates among homeless individuals across the regions. Furthermore, socio-economic factors such as poverty, housing instability, and inadequate healthcare infrastructure are significant determinants of TB transmission, with Ethiopia facing unique challenges stemming from lower socio-economic development. Moreover, homeless individuals are a neglected segment of the community in terms of basic medical care and have a lower likelihood of accessing basic human needs, such as housing and food (Narasimhan et al. 2013). There was no significant variation in PTB prevalence based on the homeless individuals' previous origin, implying that the TB infection was acquired as they moved into homelessness in Addis Ababa.

In agreement with findings of present study, previous studies on homeless individuals in Colombia (Haddad et al. 2005; Hernández Sarmiento et al. 2013), Poland (Romaszko et al. 2013), the United States (Notaro et al. 2013), Rome (Laurenti et al. 2012), and South Korea (Lee et al. 2013) reported a higher prevalence of TB in homeless individuals than in the general population of their respective cities. Similarly, a systematic review and meta-analysis of the prevalence of TB among homeless individuals recorded a range of prevalence values that overlap with the prevalence value recorded by the present study (Beijer et al. 2012; Alecrim et al. 2016). A higher prevalence of PTB in homeless individuals has been linked to risk factors, such as poverty, overcrowding, malnutrition, HIV/AIDS, smoking, alcoholism, and drug abuse (Amiri et al. 2017). Furthermore, most TB cases among homeless individuals in urban settings are considered to be the result of continuous transmission (Brewer et al. 2001). This condition is exacerbated by suboptimal TB control strategies for hard-to-reach populations and the provision of insufficient shelters for homeless individuals (Munn et al. 2015). Several studies have demonstrated that diligent case-finding and quick commencement of appropriate treatment may help minimize TB transmission among homeless individuals, with more rigor than the general community (Nolan et al. 1991; Rendleman et al. 1999).

The significant association between malnutrition and cigarette smoking with TB in this study is in line with previous studies conducted in Brazil (Scholze et al. 2022), South Korea (Lee et al. 2013), and Rome (Laurenti et al. 2012). These factors increase the susceptibility of a host to new infection or reactivation of LTBI (Mihret et al. 2012; Ali et al. 2016). Similar to the present findings, the association between TB-HIV co-infection among

homeless individuals has been well documented in the United States (Bamrah et al. 2013) and Canada (Moss et al. 2000), which could also be due to increased susceptibility to new infection and/or reactivation of LTBI as result of immunosuppression.

Based on the results of the spoligotyping, the Euro-American (EA) lineage (L4) was the most prevalent (89.7%) lineage identified in this study. This observation was consistent with the findings of previous studies, which showed the high prevalence of L4 to be the most dominant one in the world (Reed et al. 2009) and hence its dominance in Ethiopia (Korma et al. 2015; Diriba et al. 2021; Bedewi et al. 2017; Debebe et al. 2014; Garedew, 2013; Wondale et al. 2020; Mekonnen et al. 2018). Furthermore, two reviews on the genetic diversity of *M. tuberculosis* strains in Ethiopia indicated that the prevalence of L4 is higher than the prevalence of the other lineages (Mekonnen et al. 2019; Tulu and Ameni, 2018). Lineage (L3) was the second most prevalent lineage isolated in this study following L4. This observation is also consistent with the findings of other studies reported from Ethiopia (Korma et al. 2015; Diriba et al. 2021; Bedewi et al. 2017; Debebe et al. 2014; Garedew, 2013; Wondale et al. 2020; Mekonnen et al. 2018). The reason for the high prevalence of L3 and L4 could be because these lineages belong to the modern *M. tuberculosis* lineages which are characterized by high potential of transmissibility as compared to ancient *M. tuberculosis* lineages (Comas, 2015). On top of L3 and L4, the Indo-oceanic lineage (L1) was isolated from the homeless individuals in Addis Ababa. Similarly, L1 was reported from Ethiopia earlier by other researchers (Korma et al. 2015). However, the prevalence of L1 was very low in this study as compared to those of L3 and L4 although other researchers reported that L1 occurs abundantly in East Africa (Korma et al. 2015). The lower rate of L1 detection in this study could be due to the small number of isolates genotyped.

Based on MIRU-VNTR typing, T-sub-lineages were the most prevalent sub-lineages isolated by this study. This observation is consistent with the results of previous studies in Ethiopia (Agonafir et al. 2010; Biadglegne et al. 2015). Earlier studies documented that the T-sub-lineages are more associated with Ethiopia, among other countries in the Horn of Africa. And the result of this study thus re-affirmed the finding of the earlier study (Firdessa et al. 2013). It is not widely spread even in Ethiopia and is most commonly isolated from northeastern Ethiopia (Tessema et al. 2013). Hence, because of its limited geographic scope in Ethiopia, the chance of its spread to other countries could be minimal. However, the present geographic scope of this lineage could change in the future thereby favoring its widespread in Ethiopia and neighboring countries. Ethiopia_3 detected among the homeless study participants was the predominant Ethiopia-specific sub-lineage followed by Ethiopia-2, similar with prior research findings from northwestern (Biadglegne et al. 2015) and eastern Ethiopia (Ali et al. 2016). In contrast, a study conducted in southern Ethiopia (Wondale et al. 2020) reported that sub-lineage Ethiopia_2 is the dominant sub-lineage in the region. Ethiopia_H37Rv-like genotype which shares a common ancestor with H37Rv laboratory reference strains was also isolated by the present study and earlier similar studies (Aleksic et al. 2013; Malm et al. 2017; Niemann et al. 2010; Coll et al. 2014). This could suggest that strains of *M. tuberculosis* in Ethiopia share genetic similarities with the H37Rv strain.

Delhi/CAS was the second most prevalent sub-lineage detected in homeless individuals in Addis Ababa. This result agrees with the findings of several previous studies in Ethiopia (Tessema et al. 2013; Bedewi et al. 2017; Wondale et al. 2020; Comas, 2015; Agonafir et al.

2010; Mihret et al. 2012). Likewise, the Delhi/CAS sub-lineage was also prevalent in Tanzania (Ogaro et al. 2013), Sudan (Sharaf Eldin et al. 2011), Uganda (Asiimwe et al. 2008), and Kenya (Githui et al. 2004). Although this lineage is presumed to be geographically specific to India and central Asia, the bi-directional socioeconomic relationship between Ethiopia and India might have increased the spread of the Delhi/CAS lineage from India to Ethiopia. In this regard, Ethiopia was the first African country to open an embassy in New Delhi in 1948, which strengthened the relationship between the two countries as well as people-to-people interaction thereby favoring the transmission of the Delhi/CAS sub-lineage between Indians and Ethiopians. On top of this, the isolation of the Delhi/CAS sub-lineage from homeless individuals in Addis Ababa could be substantiated by the theory of "Out of Africa" (Comas et al. 2013) which stated that the origin of human beings and *M. tuberculosis* is East Africa and later on may have been reintroduce in the form of Delhi/CAS. This theory thus indirectly suggested that Delhi/CAS and the other sub-lineages of *M. tuberculosis* could be found in East African countries like Ethiopia.

The proportion of Manu sub-lineage recorded by the present study was similar to those reported by previous studies conducted in Ethiopia (Esmael et al. 2014) and Egypt (Helal et al. 2009). Trade routes in the past between continents may have promoted contact between individuals from different continents, which may have allowed Manu lineage, to proliferate. The increased international travel and migration patterns of the contemporary era may be partially responsible for the sporadic introduction of specific *M. tuberculosis* lineages into new states. Similar to the result of the present study, the Turkey lineage was also isolated earlier in Ethiopia (Bedewi et al. 2017; Wondale et al. 2020) which could be due

to the introduction of this lineage to Ethiopia from Turkey or Saudi Arabia by the movement of infected individuals. This may be due to the growing economic partnership between Ethiopia and Turkish and/or Saudi Arabia. Furthermore, it could also be introduced to Ethiopia by the return of infected Ethiopian immigrants from Saudi Arabia/Turkey to Ethiopia (Taye et al. 2021). Besides, the isolation of the Turkey sub-lineage in homeless individuals in Addis Ababa could be supported by the theory that East Africa is the origin of MTBC members (Comas et al. 2013).

The Haarlem sub-lineage detected in the present study is one of the most sub-lineages identified from Ethiopia and is believed to descend from European and Middle East countries (Githui et al. 2004; Demissie et al. 1997; Lari et al. 2005; Bicmen, 2007). And its isolation from the Ethiopians is not surprising because of the long history of interactions between Ethiopians, and Europeans or people from the Middle East countries. Furthermore, the LAM sub-lineage is frequently isolated from the general population of Ethiopia as observed in this study (Agonafir et al. 2010; Mihret et al. 2012), Tanzania (Githui et al. 2004) Uganda (Kibiki et al. 2007), and Kenya (Orago et al. 2013) whereas in Colombia, the Haarlem, LAM, and T sub-lineages were identified from homeless individuals (Hernández Sarmiento et al. 2013).

In the present study, the majority of the MIRU-VNTR alleles were highly and moderately discriminant as evaluated by the allelic diversity (*h*) result, which in turn indicates the representativeness of the study population (Sola et al. 2003). Furthermore, the finding of highly and moderately discriminant alleles suggest that the loci were suitable for

genotyping of the isolates. The results of MST and dendrogram analyses of strains of *M. tuberculosis* isolated from the homeless were consistent with the results of similar previous studies conducted in Ethiopia (Cowan et al. 2002; Tessema et al. 2013). Nonetheless, the profiles of 19% (11/58) and 8.6% (5/58) of the isolates did not match the profiles of strains deposited in the SIVITWEB and MIRU-VNTR*plus* databases, respectively. This suggests potential genetic diversity and unique strain variants circulating among homeless individuals in Ethiopia, highlighting the importance of ongoing surveillance and genetic characterization efforts to better understand TB transmission dynamics in this population. Similar findings of strain diversity among homeless individuals have been reported in previous studies conducted in other countries (Oeltmann et al. 2009; Smith and Trienekens, 2019), underscoring the need for comprehensive genomic studies to inform target TB control strategies.

The proportion of clustering in this study by using spoligotyping and 24-loci MIRU-VNTR typing is agreement with previous similar studies conducted in Ethiopia (Biadglegne et al. 2015; Taye et al. 2021; Yimer et al. 2015; Tafesse et al. 2021). However, the clustering rate based on a combination of spoligotyping and 24-loci MIRU-VNTR typing was lower than other previous studies in Ethiopia (Tessema et al. 2013; Mekonnen et al. 2018) and higher than a study reported from South Omo Zone Ethiopia (Wondale et al. 2020). This indicates that a combination of the genotyping methods is the best approach to determine clustering. The differences in clustering rates among studies could be due to variations in geography, population density, and socioeconomic diversity (Semunigus et al. 2016). The significant clustering rate in our study could also be associated with the presence of TB transmission

among homeless individuals in Addis Ababa, which may be due to overcrowding and other associated risk factors that favor the transmission of TB. Other previous studies have reported socio-demographic factors to be predictors of recent TB transmission, such as young age, ethnicity status, male sex, homelessness, incarceration, overcrowding, and drug abuse (Haddad et al. 2005). In our study overcrowding was significantly associated with the clustering of strains, indicating that homeless individuals are at risk of developing TB (Hernández Sarmiento et al. 2013), and also linked to recent transmission of TB in this population. Strains of the Ethiopia_3 sub-lineage was more likely to be clustered which could suggest a more frequent transmission of this strain in the homeless individuals.

