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Research Article

Molecular Characterization and Epidemiology of Drug-Resistant *Mycobacterium Tuberculosis* Isolates Among Pulmonary Tuberculosis Patients in Jimma Zone, South-West Ethiopia

Feyera Gemeda[1,2*], Tekle Tadesse[1]

[1]Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia
[2]School of Medicine & Department of Tropical and Infectious Diseases, Jimma University, Jimma, Ethiopia

***Corresponding Author:** Feyera Gemeda, Jimma University, Ethiopia, P.O. Box 378, Jimma, Ethiopia

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Abstract

Background: Drug-resistant tuberculosis (DR-TB) is a major public health threat, particularly in high-burden countries like Ethiopia. Local data on resistance patterns and circulating strains are crucial for effective TB control programs. This study aimed to determine the prevalence and molecular characteristics of drug-resistant Mycobacterium tuberculosis among pulmonary TB patients in Jimma Zone, South-West Ethiopia.

Methods: A health facility-based cross-sectional study was conducted from September 2018 to August 2019. A total of 286 newly diagnosed and previously treated pulmonary TB patients were enrolled consecutively from 12 selected health facilities in Jimma Zone. Sputum samples were collected and processed using GeneXpert MTB/RIF for rapid detection of Mycobacterium tuberculosis and rifampicin resistance. Line Probe Assay (GenoType® MTBDRplus VER 2.0) was performed to detect resistance to both rifampicin and isoniazid. Molecular characterization of circulating strains was conducted using spoligotyping. Data were analyzed using SPSS version 25.0, with logistic regression analysis to identify factors associated with drug resistance.

Results: Of the 286 participants, M. tuberculosis was detected in 250 (87.4%) samples by either method. Any drug resistance was observed in 28 (11.2%) isolates. Rifampicin resistance was detected in 17 (6.8%) isolates, and isoniazid resistance in 21 (8.4%) isolates. Multidrug-resistant tuberculosis (MDR-TB) was confirmed in 14 (5.6%) of the total positive cases. Among MDR-TB cases, 10 (71.4%) had the rpoB S531L mutation, and 12 (85.7%) had the katG S315T mutation. Spoligotyping revealed 21 different spoligotype patterns, with the most prevalent families being Ethiopia_3 (SIT149) (28.4%, n=71), followed by Haarlem (SIT62) (12.8%, n=32), URAL (SIT35) (9.2%, n=23), and TUR (SIT41) (7.6%, n=19). Previous TB treatment history (AOR: 4.82; 95% CI: 2.34-9.91; p<0.001), HIV co-infection (AOR: 3.17; 95% CI: 1.42-7.08; p=0.005), and contact with known TB patient (AOR: 2.68; 95% CI: 1.18-6.09; p=0.018) were significantly associated with MDR-TB.

Conclusion: The prevalence of MDR-TB in this study setting was moderate but significant, with distinct genotypic diversity of circulating strains. The predominance of the Ethiopia_3 family and specific mutation patterns provides valuable insights for regional TB control programs. These findings underscore the need for strengthened diagnostic capacity, routine drug susceptibility testing for all patients, and enhanced contact tracing. Continuous molecular surveillance is essential to monitor resistance patterns and guide evidence-based treatment regimens in the region.

Keywords: Drug-resistant tuberculosis, MDR-TB, GeneXpert, Line Probe Assay, Spoligotyping, Molecular epidemiology, Ethiopia, Jimma Zone

Introduction

Background

Tuberculosis (TB) remains one of the top infectious disease killers worldwide, despite being preventable and curable for decades. According to the World Health Organization (WHO) Global Tuberculosis Report 2023, an estimated 10.6 million people fell ill with TB in 2022, and 1.3 million died from the disease [1]. The emergence and spread of drug-resistant tuberculosis (DR-TB) have become a major threat to global TB control efforts, jeopardizing progress toward the End TB Strategy targets [2].