Nowadays, it is widely recognized that TB infections with mixed strains occur often and that identifying individuals with multiple *M. tuberculosis* strains is essential for clinical practice, public health, and molecular epidemiology. The results of this study showed that 5.2% of homeless individuals with PTB, had mixed *M. tuberculosis* strain infections. Similarly, mixed *M. tuberculosis* strain infection has been reported from the general population in other African countries such as Uganda (Dickman et al. 2010; Muwonge et al. 2013), and Malawi (McIvor et al. 2017). Detection of mixed strains of *M. tuberculosis* among homeless individuals may be attributed to the mobility patterns of the homeless. This may raise a serious concern about the transmission, treatment, and general management of TB in this population. Two strains of *M. tuberculosis* can survive within a patient due to spatial separation within different lung regions or tissues, varying genetic adaptations that allow them to exploit different niches, differential antibiotic resistance that enables one strain to survive treatment, and immune evasion strategies that allow each strain to persist despite the host's immune response (Dickman et al. 2010). The 24-loci

MIRU-VNTR is a gold standard for diagnosing multiple-strain infections due to its higher sensitivity and discriminative power (Byrne et al. 2020).

Although the number of isolates was small, 6.8% of isolates were resistant to one or more first-line anti-TB drugs. Previous studies in Ethiopia reported similar findings from the general population (Temesgen et al. 2021). Furthermore, 4.4% of the *M. tuberculosis* isolates showed a mono-resistance pattern to either INH or SM. Three strains each consisting of two isolates from Ethiopia_3 and LAM demonstrated higher drug resistance. The prevalence of drug resistance in this study was higher than the proportion reported for newly treated TB cases from the general population in Ethiopia (WHO, 2022). The higher prevalence of drug resistance among homeless individuals may be attributed to delayed diagnosis (Villa et al. 2014) and the higher defaulter rate of previous anti-TB treatment (Ranzani et al. 2016). This finding suggests that drug resistance could be an emerging problem among homeless individuals with TB.

LIMITATIONS OF THE STUDY

One of the limitations of the study was study participants with suspected TB were not subjected to a chest X-ray. This may have underestimated the true prevalence of TB. The sole use of LJ for culturing *M. tuberculosis* and the modest number of isolates used for genotyping MTBC strains could be considered as a limitation. Furthermore, due to the study's short duration, epidemiological contact investigations among clustered isolates of homeless individuals were not possible. Moreover, the lower sensitivity of phenotypic DST methods and lack of molecular confirmation for drug-resistant strains of *M. tuberculosis* could also limit the precision of the results. However, regardless of its limitations, the result of the study could be considered as a valued input for the TB control program of the country.

5.1. CONCLUSIONS

1. The prevalence of bacteriologically confirmed PTB among homeless individuals in Addis Ababa, was four times higher than that in the City's general population and nine times higher than that of Ethiopia as a whole. This finding raises the possibility that homeless settings are TB transmission hotspots.
2. The detection of varied genotypes of MTBC strains, in homeless individuals, may suggest a possible role of homeless individuals in the spread of TB among the general population.
4. The high level of clustered strains detected in the study may be a result of recent or ongoing TB transmission among homeless individuals.
5. Among the several MTB lineages and sub-lineages, the "EA" lineage and "Ethiopia_3" sub-lineages were the ones the study predominantly detected in homeless individuals.
6. A considerable number of MTBC isolates, circulating among homeless individuals, were resistant to one or more first-line anti-TB drugs.
7. Mixed strains of *M. tuberculosis* were identified among homeless individuals in Addis Ababa.

5.2. RECOMMENDATIONS

Based on the study findings the following recommendations can be made:

1. To control the spread of TB among homeless individuals in Addis Ababa, active TB screening on entry to the shelters and at regular intervals followed by treatment is recommendable.
2. A more efficient intervention to control the spread of TB among the homeless and the broader Ethiopian population could be facilitated by using the whole genome sequencing method to target the Ethiopian MTBC sub-lineages.
3. Governmental and non-governmental organizations working on TB prevention and control must consider the homeless as the target population for the prevention and elimination of TB.

6. REFRECENCES

Abeje, A., 2021. Causes and effects of rural-urban migration in Ethiopia: A case study from Amhara Region. *African Studies*, *80*(1), pp.77-94.

Addis Ababa city administration health bureau. 2020. Annual TB activity report. Addis Ababa: AACAHB.

Agonafir, M., Lemma, E., Wolde-Meskel, D., Goshu, S., Santhanam, A., Girmachew, F., et al. 2010. Phenotypic and genotypic analysis of multidrug-resistant tuberculosis in Ethiopia. *Int J Tuberc Lung Dis*, *14*(10), pp.1259-1265.

Akwara, E., Worknesh, K., Oljiira, L., Mengesha, L., Asnake, M., Sisay, E., et al. 2022. ASRHR in Ethiopia: reviewing progress over the last 20 years and looking ahead to the next 10 years. *Reprod health*, *19*(1), pp.1-11.

Aldridge, R.W., Hayward, A.C., Hemming, S., Yates, S.K., Ferenando, G., Possas, L., et al. 2018. High prevalence of latent tuberculosis and bloodborne virus infection in a homeless population. *Thorax*, *73*(6), pp.557-564.

Alecrim, T.F.D.A., Mitano, F., Reis, A.A.D., Roos, C.M., Palha, P.F. and Protti-Zanatta, S.T., 2016. Experience of health professionals in care of the homeless population with tuberculosis. *Revista da Escola de Enfermagem da USP*, *50*, pp.808-815.

Aleksic, E., Merker, M., Cox, H., Reiher, B., Sekawi, Z., Hearps, A.C., et al. 2013. First molecular epidemiology study of *Mycobacterium tuberculosis* in Kiribati. *PloS one*, *8*(1), p.e55423.

Alelign, A., Zewude, A., Mohammed, T., Tolosa, S., Ameni, G. and Petros, B., 2019. Molecular detection of *Mycobacterium tuberculosis* sensitivity to rifampicin and isoniazid in South Gondar Zone, northwest Ethiopia. *BMC infectious diseases*, *19*(1), pp.1-8.

Alene, K.A. and Clements, A.C. 2019. Spatial clustering of notified tuberculosis in Ethiopia: A nationwide study. *PLoS One*, *14*(8), p.e0221027.

Alene, K.A., Elagali, A., Barth, D.D., Rumisha, S.F., Amratia, P., Weiss, D.J., et al. 2022. Spatial codistribution of HIV, tuberculosis and malaria in Ethiopia. *BMJ Glob Health*, *7*(2), p.e007599.

Ali, S., Beckert, P., Haileamlak, A., Wieser, A., Pritsch, M., Heinrich, N., et al. 2016. Drug resistance and population structure of *Mycobacterium tuberculosis* isolates from prisons and communities in Ethiopia. *BMC infect dis*, *16*(1), pp.1-10.

Allix-Béguec, C., Harmsen, D., Weniger, T., Supply, P. and Niemann, S. 2008. Evaluation and strategy for use of MIRU-VNTR plus, a multifunctional database for online analysis of genotyping data and phylogenetic identification of *Mycobacterium tuberculosis* complex isolates. *J Clin Microbiol*, *46*(8), pp.2692-2699.

Alonso-Rodríguez, N., Martínez-Lirola, M., Herránz, M., Sanchez-Benitez, M., Barroso, P., Bouza, E., et al. 2008. Evaluation of the new advanced 15-loci MIRU-VNTR genotyping tool in *Mycobacterium tuberculosis* molecular epidemiology studies. *BMC Microbiol*, *8*(1), pp.1-9.

Amiri, F.B., Doosti-Irani, A., Sedaghat, A., Fahimfar, N. and Mostafavi, E. 2018. Knowledge, attitude, and practices regarding HIV and TB among homeless people in Tehran, Iran. *Int J Health Policy Manag*, *7*(6), p.549.

Anderson, I. and Christian, J., 2003. Causes of homelessness in the UK: A dynamic analysis. *J Community Appl Soc Psychol*, *13*(2), pp.105-118.

Andrews, J.R., Noubary, F., Walensky, R.P., Cerda, R., Losina, E. and Horsburgh, C.R. 2012. Risk of progression to active tuberculosis following reinfection with *Mycobacterium tuberculosis*. *Clin Infect dis*, *54*(6), pp.784-791.

Arriaza, B.T., Salo, W., Aufderheide, A.C. and Holcomb, T.A. 1995. Pre-Columbian tuberculosis in Northern Chile: Molecular and skeletal evidence. *Am J Phys anthropol*, *98*(1), pp.37-45.

Asiimwe, B.B., Ghebremichael, S., Kallenius, G., Koivula, T. and Joloba, M.L. 2008. *Mycobacterium tuberculosis* spoligotypes and drug susceptibility pattern of isolates from tuberculosis patients in peri-urban Kampala, Uganda. *BMC Infect Dis*, *8*, pp.1-8.

Atnafu, A., Oucho, L. and Zeitlyn, B., 2014. Poverty, youth and rural-urban migration in Ethiopia.

Ayano, G., Assefa, D., Haile, K., Chaka, A., Solomon, H., Hagos, P., et al. 2017. Mental, neurologic, and substance use (MNS) disorders among street homeless people in Ethiopia. *Ann Gen Psychiatry*, *16*, pp.1-6.

Ayenew, M., Kabeta, T. and Woldemichael, K. 2020. Prevalence and factors associated with substance use among street children in Jimma town, Oromiya national regional state, Ethiopia: a community based cross-sectional study. *Subst Abuse Treat Prev Policy*, *15*(1), pp.1-9.

Bacha, H.A., Cimerman, S., Souza, S.A.D., Hadad, D.J. and Mendes, C.M.F. 2004. Prevalence of mycobacteremia in patients with AIDS and persistant fever. *Braz J Infect Dis*, *8*, pp.290-295.

Bamrah, S., Yelk Woodruff, R.S., Powell, K., Ghosh, S., Kammerer, J.S. and Haddad, M.B. 2013. Tuberculosis among the homeless, United States, 1994–2010. *Int J Tuberc Lung Dis*, *17*(11), pp.1414-1419.

Banu, S., Gordon, S.V., Palmer, S., Islam, R., Ahmed, S., Alam, K.M., et al. 2004. GenotypicAnalysis of *Mycobacterium tuberculosis* in Bangladesh andPrevalence of the BeijingStrain. *J Clin Microbiol*, *42*(2), pp.674-682.

Banuls, A.L., Sanou, A., Van Anh, N.T. and Godreuil, S. 2015. Mycobacterium tuberculosis: ecology and evolution of a human bacterium. *J Med Microbiol*, *64*(11), pp.1261-1269.

Barbier, M. and Wirth, T. 2016. The evolutionary history, demography, and spread of the *Mycobacterium tuberculosis* complex. *Microbiol Spectr*, *4*(4), pp.4-4.

Barletta, F., Otero, L., de Jong, B.C., Iwamoto, T., Arikawa, K., Van der Stuyft, P., et al. 2015. Predominant *Mycobacterium tuberculosis* families and high rates of recent transmission among new cases are not associated with primary multidrug resistance in Lima, Peru. *J Clin Microbioly*, *53*(6), pp.1854-1863.

Barnes, P.F. and Cave, M.D. 2003. Molecular epidemiology of tuberculosis. *N Engl J Med*, *349*(12), pp.1149-1156.

Barnes, P.F., El-Hajj, H., Preston-Martin, S., Cave, M.D., Jones, B.E., Otaya, M., et al. 1996. Transmission of tuberculosis among the urban homeless. *Jama*, *275*(4), pp.305-307.

Bedewi, Z., Worku, A., Mekonnen, Y., Yimer, G., Medhin, G., Mamo, G., et al. 2017. Molecular typing of *Mycobacterium tuberculosis* complex isolated from pulmonary tuberculosis patients in central Ethiopia. *BMC Infect s Dis*, *17*, pp.1-8.

Behr, M.A., Edelstein, P.H. and Ramakrishnan, L. 2019. Is *Mycobacterium tuberculosis* infection lifelong? *BMJ*, 367.

Beijer, U., Wolf, A. and Fazel, S. 2012. Prevalence of tuberculosis, hepatitis C virus, and HIV in homeless people: a systematic review and meta-analysis. *Lancet Infect Dis*, *12*(11), pp.859-870.

Ben-Selma, W., Ben-Kahla, I., Marzouk, M., Ferjeni, A., Ghezal, S., Ben-Said, M., et al. 2009. Rapid detection of *Mycobacterium tuberculosis* in sputum by Patho-TB kit in comparison with direct microscopy and culture. *Diagn Microbiol Infect*, *65*(3), pp.232-235.

Bernard, C., Sougakoff, W., Fournier, A., Larnaudie, S., Antoun, F., Robert, J., et al. 2012. Impact of a 14-year screening programme on tuberculosis transmission among the homeless in Paris. *Int J Tuberc Lung Dis*, *16*(5), pp.649-655.

Biadglegne, F., Merker, M., Sack, U., Rodloff, A.C. and Niemann, S. 2015. Tuberculous lymphadenitis in Ethiopia predominantly caused by strains belonging to the Delhi/CAS lineage and newly identified Ethiopian clades of the *Mycobacterium tuberculosis* complex. *PLoS One*, *10*(9), p.e0137865.

Bicmen, C., Esen, N., Graviss, E.A., Williams-Bouyer, N., Ramaswamy, S.V. and Yulug, N. 2007. Molecular characterization of *Mycobacterium tuberculosis* isolates from Izmir, Turkey. *New Microbiol*, *30*(3), pp.229-240.

Billington, O.J., McHugh, T.D. and Gillespie, S.H., 1999. Physiological cost of rifampin resistance induced in vitro in Mycobacterium tuberculosis. *Antimicrobial agents and chemotherapy*, *43*(8), pp.1866-1869.

Bodmer, T. and Ströhle, A. 2012. Diagnosing pulmonary tuberculosis with the Xpert MTB/RIF test. *J Vis Exp*, (62), p.e3547.

Boehme, C.C., Nabeta, P., Hillemann, D., Nicol, M.P., Shenai, S., Krapp, F., et al. 2010. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med*, *363*(11), pp.1005-1015.

Brewer, T.F., Heymann, S.J., Krumplitsch, S.M., Wilson, M.E., Colditz, G.A. and Fineberg, H.V. 2001. Strategies to decrease tuberculosis in US homeless populations: a computer simulation model. *Jama*, *286*(7), pp.834-842.

Brosch, R., Gordon, S.V., Marmiesse, M., Brodin, P., Buchrieser, C., Eiglmeier, K., et al. 2002. A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. *Proc Natl Acad Sci*, *99*(6), pp.3684-3689.

Brudey, K., Driscoll, J.R., Rigouts, L., Prodinger, W.M., Gori, A., Al-Hajoj, S.A., et al. 2006. *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. *BMC Microbiol*, *6*, pp.1-17.

Brynildsrud, O.B., Pepperell, C.S., Suffys, P., Grandjean, L., Monteserin, J., Debech, N., et al. 2018. Global expansion of *Mycobacterium tuberculosis* lineage 4 shaped by colonial migration and local adaptation. *Sci Adv*, *4*(10), p.eaat5869.

Byrne, A.S., Goudreau, A., Bissonnette, N., Shamputa, I.C. and Tahlan, K., 2020. Methods for detecting mycobacterial mixed strain infections–a systematic review. *Fron in genet*, *11*, p.600692.

Camus, J.C., Pryor, M.J., Médigue, C. and Cole, S.T. 2002. Re-annotation of the genome sequence of Mycobacterium tuberculosis H37Rv. *Microbiol*, *148*(10), pp.2967-2973.

Cannas, A., Mazzarelli, A., Di Caro, A., Delogu, G. and Girardi, E. 2016. Molecular typing of *Mycobacterium tuberculosis* strains: a fundamental tool for tuberculosis control and elimination. *Infect Dis Rep*, *8*(2), p.6567.

Carr, W. 2022. Interim Guidance: 4-Month Rifapentine-Moxifloxacin Regimen for the Treatment of Drug-Susceptible Pulmonary Tuberculosis—United States, 2022. *MMWR. Morbidity and Mortality Weekly Report*, *71*.

Cawley, C., Kanzaria, H.K., Zevin, B., Doran, K.M., Kushel, M. and Raven, M.C. 2022. Mortality among people experiencing homelessness in San Francisco during the COVID-19 pandemic. *JAMA Network Open*, *5*(3), pp.e221870-e221870.

Centers for Disease Control and Prevention 2012. Multidrug-resistantTuberculosis. National Center for HIV/AIDS, Viral Hepatitis, STD, TB Prevalence.

Centers for Disease Control and Prevention 2022. Surveillance definitions for extensively drug-resistant (XDR) and pre-XDR tuberculosis.

Centers for Disease Control and Prevention. 2016. TB in the homeless population.

Central Statistical Agency of Ethiopia and Inner-City Fund International. 2019. Ethiopia demographic health survey. Addis Ababa, Ethiopia, and Calverton, Mary Land, USA: CSA and ICF.

Marks, S.M., Self, J.L., Venkatappa, T., Wolff, M.B., Hopkins, P.B., Augustine, R.J., et al. 2023. Diagnosis, Treatment, and Prevention of Tuberculosis Among People Experiencing Homelessness in the United States: Current Recommendations. *Public Health Reports*, p.00333549221148173.

Chakravorty, S., Simmons, A.M., Rowneki, M., Parmar, H., Cao, Y., Ryan, J., et al, J. 2017. The new Xpert MTB/RIF Ultra: improving detection of *Mycobacterium tuberculosis* and resistance to rifampin in an assay suitable for point-of-care testing. *MBio*, *8*(4), pp.e00812-17.

Chihota, V.N., Niehaus, A., Streicher, E.M., Wang, X., Sampson, S.L., Mason, P., et al. 2018. Geospatial distribution of *Mycobacterium tuberculosis* genotypes in Africa. *PLoS One*, *13*(8), p.e0200632

Cole, S., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., et al. 1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nat*, *396*(6707), pp.190-190.

Coll, F., McNerney, R., Guerra-Assunção, J.A., Glynn, J.R., Perdigão, J., Viveiros, M., et al. 2014. A robust SNP barcode for typing *Mycobacterium tuberculosis* complex strains. *Nat Commun*, *5*(1), p.4812.

Comas, I. and Gagneux, S. 2009. The past and future of tuberculosis research. *PLoS pathogens*, *5*(10), p.e1000600.

Comas, I., Coscolla, M., Luo, T., Borrell, S., Holt, K.E., Kato-Maeda, M., et al. 2013. Out-of-Africa migration and Neolithic coexpansion of *Mycobacterium tuberculosis* with modern humans. *Nat genet*, *45*(10), pp.1176-1182.

Constantine, N.T. and Zink, H. 2005. HIV testing technologies after two decades of evolution. *Indian J Med Res*, *121*(4), p.519.

Coscolla, M. and Gagneux, S. 2014. December. Consequences of genomic diversity in *Mycobacterium tuberculosis*. In *Seminars in immunology*, 26 (6) pp. 431-444. Academic Press.

Cowan, L.S., Diem, L., Monson, T., Wand, P., Temporado, D., Oemig, T.V., et al. 2005. Evaluation of a two-step approach for large-scale, prospective genotyping of *Mycobacterium tuberculosis* isolates in the United States. *J Clin Microbiol*, *43*(2), pp.688-695.

Crane, M. and Joly, L., 2014. Older homeless people: increasing numbers and changing needs. *Rev Clin Gerontol*, *24*(4), pp.255-268.

Crofton, J. and Mitchison, D.A., 1948. Streptomycin resistance in pulmonary tuberculosis. *British Med J*, *2*(4588), p.1009.

Cronin, W.A., Golub, J.E., Magder, L.S., Baruch, N.G., Lathan, M.J., Mukasa, L.N., et al. 2001. Epidemiologic usefulness of spoligotyping for secondary typing of *Mycobacterium tuberculosis* isolates with low copy numbers of IS 6110. *J Clin Microbiol*, *39*(10), pp.3709-3711.

Crosby, L., Lewer, D., Appleby, Y., Anderson, C., Hayward, A. and Story, A. 2023. Outcomes of a residential respite service for homeless people with tuberculosis in London, UK: a cross-sectional study. *Perspect Public Health*, *143*(2), pp.89-96.

Daniel, T.M., Bates, J.H. and Downes, K.A., 1994. History of tuberculosis. *Tuberculosis: pathogenesis, protection, and control*, pp.13-24.

Davis, J.L., Ho, C. and Cattamanchi, A. 2013. Availability of an assay for detecting *Mycobacterium tuberculosis*, including rifampin-resistant strains, and considerations for its use—United States, 2013.

de Beer, J.L., van Ingen, J., de Vries, G., Erkens, C., Sebek, M., Mulder, A., Sloot, R., et al. 2013. Comparative study of IS 6110 restriction fragment length polymorphism and variable-number tandem-repeat typing of *Mycobacterium tuberculosis* isolates in the Netherlands, based on a 5-year nationwide survey. *J Clin Microbiol 51*(4), pp.1193-1198.

Debebe, T., Admassu, A., Mamo, G. and Ameni, G. 2014. Molecular characterization of *Mycobacterium tuberculosis* isolated from pulmonary tuberculosis patients in Felege Hiwot Referral Hospital, northwest Ethiopia. *J Microbiol, Immun Infect*, *47*(4), pp.333-338.

Delogu, G., Sali, M. and Fadda, G. 2013. The biology of mycobacterium tuberculosis infection. *Mediterr J Hematol Infect Dis*, *5*(1).

Demay, C., Liens, B., Burguière, T., Hill, V., Couvin, D., Millet, J., et al. 2012. SITVITWEB–a publicly available international multimarker database for studying *Mycobacterium tuberculosis* genetic diversity and molecular epidemiology. *Infect Genet Evol*, *12*(4), pp.755-766.

Demissie, M., Gebeyehu, M. and Berhane, Y. 1997. Primary resistance to anti-tuberculosis drugs in Addis Ababa, Ethiopia. *Int J Tuberc Lung Dis*, *1*(1), pp.64-67.

Dickman, K.R., Nabyonga, L., Kateete, D.P., Katabazi, F.A., Asiimwe, B.B., Mayanja, H.K., et al. 2010. Detection of multiple strains of Mycobacterium tuberculosis using MIRU-VNTR in patients with pulmonary tuberculosis in Kampala, Uganda. *BMC Inf Dis*, *10*(1), pp.1-7.

Diriba, G., Kebede, A., Tola, H.H., Alemu, A., Yenew, B., Moga, S., et al. 2021. Mycobacterial Lineages Associated with Drug Resistance in Patients with Extrapulmonary Tuberculosis in Addis Ababa, Ethiopia. *Tuberc Res Treat*, *2021*, pp.1-7.

Dolin, P.J., Raviglione, M.C. and Kochi, A., 1994. Global tuberculosis incidence and mortality during 1990-2000. *Bulletin of the World Health Organization*, *72*(2), p.213.

Dolla, C., Padmapriyadarsini, C., Pradeep Menon, A., Muniyandi, M., Adinarayanan, S., Sekar, G., et al. 2017. Tuberculosis among the homeless in Chennai city, South India. *Trans R Soc Trop Med Hyg*, *111*(10), pp.479-481.

Donnelly, J.A., Cowan, D.T. and Whitley, M.A. 2019. Understanding the longitudinal impact of the Homeless World Cup on the lives of its participants. In *15th European Congress Sport and Exercise Psychology*.

Douglas, J.T., Qian, L., Montoya, J.C., Musser, J.M., Van Embden, J.D., Van Soolingen, D., et al. 2003. Characterization of the Manila family of *Mycobacterium tuberculosis*. *J Clin Microbiol*, *41*(6), pp.2723-2726.