Drug-resistant TB occurs when Mycobacterium tuberculosis strains develop resistance to the most effective first-line anti-TB drugs. Multidrug-resistant tuberculosis (MDR-TB), defined as resistance to at least rifampicin and isoniazid—the two most potent first-line drugs—requires longer, more toxic, and expensive treatment regimens with poorer outcomes [3]. In 2022, an estimated 410,000 people developed MDR-TB or rifampicin-resistant TB (RR-TB) globally, with only about two-thirds of these cases being diagnosed and enrolled on treatment [1].

The Situation in Ethiopia

Ethiopia is among the 30 high TB and TB/HIV burden countries, with an estimated TB incidence of 119 per 100,000 population in 2022 [1]. The country has made significant progress in TB control over the past decade, achieving a treatment success rate of 90% for drug-susceptible TB. However, drug-resistant TB poses an increasing challenge. The national prevalence of MDR-TB among new cases is estimated at 1.4% and among previously treated cases at 12% [1,4].

Regional variations in drug resistance patterns exist across Ethiopia due to differences in TB control program implementation, population movement, HIV prevalence, and circulating M. tuberculosis strains. The Jimma Zone in South-West Ethiopia is particularly important to study due to its unique characteristics: it serves as a major commercial hub with significant population movement, has areas with high HIV prevalence, and includes both urban and remote rural communities with varying access to healthcare services [5].

Molecular Epidemiology of TB

Molecular epidemiology combines conventional epidemiological methods with molecular typing techniques to understand the transmission dynamics and strain diversity of M. tuberculosis [6]. Various genotyping methods have been developed, including spoligotyping, mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing, and whole-genome sequencing (WGS). Spoligotyping, while having lower discriminatory power than other methods, remains valuable for phylogenetic studies and initial screening due to its relative simplicity, reproducibility, and ability to assign strains to known lineages and families [7].

Different M. tuberculosis lineages and families have been associated with varying degrees of transmissibility, virulence, and propensity to develop drug resistance [8]. Understanding which strain families circulate in a given region and their association with drug resistance can provide insights into transmission dynamics and inform targeted interventions. The Ethiopia_3 family (also known as Central Asian Strain or CAS1-Delhi) has been reported as a predominant lineage in various parts of Ethiopia, but regional data from Jimma Zone remain limited [9,10].

Molecular Mechanisms of Drug Resistance

Resistance to rifampicin, the most powerful first-line anti-TB drug, is primarily caused by mutations in an 81-base pair core region of the rpoB gene encoding the β-subunit of RNA polymerase [11]. The most common mutations occur at codons 531 (S531L), 526 (H526Y/D/V), and 516 (D516V) [12].

Resistance to isoniazid is more complex, involving mutations in several genes, most commonly the katG gene (particularly codon 315, S315T) and the promoter region of the inhA gene [13]. The katG S315T mutation confers high-level isoniazid resistance while preserving catalase-peroxidase activity necessary for bacterial fitness, explaining its prevalence in clinical isolates [14].

Line Probe Assays (LPAs), such as GenoType® MTBDRplus, detect these common mutations rapidly, allowing for timely diagnosis of MDR-TB and appropriate treatment initiation [15]. The WHO strongly recommends LPAs as the initial test for detecting rifampicin and isoniazid resistance in sputum smear-positive specimens or cultured isolates [16].

Rationale for the Study

Despite the national estimates of drug-resistant TB in Ethiopia, there is a paucity of local data from the Jimma Zone regarding the prevalence of drug resistance, the specific mutations conferring resistance, and the molecular characteristics of circulating strains. Such information is essential for:

1. Informing local treatment guidelines: Understanding local resistance patterns helps clinicians choose appropriate empiric treatment regimens while awaiting drug susceptibility testing (DST) results.
2. Strengthening the TB control program: Identifying transmission hotspots and risk groups enables targeted interventions.
3. Monitoring the effectiveness of control measures: Baseline data allow for assessment of future interventions.
4. Contributing to the global knowledge base: Data from understudied regions enrich our understanding of TB epidemiology and evolution.

Research Questions

1. What is the prevalence of rifampicin resistance, isoniazid resistance, and MDR-TB among pulmonary TB patients

in Jimma Zone?
2. What are the specific mutations in the rpoB, katG, and inhA genes associated with drug resistance in these isolates?
3. What are the circulating M. tuberculosis strain families in Jimma Zone, and what is their phylogenetic diversity?
4. What demographic and clinical factors are associated with drug-resistant TB in this population?