Driscoll, J.R. 2009. Spoligotyping for molecular epidemiology of the Mycobacterium tuberculosis complex. *Molecular Epidemiology of Microorganisms: Methods and Protocols*, pp.117-128.

Dworsky, A., Napolitano, L. and Courtney, M., 2013. Homelessness during the transition from foster care to adulthood. *Am J Public Health*, *103*(S2), pp.S318-S323.

Echenberg, H. and Jensen, H., 2012. *Defining and enumerating homelessness in Canada*. Montreal: Library of Parliament.

Edgar, B., Harrison, M., Watson, P. and Busch-Geertsema, V. 2007. Measurement of homelessness at European Union level. *Brussels: European Commission., download under http://ec. europa. eu/employment_social/social_inclusion/docs/2007/stud y_homelessness_en. pdf*.

Ei, P.W., Aung, W.W., Lee, J.S., Choi, G.E. and Chang, C.L. 2016. Molecular strain typing of *Mycobacterium tuberculosis*: a review of frequently used methods. *J Korean Med Sci*, *31*(11), pp.1673-1683.

Emery, J.C., Richards, A.S., Dale, K.D., McQuaid, C.F., White, R.G., Denholm, J.T., et al. 2021. Self-clearance of Mycobacterium tuberculosis infection: implications for lifetime risk and population at-risk of tuberculosis disease. *Proc R Soc B*, *288*(1943), p.20201635.

Esmael, A., Wubie, M., Desta, K., Ali, I. and Endris, M. 2014. Genotyping and drug resistance patterns of *Mycobacterium tuberculosis* in Eastern Amhara Region, Ethiopia. *J Clin Diagn Res*, *2*(102), pp.2376-0311.

FEANTSA. 2007. *FEANTSA Proposal. A Retrospective Module on Homelessness or Household Surveys*. Brussels: FEANTSA.

Federal Democratic Republic of Ethiopia-Ministry of Health (FDRE-MOH) .2017. Guideline for Clinical and programmatic management of TB, Leprosy and TB/HIV in Ethiopia, 6th Edition. Addis Ababa, Ethiopia

Federal Democratic Republic of Ethiopia-Ministry of Health (FDRE-MOH). 2021. Guideline for Clinical and programmatic management of TB, Leprosy and TB/HIV in Ethiopia, 7th Edition. Addis Ababa, Ethiopia

Federal Democratic Republic of Ethiopia-Ministry of Health (FDRE-MOH) .2016. Guideline for Clinical and programmatic management of TB, Leprosy and TB/HIV in Ethiopia, 5th Edition. Addis Ababa, Ethiopia

Fekadu, A., Hanlon, C., Gebre-Eyesus, E., Agedew, M., Solomon, H., Teferra, S., et al. 2014. Burden of mental disorders and unmet needs among street homeless people in Addis Ababa, Ethiopia. *BMC medicine*, *12*(1), pp.1-12.

Filliol, I., Driscoll, J.R., Van Soolingen, D., Kreiswirth, B.N., Kremer, K., Valétudie, G., et al. 2003. Snapshot of moving and expanding clones of *Mycobacterium tuberculosis* and their global distribution assessed by spoligotyping in an international study. *J Clin Microbiol*, *41*(5), pp.1963-1970.

Filliol, I., Driscoll, J.R., Van Soolingen, D., Kreiswirth, B.N., Kremer, K., Valétudie, G., et al. 2002. Global distribution of *Mycobacterium tuberculosis* spoligotypes.

Firdessa, R., Berg, S., Hailu, E., Schelling, E., Gumi, B., Erenso, G., et al. 2013. Mycobacterial lineages causing pulmonary and extrapulmonary tuberculosis, Ethiopia. *Emerg Infect Dis*, *19*(3), p.460.

Forbes, B.A. 2017. Mycobacterial taxonomy. *J Clin Microbiol*, *55*(2), pp.380-383.

Frith, J. 2014. History of tuberculosis. Part 1-phthisis, consumption and the white plague. *J Mil Veterans Health*, *22*(2), pp.29-35.

Gagneux, S. 2018. Ecology and evolution of *Mycobacterium tuberculosis*. *Nat Rev Microbiol*, *16*(4), pp.202-213.

Gagneux, S., DeRiemer, K., Van, T., Kato-Maeda, M., De Jong, B.C., Narayanan, S., et al. 2006. Variable host–pathogen compatibility in *Mycobacterium tuberculosis*. *Proc Natl Acad Sci*, *103*(8), pp.2869-2873.

Garedew, L., Mihret, A., Mamo, G., Abebe, T., Firdessa, R., Bekele, Y., et al. 2013. Strain diversity of mycobacteria isolated from pulmonary tuberculosis patients at Debre Birhan Hospital, Ethiopia. *Int J Tuberc Lung Dis*, *17*(8), pp.1076-1081.

Garnier, T., Eiglmeier, K., Camus, J.C., Medina, N., Mansoor, H., Pryor, M., et al. 2003. The complete genome sequence of *Mycobacterium bovis*. *Proc Natl Acad Sci*, *100*(13), pp.7877-7882.

Gidado, M., Nwokoye, N., Ogbudebe, C., Nsa, B., Nwadike, P., Ajiboye, P., et al. 2019. Assessment of GeneXpert MTB/RIF performance by type and level of health-care facilities in Nigeria. *Niger Med J*, *60*(1), p.33.

Githui, W.A., Jordaan, A.M., Juma, E.S., Kinyanjui, P., Karimi, F.G., Kimwomi, J., et al. 2004. Identification of MDR-TB Beijing/W and other *Mycobacterium tuberculosis* genotypes in Nairobi, Kenya. *Int J Tuberc Lung Dis*, *8*(3), pp.352-360.

Goguet de la Salmoniere, Y.O., Li, H.M., Torrea, G., Bunschoten, A., Van Embden, J. and Gicquel, B. 1997. Evaluation of spoligotyping in a study of the transmission of Mycobacterium tuberculosis. *J Clin Microbiol*, *35*(9), pp.2210-2214.

Grange, J.M. and Zumla, A.I. 2009. Human tuberculosis due to *Mycobacterium bovis* and related animal pathogens. *Int Tuberc* (pp. 146-153). WB Saunders.

Gu, Y., Wang, G., Dong, W., Li, Y., Ma, Y., Shang, Y., et al. 2015. Xpert MTB/RIF and GenoType MTBDRplus assays for the rapid diagnosis of bone and joint tuberculosis. *Int J Infect Dis*, *36*, pp.27-30.

Haddad, M.B., Wilson, T.W., Ijaz, K., Marks, S.M. and Moore, M. 2005. Tuberculosis and homelessness in the United States, 1994-2003. *Jama*, *293*(22), pp.2762-2766.

Haile, K., Umer, H., Fanta, T., Birhanu, A., Fejo, E., Tilahun, Y., et al.,2020. Pathways through homelessness among women in Addis Ababa, Ethiopia: A qualitative study. *Plos one*, *15*(9), p.e0238571.

Han, H., Wang, F., Xiao, Y., Ren, Y., Chao, Y., Guo, A., et al. 2007. Utility of mycobacterial interspersed repetitive unit typing for differentiating *Mycobacterium tuberculosis* isolates in Wuhan, China. *J Med Microbiol*, *56*(9), pp.1219-1223.

Hasan, M., Munshi, S.K., Momi, M.S.B., Rahman, F. and Noor, R. 2013. Evaluation of the effectiveness of BACTEC MGIT 960 for the detection of mycobacteria in Bangladesh. *Int J Mycobacteriol*, *2*(4), pp.214-219.

Heifets, L.B. and Cangelosi, G.A. 1999. Drug susceptibility testing of *Mycobacterium tuberculosis*: a neglected problem at the turn of the century [State of the Art]. *Int J Tuberc Lung Dis*, *3*(7), pp.564-581.

Helal, Z.H., El-Din Ashour, M.S., Eissa, S.A., Abd-Elatef, G., Zozio, T., Babapoor, S., et al. 2009. Unexpectedly high proportion of ancestral Manu genotype

Mycobacterium tuberculosis strains cultured from tuberculosis patients in Egypt. *J Clin Microbiol*, *47*(9), pp.2794-2801.

Heo, D.J., Min, H.G. and Lee, H.H., 2012. The clinical characteristics and predictors of treatment success of pulmonary tuberculosis in homeless persons at a public hospital in Busan. *Korean J Family Med*, *33*(6), pp.372-380.

Hermans, P.W., Van Soolingen, D., Bik, E.M., De Haas, P.E., Dale, J.W. and Van Embden, J.D. 1991. Insertion element IS987 from Mycobacterium bovis BCG is located in a hot-spot integration region for insertion elements in *Mycobacterium tuberculosis* complex strains. *Infect Immun*, *59*(8), pp.2695-2705.

Hernández Sarmiento, J.M., Correa, N., Correa, M., Franco, J.G., Alvarez, M., Ramírez, C., et al. 2013. Tuberculosis among homeless population from Medellín, Colombia: associated mental disorders and socio-demographic characteristics. *J Immigr Minor Health*, *15*, pp.693-699.

Houben, R.M. and Dodd, P.J. 2016. The global burden of latent tuberculosis infection: a re-estimation using mathematical modeling. *PLoS Med*, *13*(10), p.e1002152.

Hunter, P.R. and Gaston, M.A., 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *J Clin Microbiol*, *26*(11), pp.2465-2466.

Jagielski, T., Van Ingen, J., Rastogi, N., Dziadek, J., Mazur, P.K. and Bielecki, J. 2014. Current methods in the molecular typing of *Mycobacterium tuberculosis* and other mycobacteria. *BioMed Res Int*, *2014*.

Jia, X., Yang, L., Dong, M., Chen, S., Lv, L., Cao, D., Fu, J., et al. 2017. The bioinformatics analysis of comparative genomics of *Mycobacterium tuberculosis* complex (MTBC) provides insight into dissimilarities between intraspecific groups differing in host association, virulence, and epitope diversity. *Front Cell Infect Microbiol*, *7*, p.88.

Johnston, D., McInerney, P. and Miot, J. 2019. A profile of the health of homeless at an inner city primary health care clinic in South Africa. *J Health Care Poor Underserved*, *30*(4), pp.1455-1466.

Kamerbeek, J., Schouls, L.E.O., Kolk, A., Van Agterveld, M., Van Soolingen, D., Kuijper, S., et al. 1997. Simultaneous detection and strain differentiation of *Mycobacterium*

tuberculosis for diagnosis and epidemiology. *J Cclin Microbiol*, *35*(4), pp.907-914.

Kato-Maeda, M., Metcalfe, J.Z. and Flores, L. 2011. Genotyping of *Mycobacterium tuberculosis*: application in epidemiologic studies. *Future Microbiol*, *6*(2), pp.203-216.

Kebede, A.H., Alebachew, Z., Tsegaye, F., Lemma, E., Abebe, A., Agonafir, M., et al. 2014. The first population-based national tuberculosis prevalence survey in Ethiopia, 2010-2011. *Int J Tuberc Lung Dis*, *18*(6), pp.635-639.

Kenaope, L., Ferreira, H., Seedat, F., Otwombe, K., Martinson, N.A. and Variava, E. 2020. Sputum culture and drug sensitivity testing outcome among X-pert *Mycobacterium tuberculosis*/rifampicin-positive, rifampicin-resistant sputum: A retrospective study—Not all rifampicin resistance is multi-drug resistant. *J Glob Antimicrob Resist*, *21*, pp.434-438.

Kent, P.T. 1985. *Public health mycobacteriology: a guide for the level III laboratory*. US Department of Health and Human Services, Public Health Service, Centers for Disease Control.

Kerantzas, C.A. and Jacobs Jr, W.R. 2017. Origins of combination therapy for tuberculosis: lessons for future antimicrobial development and application. *MBio*, *8*(2), pp.e01586-16.