Objectives

General Objective:

To determine the prevalence and molecular characteristics of drug-resistant Mycobacterium tuberculosis isolates among pulmonary tuberculosis patients in Jimma Zone, South-West Ethiopia.

Specific Objectives:

1. To determine the prevalence of rifampicin resistance, isoniazid resistance, and MDR-TB using GeneXpert MTB/RIF and Line Probe Assay.
2. To characterize the molecular mechanisms of resistance by identifying mutations in rpoB, katG, and inhA genes.
3. To identify the circulating M. tuberculosis strain families using spoligotyping.
4. To assess demographic and clinical factors associated with drug-resistant TB.
5. To compare the diagnostic performance of GeneXpert MTB/RIF and LPA for detecting drug resistance.

Materials And Methods

Study Design and Period

A health facility-based cross-sectional study was conducted from September 2018 to August 2019 (12 months). This design was chosen to estimate the prevalence of drug-resistant TB at a single point in time and to identify associated factors.

Study Area

The study was conducted in Jimma Zone, Oromia Regional State, South-West Ethiopia. Jimma Zone is located approximately 350 km southwest of Addis Ababa, covering an area of 15,568.58 km². According to the 2017 Central Statistical Agency projection, the zone has a population of approximately 3.2 million people, with 88% living in rural areas engaged primarily in subsistence agriculture and coffee cultivation [17].

The zone has one tertiary teaching hospital (Jimma University Medical Center), three general hospitals, eight primary hospitals, and 96 health centers. TB diagnosis and treatment services are available at all public health facilities, following the national TB program guidelines. The zone has a TB notification rate of 145 per 100,000 population, slightly above the national average [18].

Source and Study Population

Source Population: All pulmonary TB patients attending public health facilities in Jimma Zone during the study period.

Study Population: Newly diagnosed and previously treated smear-positive or clinically diagnosed pulmonary TB patients aged ≥18 years who were enrolled at selected health facilities during the study period.

Eligibility Criteria

Inclusion Criteria:

- Age ≥18 years
- Presumptive pulmonary TB patients with at least one positive smear microscopy (acid-fast bacilli, AFB) or positive GeneXpert result
- Willingness to provide sputum sample and participate in the study
- Provision of written informed consent
- Residence in Jimma Zone for at least 6 months prior to enrollment

Exclusion Criteria:

- Patients on anti-TB treatment for more than 7 days at the time of enrollment
- Patients unable to produce sputum
- Extrapulmonary TB cases without pulmonary involvement
- Critically ill patients unable to provide consent
- Previous enrollment in this study (to avoid duplication)

Sample Size Determination

The sample size was calculated using single population proportion formula:

$n = (Z^2 \times p \times (1-p)) / d^2$

Where:

- Z = 1.96 (95% confidence level)
- p = expected prevalence of MDR-TB among pulmonary TB patients = 0.05 (based on previous Ethiopian studies showing MDR-TB prevalence ranging from 1.6% to 12.8% [19,20], we used 5% as a conservative estimate)
- d = margin of error = 0.025

$n = (1.96^2 \times 0.05 \times 0.95) / 0.025^2 = 292$

Adding 10% for non-response and inadequate samples, the final sample size was 322. However, during the study period, 286 eligible patients were enrolled due to logistical constraints and the end of the study period. Post-hoc power analysis indicated that this sample size was sufficient to detect an MDR-TB prevalence of 5% with 80% power at 95% confidence.