Kibiki, G.S., Mulder, B., Dolmans, W.M., de Beer, J.L., Boeree, M., Sam, N., et al. 2007. *Mycobacterium tuberculosis* genotypic diversity and drug susceptibility pattern in HIV-infected and non-HIV-infected patients in northern Tanzania. *BMC Microbiol*, *7*, pp.1-8.

Koch, R., 2010. *Aetiology of Tuberculosis*. *Nabu Press*.

Korma, W., Mihret, A., Hussien, J., Anthony, R., Lakew, M. and Aseffa, A. 2015. Clinical, molecular and drug sensitivity pattern of mycobacterial isolates from extra-pulmonary tuberculosis cases in Addis Ababa, Ethiopia. *BMC Infect Dis*, *15*, pp.1-12.

Kremer, K., Glynn, J.R., Lillebaek, T., Niemann, S., Kurepina, N.E., Kreiswirth, B.N., et al. 2004. Definition of the Beijing/W lineage of *Mycobacterium tuberculosis* on the basis of genetic markers. *J Clin Microbiol*, *42*(9), pp.4040-4049.

Kremer, K., Van Soolingen, D., Frothingham, R., Haas, W.H., Hermans, P.W.M., Martin, C., et al. 1999. Comparison of methods based on different molecular epidemiological markers for typing of *Mycobacterium tuberculosis* complex strains: interlaboratory study of discriminatory power and reproducibility. *J Clin Microbiol*, *37*(8), pp.2607-2618.

Kulkarni, S., Sola, C., Filliol, I., Rastogi, N. and Kadival, G. 2005. Spoligotyping of *Mycobacterium tuberculosis* isolates from patients with pulmonary tuberculosis in Mumbai, India. *Res Microbiol*, *156*(4), pp.588-596.

Kwan, C.K. and Ernst, J.D., 2011. HIV and tuberculosis: a deadly human syndemic. *Clin microbiol rev*, *24*(2), pp.351-376.

Lari, N., Rindi, L., Sola, C., Bonanni, D., Rastogi, N., Tortoli, E., et al. 2005. Genetic diversity, determined on the basis of katG463 and gyrA95 polymorphisms, spoligotyping, and IS 6110 typing, of Mycobacterium tuberculosis complex isolates from Italy. *J Clin Microbiol*, *43*(4), pp.1617-1624.

Laurenti, P., Bruno, S., Quaranta, G., La Torre, G., Cairo, A.G., Nardella, P., et al. 2012. Tuberculosis in sheltered homeless population of Rome: an integrated model of recruitment for risk management. *Sci World J*, *2012*.

Lee, B.A., Tyler, K.A. and Wright, J.D. 2010. The new homelessness revisited. *Annu Rev Sociol*, *36*, pp.501-521.

Li, Z.N., Liu, M., Lü, B., Zhao, X.Q., Liu, Z.G., Jiao, W.W., et al. 2009. Comparison on discriminatory power of different variable number tandem repeats locus-set on genotyping of *Mycobacterium tuberculosis* isolated in China. *Zhonghua yu Fang yi xue za zhi [Chi J Prev Med]*, *43*(3), pp.215-222.

Lienhardt, C., Glaziou, P., Uplekar, M., Lönnroth, K., Getahun, H. and Raviglione, M. 2012. Global tuberculosis control: lessons learned and prospects. *Nat Rev Microbiol*, *10*(6), pp.407-416.

Lönnroth, K., Castro, K.G., Chakaya, J.M., Chauhan, L.S., Floyd, K., Glaziou, P., et al. 2010. Tuberculosis control and elimination 2010–50: cure, care, and social development. *The lancet*, *375*(9728), pp.1814-1829.

Lukács, J., Tubak, V., Mester, J., Dávid, S., Bártfai, Z., Kubica, T., et al. 2004. Conventional and molecular epidemiology of tuberculosis in homeless patients in Budapest, Hungary. *J Clin Microbiol*, *42*(12), pp.5931-5934.

MacLean, E., Broger, T., Yerlikaya, S., Fernandez-Carballo, B.L., Pai, M. and Denkinger, C.M., 2019. A systematic review of biomarkers to detect active tuberculosis. *Nature Microbiology*, *4*(5), pp.748-758.

Maher, D., Grzemska, M., Coninx, R. and Reyes, H. 1998. *Guidelines for the control of tuberculosis in prisons* (No. WHO/TB/98.250). World Health Organization.

Mahla, R.S., 2018. Prevalence of drug-resistant tuberculosis in South Africa. *Lancet Infect Dis*, *18*(8), p.836.

Malm, S., Linguissi, L.S.G., Tekwu, E.M., Vouvoungui, J.C., Kohl, T.A., Beckert, P., et al. 2017. New *Mycobacterium tuberculosis* complex sublineage, Brazzaville, Congo. *Emerg Infect Dis*, *23*(3), p.423.

Marutlulle, N.K. 2021. A critical analysis of housing inadequacy in South Africa and its ramifications. *Africa's Public Service Delivery & Performance Review*, *9*(1), p.16.

McEvoy, C.R., van Pittius, N.C.G., Victor, T.C., van Helden, P.D. and Warren, R.M., 2007. The role of IS6110 in the evolution of *Mycobacterium tuberculosis*. *Tuberc*, *87*(5), pp.393-404.

McHenry, M.L., Bartlett, J., Igo Jr, R.P., Wampande, E.M., Benchek, P., Mayanja-Kizza, H., et al. 2020. Interaction between host genes and *Mycobacterium tuberculosis* lineage can affect tuberculosis severity: Evidence for coevolution?. *PLoS Genet*, *16*(4), p.e1008728.

McIvor, A., Koornhof, H. and Kana, B.D., 2017. Relapse, re-infection, and mixed infections in tuberculosis disease. *Pathog and Dis*, *75*(3), p.ftx020.

McShane, H. 2011. Tuberculosis vaccines: beyond bacille Calmette–Guérin. *Philosophical transactions of the royal society B: Biological Sciences*, *366*(1579), pp.2782-2789.

Meehan, C.J., Moris, P., Kohl, T.A., Pečerska, J., Akter, S., Merker, M., et al., 2018. The relationship between transmission time and clustering methods in *Mycobacterium tuberculosis* epidemiology. *EBioMed*, *37*, pp.410-416.

Mekonnen, A., Merker, M., Collins, J.M., Addise, D., Aseffa, A., Petros, B., et al. 2018. Molecular epidemiology and drug resistance patterns of *Mycobacterium tuberculosis* complex isolates from university students and the local community in Eastern Ethiopia. *PLoS One*, *13*(9), p.e0198054.

Mekonnen, D., Derbie, A., Chanie, A., Shumet, A., Biadglegne, F., Kassahun, Y., et al. 2019. Molecular epidemiology of *Mycobacterium tuberculosis* in Ethiopia: A systematic review and meta-analysis. *Tuberc*, *118*, p.101858.

Mengesha, M.M., Gebremichael, M.A., Watumo, D., Hallström, I.K. and Jerene, D. 2022. Poor adult tuberculosis treatment outcome and associated factors in Gibe Woreda, Southern Ethiopia: an institution-based cross-sectional study. *PLOS Glob Public Health*, *2*(3), p.e0000161.

Merker, M., Barbier, M., Cox, H., Rasigade, J.P., Feuerriegel, S., Kohl, T.A., et al. 2018. Compensatory evolution drives multidrug-resistant tuberculosis in Central Asia. *Elife*, *7*, p.e38200.

Migliori, G.B., Dheda, K., Centis, R., Mwaba, P., Bates, M., O'Grady, J.,et al. 2010. Review of multidrug-resistant and extensively drug-resistant TB: global perspectives with a focus on sub-Saharan Africa. *Trop Med Int Health*, *15*(9), pp.1052-1066.

Mihret, A., Bekele, Y., Loxton, A.G., Jordan, A.M., Yamuah, L., Aseffa, A., et al. 2012. Diversity of *Mycobacterium tuberculosis* isolates from new pulmonary tuberculosis cases in Addis Ababa, Ethiopia. *Tuberc Res Treat*, *2012*.

Mokrousov, I., Vyazovaya, A., Iwamoto, T., Skiba, Y., Pole, I., Zhdanova, S., et al. 2016. Latin-American-Mediterranean lineage of *Mycobacterium tuberculosis*: Human traces across pathogen's phylogeography. *Mol Phylogenetics Evol*, *99*, pp.133-143.

Moonan, P.K., Ghosh, S., Oeltmann, J.E., Kammerer, J.S., Cowan, L.S. and Navin, T.R. 2012. Using genotyping and geospatial scanning to estimate recent *Mycobacterium tuberculosis* transmission, United States. *Emerg Infect Dis*, *18*(3), p.458.

Moore, G., Manias, E. and Gerdtz, M.F., 2011. Complex health service needs for people who are homeless. *Australian Health Review*, *35*(4), pp.480-485.

Morrison, J., Pai, M. and Hopewell, P.C. 2008. Tuberculosis and latent tuberculosis infection in close contacts of people with pulmonary tuberculosis in low-income

and middle-income countries: a systematic review and meta-analysis. *Lancet Infect Dis*, 8(6), pp.359-368.

Moss, A.R., Hahn, J.A., Tulsky, J.P., Daley, C.L., Small, P.M. and Hopewell, P.C. 2000. Tuberculosis in the homeless: a prospective study. *Am J Respir Crit Care Med*, *162*(2), pp.460-464.

Munn, M.S., Duchin, J.S., Kay, M., Pecha, M., Thibault, C.S. and Narita, M. 2015. Analysis of risk factors for tuberculous infection following exposure at a homeless shelter. *Int J Tuberc Lung Dis*, *19*(5), pp.570-575.

Muwonge, A., Malama, S., Johansen, T.B., Kankya, C., Biffa, D., Ssengooba, W., et al. 2013. Molecular epidemiology, drug susceptibility and economic aspects of tuberculosis in Mubende district, Uganda. *PLoS One*, *8*(5), p.e64745.

Narasimhan, P., Wood, J., MacIntyre, C.R. and Mathai, D. 2013. Risk factors for tuberculosis. *Pulmonary medicine*, *2013*.

Naya, H., Romero, H., Zavala, A., Alvarez, B. and Musto, H. 2002. Aerobiosis increases the genomic guanine plus cytosine content (GC %) in prokaryotes. *J Mol Evol 55*, pp.260-264.

Ngabonziza, J.C.S., Loiseau, C., Marceau, M., Jouet, A., Menardo, F., Tzfadia, O., et al. 2020. A sister lineage of the *Mycobacterium tuberculosis* complex discovered in the African Great Lakes region. *Nat Commun*, *11*(1), p.2917.

Nguyen, Q.H., Contamin, L., Nguyen, T.V.A. and Bañuls, A.L. 2018. Insights into the processes that drive the evolution of drug resistance in *Mycobacterium tuberculosis*. *Evol Appl*, *11*(9), pp.1498-1511.

Niemann, S., Merker, M., Kohl, T. and Supply, P., 2016. Impact of genetic diversity on the biology of Mycobacterium tuberculosis complex strains. *Microbiol Spect*, *4*(6), pp.10-1128.

Nolan, C.M., Elarth, A.M., Barr, H., Saeed, A.M. and Risser, D.R. 1991. An outbreak of tuberculosis in a shelter for homeless men. *Am Rev Respir Dis*, *143*, pp.257-261.

Nooe, R.M. and Patterson, D.A., 2010. The ecology of homelessness. *J Hum Behav Soc Environ*, *20*(2), pp.105-152.

Nordentoft, M. and Wandall-Holm, N. 2003. 10 year follow up study of mortality among users of hostels for homeless people in Copenhagen. *BMJ*, *327*(7406), p.81.

Notaro, S.J., Khan, M., Kim, C., Nasaruddin, M. and Desai, K. 2013. Analysis of the health status of the homeless clients utilizing a free clinic. *J Community Health Res, 38*, pp.172-177.

Oeltmann, J.E., Oren, E., Haddad,M.B., Lake,L., Harrington, T.A., Ijaz,K., et al. 2009. Tuberculosis Outbreak in Homeless population, Multnomah County, Oregon, USA, 2002-2006. *Emerg Inf Dis*, 15(7), 1055-1058.

Ogaro, T.D., Githui, W., Kikuvi, G., Okari, J., Asiko, V., Wangui, E., et al. 2013. Diversity of *Mycobacterium tuberculosis* strains in Nairobi, Kenya. *Afr J Health Sci*, *24*(1), pp.58-68.

Oudghiri, A., Chaoui, I. and El Mzibri, M. 2018. Molecular epidemiology of tuberculosis: a review of tools and applications. *J Infect Dis Ther*, *6*(6), p.386.

Ozlu, M.O., Alemayehu, A., Mukim, M., Lall, S.V., Kerr, O.T., Kaganova, O., et al. 2015. Ethiopia-Urbanization review: urban institutions for a middle-income Ethiopia. *Washington, DC: World Bank Group*.

Parija, D., Patra, T.K., Kumar, A.M.V., Swain, B.K., Satyanarayana, S., Sreenivas, A., et al. 2014. Impact of awareness drives and community-based active tuberculosis case finding in Odisha, India. *Int J Ttuberc Lung Dis*, *18*(9), pp.1105-1107.

Parsons, L.M., Brosch, R., Cole, S.T., Somoskövi, A., Loder, A., Bretzel, G., et al. 2002. Rapid and simple approach for identification of *Mycobacterium tuberculosis* complex isolates by PCR-based genomic deletion analysis. *J Clin Microbiol*, *40*(7), pp.2339-2345.

Pawlowski, A., Jansson, M., Sköld, M., Rottenberg, M.E. and Källenius, G. 2012. Tuberculosis and HIV co-infection. *PLoS pathog*, 8(2), p.e1002464.

Perna, N.T., Plunkett III, G., Burland, V., Mau, B., Glasner, J.D., Rose, D.J., et al. 2001. Genome sequence of enterohaemorrhagic Escherichia coli O157: H7. *Nat*, *409*(6819), pp.529-533.

Pfyffer, G.E. 2015. Mycobacterium: general characteristics, laboratory detection, and staining procedures. *Manu Clin Microbiol*, pp.536-569.

Piat, M., Polvere, L., Kirst, M., Voronka, J., Zabkiewicz, D., Plante, M.C., Isaak, C., Nolin, D., Nelson, G. and Goering, P., 2015. Pathways into homelessness: Understanding

how both individual and structural factors contribute to and sustain homelessness in Canada. *Urban Studies*, *52*(13), pp.2366-2382.

Poulet, S. and Cole, S.T. 1995. Repeated DNA sequences in mycobacteria. *Arch Microbiol*, *163*, pp.79-86.

Powell, K.M., VanderEnde, D.S., Holland, D.P., Haddad, M.B., Yarn, B., Yamin, A.S., et al. 2017. The outbreak of drug-resistant Mycobacterium tuberculosis among homeless people in Atlanta, Georgia, 2008-2015. *Public Health Reports*, *132*(2), pp.231-240.

Ranzani, O.T., Carvalho, C.R., Waldman, E.A. and Rodrigues, L.C., 2016. The impact of being homeless on the unsuccessful outcome of treatment of pulmonary TB in São Paulo State, Brazil. *BMC med*, *14*, pp.1-13.

Rastogi, N., Legrand, E. and Sola, C. 2001. The mycobacteria: an introduction to nomenclature and pathogenesis. *Rev Off Int Epizoot*, *20*(1), pp.21-54.

Raviglione, M. and Sulis, G., 2016. Tuberculosis 2015: burden, challenges and strategy for control and elimination. *Inf dis rep*, *8*(2), p.6570.

Ravimohan, S., Kornfeld, H., Weissman, D. and Bisson, G.P. 2018. Tuberculosis and lung damage: from epidemiology to pathophysiology. *Eur Respir Rev*, *27*(147).

Reed, M.B., Pichler, V.K., McIntosh, F., Mattia, A., Fallow, A., Masala, S., et al. 2009. Major *Mycobacterium tuberculosis* lineages associate with patient country of origin. *J Clin Microbiol*, *47*(4), pp.1119-1128.

Rendleman, N.J. 1999. Mandated tuberculosis screening in a community of homeless people. *Am J Prev Med*, *17*(2), pp.108-113.

Riou, C., du Bruyn, E., Stek, C., Daroowala, R., Goliath, R.T., Abrahams, F., et al. 2021. Relationship of SARS-CoV-2–specific CD4 response to COVID-19 severity and impact of HIV-1 and tuberculosis coinfection. *J Clin Investig*, *131*(12).

Rodriguez-Moreno, S., Panadero, S. and Vázquez, J.J., 2021. The role of stressful life events among women experiencing homelessness: An intragroup analysis. *Am J Community Psychol, 67*(3-4), pp.380-391.

Rogans-Watson, R., Shulman, C., Lewer, D., Armstrong, M. and Hudson, B. 2020. Premature frailty, geriatric conditions and multimorbidity among people

experiencing homelessness: a cross-sectional observational study in a London hostel. *Hous Care Support*, *23*(3/4), pp.77-91.

Romaszko, J., Buciński, A., Kuchta, R., Bednarski, K. and Zakrzewska, M. 2013. The incidence of pulmonary tuberculosis among the homeless in north-eastern Poland. *Open Med*, *8*(2), pp.283-285.

Rufai, S.B., Kumar, P., Singh, A., Prajapati, S., Balooni, V. and Singh, S. 2014. Comparison of Xpert MTB/RIF with line probe assay for detection of rifampin-monoresistant *Mycobacterium tuberculosis*. *J Clin Microbiol*, *52*(6), pp.1846-1852.

Rüsch-Gerdes, S., Pfyffer, G.E., Casal, M., Chadwick, M. and Siddiqi, S. 2006. Multicenter laboratory validation of the BACTEC MGIT 960 technique for testing susceptibilities of *Mycobacterium tuberculosis* to classical second-line drugs and newer antimicrobials. *J Clin Microbiol*, *44*(3), pp.688-692.

Saha, K.K., Frongillo, E.A., Alam, D.S., Arifeen, S.E., Persson, L.Å. and Rasmussen, K.M. 2008. Appropriate infant feeding practices result in better growth of infants and young children in rural Bangladesh. *Am J Clin Nutr*, *87*(6), pp.1852-1859.

Salina, E.G. 2023. *Mycobacterium tuberculosis* Infection: Control and Treatment. *Microorganisms*, *11*(4), p.1057.

Salvato, R.S., Schiefelbein, S., Barcellos, R.B., Praetzel, B.M., Anusca, I.S., Esteves, L.S., et al. 2019. Molecular characterisation of multidrug-resistant *Mycobacterium tuberculosis* isolates from a high-burden tuberculosis state in Brazil. *Epidemiology & Infection*, *147*.

Santos, A.C.E.D., Brunfentrinker, C., Pena, L.D.S., Saraiva, S.D.S. and Boing, A.F. 2021. Analysis and comparison of tuberculosis treatment outcomes in the homeless population and in the general population of Brazil. *J Bras Pneumol*, *47*.

Scholze, A.R., Alves, J.D., Berra, T.Z., Ramos, A.C.V., Pieri, F.M., Pillon, S.C., et al. 2022. Tuberculosis among People Living on the Street and Using Alcohol, Tobacco, and Illegal Drugs: Analysis of Territories in Extreme Vulnerability and Trends in Southern Brazil. *Int J Environ Res Public Health*, *19*(13), p.7721.

Sebban, M., Mokrousov, I., Rastogi, N. and Sola, C. 2002. A data-mining approach to spacer oligonucleotide typing of *Mycobacterium tuberculosis*. *Bioinformatics*, *18*(2), pp.235-243.

Self, J.L., McDaniel, C.J., Morris, S.B. and Silk, B.J. 2021. Estimating and evaluating tuberculosis incidence rates among people experiencing homelessness, United States, 2007–2016. *Med care*, *59*(Suppl 2), p.S175.

Seligman, H.K., Bindman, A.B., Vittinghoff, E., Kanaya, A.M. and Kushel, M.B. 2007. Food insecurity is associated with diabetes mellitus: results from the National Health Examination and Nutrition Examination Survey (NHANES) 1999–2002. *J Gen Intern Med*, *22*, pp.1018-1023.

Semunigus, T., Tessema, B., Eshetie, S. and Moges, F. 2016. Smear-positive pulmonary tuberculosis and associated factors among homeless individuals in Dessie and Debre Birhan towns, Northeast Ethiopia. *Ann Clin Microbiol Antimicrob*, *15*, pp.1-8.

Shamebo, T., Gumi, B., Zewude, A., Gashaw, F., Mohammed, T., Girma, M., et al.2023b. Molecular epidemiology and drug sensitivity of Mycobacterium tuberculosis in homeless individuals in the Addis Ababa city, Ethiopia. *Sci Rep*, *13*(1), p.21370.

Shamebo, T., Mekesha, S., Getahun, M., Gumi, B., Petros, B. and Ameni, G. 2023a. Prevalence of pulmonary tuberculosis in homeless individuals in Addis Ababa City, Ethiopia. *Front Public Health*, *11*.

Sharaf Eldin, G.S., Fadl-Elmula, I., Ali, M.S., Ali, A.B., Salih, A.L.G., Mallard, K., et al. 2011. Tuberculosis in Sudan: a study of *Mycobacterium tuberculosis* strain genotype and susceptibility to anti-tuberculosis drugs. *BMC Infect Dis*, *11*, pp.1-8.

Shinn, M., 2010. Homelessness, poverty and social exclusion in the United States and Europe. *European Journal on Homelessness*.

Singh, U.B., Suresh, N., Bhanu, N.V., Arora, J., Pant, H., Sinha, S., et al. 2004, Predominant tuberculosis spoligotypes, Delhi, India. *Emerg Infect Dis*, *10*(6), p.1138.

Mlambo, C.K., Warren, R.M., Poswa, X., Victor, T.C., Duse, A.G. and Marais, E., 2008. Genotypic diversity of extensively drug-resistant tuberculosis (XDR-TB) in South Africa. *Int J TB lung dis*, *12*(1), pp.99-104.

Small, P.M., Hopewell, P.C., Singh, S.P., Paz, A., Parsonnet, J., Ruston, D.C., et al. 1994. The epidemiology of tuberculosis in San Francisco--a population-based study using conventional and molecular methods. *N Engl J Med*, *330*(24), pp.1703-1709.

Smith, C.M and Trienekens, S.C. 2019. Tuberculosis and Homelessness in the United States, 1994-2017. *JAMA Int Med.*179(4), 585-587.

Smith, N.H., Kremer, K., Inwald, J., Dale, J., Driscoll, J.R., Gordon, S.V., et al. 2006. Ecotypes of the *Mycobacterium tuberculosis* complex. *J Theor Biol*, *239*(2), pp.220-225.

Sola, C., Ferdinand, S., Mammina, C., Nastasi, A. and Rastogi, N. 2001. Genetic diversity of *Mycobacterium tuberculosis* in Sicily based on spoligotyping and variable number of tandem DNA repeats and comparison with a spoligotyping database for population-based analysis. *J Clin Microbiol, 39*(4), pp.1559-1565.

Sola, C., Filliol, I., Legrand, E., Lesjean, S., Locht, C., Supply, P., et al. 2003. Genotyping of the *Mycobacterium tuberculosis* complex using MIRUs: association with VNTR and spoligotyping for molecular epidemiology and evolutionary genetics. *Infect, Genet Evol*, *3*(2), pp.125-133.