Sampling Technique and Health Facility Selection

A multi-stage sampling approach was employed:

Stage 1: Health Facility Selection

Twelve health facilities were purposively selected based on:

- High patient volume (≥50 TB patients diagnosed annually)
- Geographic representation (covering urban and rural areas across the zone)

- Availability of TB diagnostic services (microscopy and GeneXpert)
- Accessibility and security considerations

Selected facilities included:

1. Jimma University Medical Center (tertiary hospital, urban)
2. Shenen Gibe General Hospital (urban)
3. Limu Genet General Hospital (semi-urban)
4. Agaro Primary Hospital (semi-urban)
5. Seka Primary Hospital (rural)
6. Serbo Health Center (rural)
7. Shebe Health Center (rural)
8. Yebu Health Center (semi-urban)
9. Omo Nada Health Center (rural)
10. Gera Health Center (rural)
11. Chora Botor Health Center (rural)
12. Manna Health Center (rural)

Stage 2: Patient Enrollment

Consecutive sampling was used to enroll all eligible patients presenting at the selected facilities during the study period until the desired sample size was approached. This method was chosen to minimize selection bias and ensure representation of all patient types.

Data Collection Procedures

Questionnaire Administration

A structured, pretested questionnaire was developed in English, translated into Afan Oromo and Amharic (the local languages), and back-translated to ensure consistency. Trained data collectors (Bachelor of Science degree holder nurses or public health officers) administered the questionnaire through face-to-face interviews.

Data collected included:

- Socio-demographic characteristics: age, sex, residence, marital status, education level, occupation, monthly income, household size
- Clinical characteristics: TB treatment history (new/retreatment), previous TB episodes, HIV status, diabetes mellitus, other comorbidities
- Behavioral factors: smoking history, alcohol use, khat chewing
- Exposure history: contact with known TB patient, household crowding, ventilation, history of incarceration, institutionalization
- Health-seeking behavior: time from symptom onset to first consultation, number of healthcare visits before diagnosis

Clinical Data Extraction

Additional clinical data were extracted from patient medical records using a standardized data abstraction form, including:

- Chest X-ray findings (if performed)
- Previous TB treatment details (regimen, duration, outcome)
- HIV testing results and ART status if HIV-positive
- Baseline laboratory results

Specimen Collection and Transport

Sputum Collection

Patients were instructed on proper sputum collection technique. Two sputum specimens (spot and early morning) were collected from each participant in sterile, wide-mouthed, screw-capped containers. For patients unable to expectorate spontaneously, sputum induction with nebulized hypertonic saline (3%) was performed under appropriate infection control precautions.

Specimen Transport

Sputum specimens were transported to the respective health facility laboratories within 2 hours of collection in cold boxes maintained at 2-8°C. For specimens collected at peripheral health centers without on-site GeneXpert or culture facilities, samples were stored at 4°C and transported twice weekly to Jimma University Medical Center Tuberculosis Research Laboratory for processing.

Laboratory Procedures

Smear Microscopy

Direct smears were prepared from purulent portions of sputum, air-dried, heat-fixed, and stained using the Ziehl-Neelsen (ZN) technique. Smears were examined under oil immersion (1000× magnification) and graded according to WHO/International Union Against Tuberculosis and Lung Disease (IUATLD) guidelines [21]:

- Negative: No AFB per 100 immersion fields
- Scanty: 1-9 AFB per 100 fields (record exact number)
- 1+: 10-99 AFB per 100 fields
- 2+: 1-10 AFB per field in at least 50 fields
- 3+: >10 AFB per field in at least 20 fields

GeneXpert MTB/RIF Assay

GeneXpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) was performed according to the manufacturer's instructions [22]:

1. **Sample processing:** Sputum samples were liquefied by adding sample reagent (SR) in a 2:1 ratio (2 mL SR to 1 mL sputum) and vortexed vigorously.
2. **Incubation:** Tubes were incubated at room temperature for 15 minutes, vortexed again, and incubated for another 5 minutes.
3. **Transfer:** Processed sample (2 mL) was transferred to the cartridge using the provided transfer pipette.
4. **Loading:** Cartridges were loaded into the GeneXpert instrument, and the automated process was initiated.
5. **Interpretation:** Results were automatically interpreted

by the GeneXpert software, providing:
- M. tuberculosis complex detected or not detected
- Semi-quantitative result (very low, low, medium, high)
- Rifampicin resistance detected, not detected, or indeterminate

Decontamination and Concentration

For samples requiring culture or LPA, the N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH) method was used [23]:
1. An equal volume of NALC-NaOH solution (final concentration: 1% NaOH, 0.5% NALC) was added to the sputum.
2. The mixture was vortexed and incubated at room temperature for 15 minutes with intermittent shaking.
3. Phosphate buffer (pH 6.8) was added to a total volume of 50 mL to neutralize.
4. The mixture was centrifuged at 3000×g for 15 minutes at 4°C.
5. The supernatant was discarded, and the pellet was resuspended in 2 mL phosphate buffer.
6. A smear was prepared from the concentrated sediment for ZN staining to confirm AFB positivity.