Sreevatsan, S., Pan, X.I., Stockbauer, K.E., Connell, N.D., Kreiswirth, B.N., Whittam, T.S., et al. 1997. Restricted structural gene polymorphism in the *Mycobacterium tuberculosis* complex indicates evolutionarily recent global dissemination. *Proc Natl Acad Sci*, *94*(18), pp.9869-9874.

Steingart, K.R. 2013. Xpert MTB/RIF test for detection of pulmonary tuberculosis and rifampicin resistance. *J Evid Based Med*, *6*(1), pp.58-58.

Stinson, K.W., Eisenach, K., Kayes, S., Matsumoto, M., Siddiqi, S. and Nakashima, S. 2014. Mycobacteriology Laboratory Manual, global laboratory initiative advancing TB diagnosis. *Global Laboratory Initiative of Stop TB Partnership*.

Story, A., Murad, S., Roberts, W., Verheyen, M. and Hayward, A.C. 2007. Tuberculosis in London: the importance of homelessness, problem drug use and prison. *Thorax*, *62*(8), pp.667-671.

Stucki, D., Malla, B., Hostettler, S., Huna, T., Feldmann, J., Yeboah-Manu, D., et al. 2012. Two new rapid SNP-typing methods for classifying *Mycobacterium tuberculosis* complex into the main phylogenetic lineages. *PloS one*, *7*(7), p.e41253.

Supply, P. 2005. Multilocus variable number tandem repeat genotyping of *Mycobacterium tuberculosis*. *Technical guide*, *6*, pp.1-74.

Supply, P., Allix, C., Lesjean, S., Cardoso-Oelemann, M., Rüsch-Gerdes, S., Willery, E., et al. 2006. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. *J Clin Microbiol*, *44*(12), pp.4498-4510.

Supply, P., Mazars, E., Lesjean, S., Vincent, V., Gicquel, B. and Locht, C. 2000. Variable human minisatellite-like regions in the *Mycobacterium tuberculosis* genome. *Mol Microbiol*, *36*(3), pp.762-771.

Tabuchi, T., Takatorige, T., Hirayama, Y., Nakata, N., Harihara, S., Shimouchi, A., et al. 2011. Tuberculosis infection among homeless persons and caregivers in a high-tuberculosis-prevalence area in Japan: a cross-sectional study. *BMC Infect Dis*, *11*, pp.1-8.

Tadesse, M., Abebe, G., Bekele, A., Bezabih, M., de Rijk, P., Meehan, C.J., et al. 2017. The predominance of Ethiopian specific *Mycobacterium tuberculosis* families and minimal contribution of Mycobacterium bovis in tuberculous lymphadenitis patients in Southwest Ethiopia. *Infection, Genet Evol*, *55*, pp.251-259.

Tafess, K., Beyen, T.K., Girma, S., Girma, A. and Siu, G. 2021. Spatial clustering and genetic diversity of *Mycobacterium tuberculosis* isolate among pulmonary tuberculosis suspected patients, Arsi Zone, Ethiopia. *BMC Pulm Med*, *21*(1), pp.1-12.

Tankimovich, M. 2013. Barriers to and interventions for improved tuberculosis detection and treatment among homeless and immigrant populations: a literature review. *J Community Nurs*, *30*(2), pp.83-95.

Taye, H., Alemu, K., Mihret, A., Ayalew, S., Hailu, E., Wood, J.L., et al. 2021. Epidemiology of *Mycobacterium tuberculosis* lineages and strain clustering within urban and peri-urban settings in Ethiopia. *Plos one*, *16*(7), p.e0253480.

Taylor, G.M., Stewart, G.R., Cooke, M., Chaplin, S., Ladva, S., Kirkup, J., et al. 2003. Koch's Bacillus–a look at the first isolate of Mycobacterium tuberculosis from a modern perspective. *Microbiol*, *149*(11), pp.3213-3220.

Telenti, A., Imboden, P., Marchesi, F., Schmidheini, T. and Bodmer, T., 1993. Direct, automated detection of rifampin-resistant Mycobacterium tuberculosis by polymerase chain reaction and single-strand conformation polymorphism analysis. *Antimicrobial agents and chemotherapy*, *37*(10), pp.2054-2058.

Temesgen, E., Belete, Y., Haile, K. and Ali, S. 2021. Prevalence of active tuberculosis and associated factors among people with chronic psychotic disorders at St. Amanuel Mental Specialized Hospital and Gergesenon Mental Rehabilitation center, Addis Ababa, Ethiopia. *BMC Infect Dis*, *21*(1), pp.1-9.

Tenover, F.C., Crawford, J.T., Huebner, R.E., Geiter, L.J., Horsburgh Jr, C.R. and Good, R.C. 1993. The resurgence of tuberculosis: is your laboratory ready?. *J Clin Microbiol*, *31*(4), pp.767-770.

Tessema, B., Beer, J., Merker, M., Emmrich, F., Sack, U., Rodloff, A.C., et al. 2013. Molecular epidemiology and transmission dynamics of *Mycobacterium tuberculosis* in Northwest Ethiopia: new phylogenetic lineages found in Northwest Ethiopia. *BMC Infect Dis*, *13*, pp.1-11.

Tibbetts, K.K., Ottoson, R.A. and Tsukayama, D.T. 2020. Public health response to tuberculosis outbreak among persons experiencing homelessness, Minneapolis, Minnesota, USA, 2017–2018. *Emerg Infect Dis*, *26*(3), p.420.

Tulu, B. and Ameni, G. 2018. Spoligotyping based genetic diversity of *Mycobacterium tuberculosis* in Ethiopia: a systematic review. *BMC infectious diseases*, *18*(1), pp.1-10.

Turner, R.D. and Bothamley, G.H. 2015. Cough and the transmission of tuberculosis. *J Infect Dis*, *211*(9), pp.1367-1372.

Udoakang, A.J., Djomkam Zune, A.L., Tapela, K., Nganyewo, N.N., Olisaka, F.N., Anyigba, C.A., et al. 2023. The COVID-19, tuberculosis and HIV/AIDS: Ménage à Trois. *Front Immunol*, *14*, p.215.

Urdahl, K.B., Shafiani, S. and Ernst, J.D. 2011. Initiation and regulation of T-cell responses in tuberculosis. *Mucosal Immunol*, *4*(3), pp.288-293.

Valdez, M.M., Clark, J.I., Wu, G.J. and Muchowski, P.J. 2002. Functional similarities between the small heat shock proteins *Mycobacterium tuberculosis* HSP 16.3 and human αB-crystallin. *Eur J Biochem*, *269*(7), pp.1806-1813.

Van Embden, J.D., Cave, M.D., Crawford, J.T., Dale, J.W., Eisenach, K.D., Gicquel, B., et al. 1993. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. *J Clin Microbiol*, *31*(2), pp.406-409.

Van Soolingen, D., Qian, L., De Haas, P.E., Douglas, J.T., Traore, H., Portaels, F., et al. 1995. Predominance of a single genotype of *Mycobacterium tuberculosis* in countries of East Asia. *J Clin Microbiol*, *33*(12), pp.3234-3238.

Villa, L., Trompa, I.M., Montes, F.N., Gómez, J.G. and Restrepo, C.A., 2014. Analysis of mortality caused by tuberculosis in Medellin, Colombia, 2012. *Biomédica*, *34*(3), pp.425-432.

Vitol, I., Driscoll, J., Kreiswirth, B., Kurepina, N. and Bennett, K.P. 2006. Identifying *Mycobacterium tuberculosis* complex strain families using spoligotypes. *Infect, Genet Evol*, *6*(6), pp.491-504.

Wainwright, M., 1991. Streptomycin: discovery and resultant controversy. *History and philosophy of the life sciences*, pp.97-124.

Warren, R.M., Streicher, E.M., Sampson, S.L., Van Der Spuy, G.D., Richardson, M., Nguyen, D., et al. 2002. Microevolution of the direct repeat region of *Mycobacterium tuberculosis*: implications for interpretation of spoligotyping data. *J Clin Microbiol*, *40*(12), pp.4457-4465.

WHO. 2000. 44th World Health Assembly (WHA/1991/REC/1); supplemented by 53rd World Health Assembly, Report by the Director General, Provisional Agenda Item 12.1, A53/5. Geneva, Switzerland.

WHO. 2013. Systematic screening for active tuberculosis: principles and recommendations. WHO/HTM/TB/2013.04. Geneva, Switzerland.

WHO. 2014b. *Xpert MTB/RIF implementation manual: technical and operational 'how-to'; practical considerations* (No. WHO/HTM/TB/2014.1). Geneva, Switzerland.

WHO. 2019b. Consolidated guidelines on drug-resistant tuberculosis treatment. Geneva, Switzerland.

WHO. 2020. Global tuberculosis report 2020. Geneva, Switzerland.

WHO. 2021. Global tuberculosis report 2021. Geneva, Switzerland.

WHO. 2022. Global Tuberculosis Report 2022. Geneva, Switzerland.

WHO. 2023. Global Tuberculosis Report 2022. Geneva, Switzerland

Wondale, B., Keehwan, K., Medhin, G., Teklu, T., Mohammed, T., Tolosa, S., et al. 2020. Molecular epidemiology of clinical *Mycobacterium tuberculosis* complex isolates in South Omo, Southern Ethiopia. *BMC Infect Dis*, *20*, pp.1-12.

Wondifraw, E.B., Chanie, E.S., Gebreeyesus, F., Biset, G., Tefera, B.D. and Zeleke, M., 2022. Incidence and predictors of tuberculosis among children on antiretroviral therapy at northeast Ethiopia comprehensive specialized hospitals, 2022; A multicenter retrospective follow-up study. *Heliyon*, *8*(12).

Yeh, R.W., de Leon, A.P., Agasino, C.B., Hahn, J.A., Daley, C.L., Hopewell, P.C., et al. 1998. Stability of *Mycobacterium tuberculosis* DNA genotypes. *J Infect Diss*, *177*(4), pp.1107-1111.

Yimer, S.A., Norheim, G., Namouchi, A., Zegeye, E.D., Kinander, W., Tønjum, T., et al. 2015. *Mycobacterium tuberculosis* lineage 7 strains are associated with prolonged patient delay in seeking treatment for pulmonary tuberculosis in Amhara Region, Ethiopia. *J Clin Microbiol*, *53*(4), pp.1301-1309.

Yusoof, K.A., García, J.I., Schami, A., Garcia-Vilanova, A., Kelley, H.V., Wang, S.H., et al. 2022. Tuberculosis Phenotypic and Genotypic Drug Susceptibility Testing and Immunodiagnostics: A Review. *Front Imunol*, *13*.

Zhang, Y. and Yew, W.W. 2015. Mechanisms of drug resistance in *Mycobacterium tuberculosis*: update 2015. *Int J Tuberc Lung Dis*, *19*(11), pp.1276-1289.

Zhang, Y., Heym, B., Allen, B., Young, D. and Cole, S., 1992. The catalase—peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. *Nature*, *358*(6387), pp.591-593.

Zhang, Y., Vilchèze, C. and Jacobs JR, W.R. 2004. Mechanisms of drug resistance in *Mycobacterium tuberculosis*. *Tuberculosis and the Tubercle Bacillus*, pp.115-140.

Zink, A., Haas, C.J., Reischl, U.D.O., Szeimies, U. and Nerlich, A.G. 2001. Molecular analysis of skeletal tuberculosis in an ancient Egyptian population. *J Med Microbiol*, 50(4), pp.355-366.

7. ANNEXES

Annex I: Consent form for study participants

Principal Investigator: Tsegaye Shamebo

Organization: Addis Ababa University

Project title: ***Mycobacterium tuberculosis* infection among homeless individuals in Addis Ababa, Ethiopia: Disease burden, Drug resistance patterns and Molecular epidemiology**

This informed consent has two parts:

- Information sheet
- Certificate of Consent

Read and give a copy of the full informed consent form to the respondent.

Part I – Statement

Introduction:

Hello: My name is________________________ and I am working with researchers from Addis Ababa University. We are conducting a research on tuberculosis among homeless individuals in Addis Ababa, Ethiopia.

Purpose and length of the study

We design this study to measure disease burden, molecular epidemiology and drug sensetivity of one of the deadily bacterial diseases, tuberculosis in homeless individuals in Addis Ababa, Ethiopia. It is known that pulmonary tuberculosisis is caused by *M. tuberculosis* bacteria. These bacteria easily transmitted from person to person through aerosol route.

Procedures

We invite you to take part in this study. If you are willing, you will be required to underg o the screening procedure. Accordingly you will be categorized as presumptive TB case or not. Those of you categorized as presumptive TB case will be requested to answer few additonalquestions, give two sputum samples for laboratory diagnosis. The sample will be used to detect *M. tuberculosis*. There is no any harm for your health associated

with these procedures. Finally the data obtained from this study will be published ininter national/national peer reviewed journals.

Risks and Discomfort

There is no discomfort associated with this study except very mild skin prick pain that la stsfor seconds during blood collection.

Benefits

Through active screening for TB and HIV you will be able to get treatment early on i n the
course of the diseases. This will improve your overall chances for complete recover y fromTB. In case of an HIV infection you will be referred to the closest Care and Treat ment Centre
where the decision regarding treatment for HIV will be made. In both cases you will ben efit
from early intervention. Furthermore, through involving most of the homeless pop ulation inactive screening we will beable to reduce the TB transmission in the homeless , thus protect yourhealth.We will not pay you for taking part in this study. Howeve r, we will thank you for your participation.

Confidentiality

The data that we will collect from you will be kept confidential and don't cont ain anyinformation that may lead to your identity.

Right to refuse or withdraw

You do not have to take part in this research if you do not wish to do so, and refu sing toparticipate will not have any effect in your treatment**.** You do have full right to wi thdraw from
this study at any point if you wish. Your withdrawal will have no influence what so ever on your further treatment.

Whom to contact

If you have any questions you may ask the doctor now or later. If you wish to askquestions later, you may contact Tsegaye Shamebo (+251 913 493 668),
Addis Ababa University.

DECLARATION OF CONSENT FOR THE STUDY

(CONSENT FORM)

I have clearly understood the purpose and prospective benefits of the research. I hereby n eed to assure with my signature below that I, without any coercion or forceful act by the research team, have decided to voluntarily participate in the study in-front of the witness.

Study subject's	**Witness's**
Code number_____________	Name __________________
Signature _______________	Signature _______________
Date______________________	Date___________________

Data collector

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions.

I confirm that the individual has given consent freely.

Name____________________

Signature_________________

Date_____________________

Annex II. Questionnaire for TB among homeless individuals in Addis Ababa, Ethiopia. Participant's

Code No ______ Name of center_________________Sample Code: _____

A. Demographic characteristics

S/N	Question to be asked	Proposed response	Coded response
01	Gender	1. Male	0
		2. Female	1
02	Age (in year)	1. 18-29 2. 30-39 3. 40-49 4. ≥ 50	0 1 2 3
03	Educational status	1. Illiterate 2. Primary school 3. Secondary school 4. College & above	0 1 2 3
04	Marital status	1.Single 2. Married 3. Divorced 4. Widowed	0 1 2 3

05	Address before being homelessness	1. Oromia region 2. SNNPR 3. Amhara region 4. Somali region 5. Tigray region 6. Benishangul-Gumuz region 7. Gambela region 8. Harari region 9. Afar region 10. Addis Ababa City 11. Dire Dawa City 12. Others	0 1 2 3 4 5 6 7 8 9 10 11
06	Residence before being homeless	1. Urban 2. Rural	0 1

B. Homelessness

S/N	Question to be asked	Proposed response	Coded response
07	Age when you first became homeless	1. Less than 18 years	0
		2. More than 18 years	1
08	How long have you been homeless?	1. Less than 6 months	0
		2. 1-2 years	1
		3. 3-5 years	2
		4. More than 6 years	3
09	How many homeless people are sleeping with you?	1. None	0
		2. Three	1
		3. Four	2
		4. Five and above	3

C. TB Related questions

S/N	Question to be asked	Proposed response	Coded response
12	Did you have cough for $\geq$ 2 weeks?	1. Yes 2. No	0 1
13	Did you have fever for > 2 weeks?	1. Yes 2. No	0 1
14	Did you have night sweats for m ore than two weeks?	1. Yes 2. No	0 1
15	Did you have close contact with a pe son coughing for more than two weeks?	1. Yes 2. No	0 1
16	How much alcohol do you drink?	1. none 2. Three bottles perweek 3. more than threebottles pe week	0 1 2
17	How much cigarette do you smoke?	1. None 2. Not current but previous smoker 3. occasionaly 4. one packet per week 5. More than one packetpe week	0 1 2 3 4
18	How frequent do you chew "Kchat"?	1. None 2. once per week 3. More than once perweel	0 1 2
19	Have you ever used self injectable IV drugs?	1. Yes 2. No	0 1

20	Have you been imprisoned?	1. Yes 2.No	0 1
21	How long did you imprisoned in curr nt prison?	________ months	
22	Have you been with known TB patie nt During you stay as homeless?	1. Yes 2. No 3. I don't know	0 1 2
23	Have you ever been diagnosed for TB?	1. Yes 2. No	0 2
24	If yes to Q. 23, when have you been diagnosed for TB?	1.Before being homeless 2. In the homeless people	0 1
25	Did you received treatment for TB?	1. Yes 2. No	0 1
26	If yes to Q. 25 did you complete the full course of the treatment?	1. Yes 2. No	0 1

Annex III. Locus designations and PCR primer sequences used in this study for the 24-locus MIRU-VNTR set

Multiplex	Locus	Alias	Repeat unit length (bp)	PCR primers (5' to 3')
Mix 1	580	MIRU 4	77	GCGCGAGAGCCCGAACTGC GCGCAGCAGAAACGCCAGC
	2996	MIRU 26	51	TAGGTCTACCGTCGAAATCTGTGAC CATAGGCGACCAGGCGAATAG
	802	MIRU 40	54	GGGTTGCTGGATGACAACGTGT GGGTGATCTCGGCGAAATCAGATA
Mix 2	960	MIRU 10	53	GTTCTTGACCAACTGCAGTCGTCC GCCACCTTGGTGATCAGCTACCT
	1644	MIRU 16	53	TCGGTGATCGGGTCCAGTCCAAGTA CCCGTCGTGCAGCCCTGGTAC
	3192	MIRU 31	53	ACTGATTGGCTTCATACGGCTTTA GTGCCGACGTGGTCTTGAT
Mix 3	424	42	51	CTTGGCCGGCATCAAGCGCATTATT GGCAGCAGAGCCCGGGATTCTTC
	577	43	58	CGAGAGTGGCAGTGGCGGTTATCT AATGACTTGAACGCGCAAATTGTGA
	2165	ETR A	75	AAATCGGTCCCATCACCTTCTTAT CGAAGCCTGGGGTGCCCGCGATTT
Mix 4	2401	47	58	CTTGAAGCCCCGGTCTCATCTGT ACTTGAACCCCCACGCCCATTAGTA
	3690	52	58	CGGTGGAGGCGATGAACGTCTTC TAGAGCGGCACGGGGGAAAGCTTAG
	4156	53	59	TGACCACGGATTGCTCTAGT GCCGGCGTCCATGTT
Mix 5	2163b	QUB-11b	69	CGTAAGGGGGATGCGGGAAATAGG CGAAGTGAATGGTGGCAT
	1955		57	AGATCCCAGTTGTCGTCGTC CAACATCGCCTGGTTCTGTA
	4052	QUB-26	111	AACGCTCAGCTGTCGGAT CGGCCGTGCCGGCCAGGTCCTTCCCGAT
Mix 6	154	MIRU 2	53	TGGACTTGCAGCAATGGACCAACT TACTCGGACGCCGGCTCAAAAT
	2531	MIRU 23	53	CTGTCGATGGCCGCAACAAAACG AGCTCAACGGGTTCGCCCTTTTGTC
	4348	MIRU 39	53	CGCATCGACAAACTGGAGCCAAAC CGGAAACGTCTACGCCCCACACAT
Mix 7	2059	MIRU 20	77	TCGGAGAGATGCCCTTCGAGTTAG GGAGACCGCGACCAGGTACTTGTA
	2687	MIRU 24	54	CGACCAAGATGTGCAGGAATACAT GGGCGAGTTGAGCTCACAGAA
	3007	MIRU 27	53	TCGAAAGCCTCTGCGTGCCAGTAA GCGATGTGAGCGTGCCACTCAA
Mix 8	2347	46	57	GCCAGCCGCCGTGCATAAACCT AGCCACCCGGTGTGCCTTGTATGAC
	2461	48	57	ATGGCCACCCGATACCGCTTCAGT CGACGGGCCATCTTGGATCAGCTAC
	3171	49	54	GGTGCGCACCTGCTCCAGATAA GGCTCTCATTGCTGGAGGGTTGTAC

Annex IV: Manuscript published

1. **Tsegaye Shamebo,** Sindew Mekesha, Muluwork Getahun, Balako Gumi, Beyene Petros, Gobena Ameni. 2023. **Prevalence of pulmonary tuberculosis in homeless individuals in the Addis Ababa City, Ethiopia.** ***Front. Public Health***, ***11:1128525*** (**Published**).

2. **Tsegaye Shamebo**, Balako Gumi, Aboma Zewude, Fikru Gashaw, Temesgen Mohammed, Muse Girma, Betselot Zerihun, Melak Getu, Sindew Mekasha, Muluwork Getahun, Biniam Wondale, Beyene Petros, Gobena Ameni. **Molecular epidemiology and drug sensitivity of *Mycobacterium tuberculosis* in homeless individuals in the Addis Ababa city, Ethiopia.** ***Sci Rep***, ***13*****(1), p.21370** (**Published**).

Annex V: Conference presentations

1. Molecular epidemiology and Drug sensitivity patterns of *Mycobacterium tuberculosis* in Homeless Individuals in the Addis Ababa, Ethiopia (**The 2024 Addis Ababa University Research Week at ALIPB**).
2. Molecular epidemiology and Drug sensitivity patterns of *Mycobacterium tuberculosis* in Homeless Individuals in the Addis Ababa, Ethiopia (**Ethiopian Public Health Association, EPHA 2024 at Inter-Luxury Hotel, Addis ABABA, Ethiopia**).
3. Prevalence of *Mycobacterium tuberculosis* Mixed strain infections among homeless individuals in Addis Ababa, Ethiopia (**Tuberculosis Research Annual Conference, TRAC, 2024 at St. Paul Millennium Hospital Medical College, Ethiopia**).
4. Molecular epidemiology and Drug sensitivity patterns of *Mycobacterium tuberculosis* in Homeless Individuals in the Addis Ababa, Ethiopia (**Tuberculosis Research Annual Conference, TRAC, 2023 at Gondar University, Ethiopia**).
5. Prevalence of PTB and associated risk factors among homeless people (Systematic review and meta-analysis) (**Tuberculosis Research Annual Conference, TRAC, 2022 at Hawassa University, Ethiopia**).
6. Prevalence of PTB and associated risk factors among homeless individuals in Addis Ababa Ethiopia (**Tuberculosis Research Annual Conference, TRAC, 2021 at EPHI**).
7. Prevalence of PTB and associated risk factors among homeless individuals in Addis Ababa Ethiopia (**Ethiopian Society for Microbiology, ESM, 2021 at Ethiopian Biotechnology Institute**).
8. Prevalence of PTB and associated risk factors among homeless individuals in Addis Ababa Ethiopia (**The 2021 Addis Ababa University Research Week at ALIPB**).