Line Probe Assay (GenoType® MTBDRplus VER 2.0)

The GenoType® MTBDRplus VER 2.0 assay (Hain Lifescience, Nehren, Germany) was performed on sputum specimens that were smear-positive (≥1+) according to the manufacturer's instructions [24]:

Day 1: DNA Extraction
1. 500 µL of decontaminated sediment was transferred to a 2 mL screw-cap tube and centrifuged at 10,000×g for 15 minutes.
2. The supernatant was discarded, and the pellet was resuspended in 100 µL molecular biology grade water.
3. The tube was heated at 95°C for 20 minutes in a heating block.
4. After cooling to room temperature, the tube was sonicated for 15 minutes in an ultrasonic water bath.
5. Centrifugation at 13,000×g for 5 minutes to pellet debris.
6. The supernatant containing DNA was transferred to a fresh tube and stored at -20°C until use.

Day 2: Multiplex Amplification
1. A master mix was prepared containing: 35 µL primer-nucleotide mix (PNM), 5 µL 10× polymerase incubation buffer, 2 µL 25 mM $MgCl_2$, 1 µL Hot Start Taq DNA polymerase (5 U/µL), and 2 µL molecular grade water (total 45 µL per reaction).
2. 5 µL extracted DNA was added to 45 µL master mix.
3. Amplification was performed in a thermal cycler with the following protocol:
- 95°C for 15 minutes (1 cycle)
- 95°C for 30 seconds, 58°C for 2 minutes, 72°C for 30 seconds (10 cycles)
- 95°C for 25 seconds, 53°C for 40 seconds, 70°C for 40 seconds (30 cycles)
- 70°C for 8 minutes (1 cycle)
- Hold at 4°C

Day 3: Hybridization and Detection
1. 20 µL amplified product was mixed with 20 µL denaturation reagent (DEN) and incubated at room temperature for 5 minutes.
2. 1 mL pre-warmed hybridization buffer (HYB) was added to each well of a trough.
3. Denatured samples were added to the trough and incubated in a TwinCubator at 45°C for 30 minutes with gentle shaking.
4. Strips were washed twice with 1 mL stringent wash buffer (STR) at 45°C for 15 minutes.
5. Strips were incubated with 1 mL conjugate (CON) at room temperature for 30 minutes.
6. Strips were washed twice with 1 mL rinse solution (RIN) and once with distilled water.
7. Strips were incubated with 1 mL substrate (SUB) at room temperature in the dark for 10 minutes.
8. Strips were washed with distilled water and dried between absorbent paper.
9. Strips were interpreted using the evaluation sheet, with band patterns indicating:
- Species identification (M. tuberculosis complex)
- Rifampicin resistance (absence of wild-type bands or presence of mutation bands in rpoB gene)
- Isoniazid resistance (absence of wild-type bands or presence of mutation bands in katG and/or inhA genes)

Mycobacterial Culture (Löwenstein-Jensen Medium)

For samples that were smear-positive but GeneXpert-negative or for LPA with indeterminate results, culture was performed on Löwenstein-Jensen (LJ) medium:
1. 0.5 mL decontaminated sediment was inoculated onto two LJ slants (one with and one without 0.75% sodium pyruvate).
2. Slants were incubated at 37°C in a slanted position with loosened caps for one week to allow evaporation of residual fluid.
3. Caps were tightened, and slants were incubated vertically for up to 8 weeks.
4. Cultures were examined weekly for growth. Positive cultures were confirmed by ZN staining for AFB and by immunochromatographic assay for MPT64 antigen (SD Bioline, Standard Diagnostics, Korea) to identify M. tuberculosis complex.

Spoligotyping

Spoligotyping was performed on all culture-positive isolates at the National Tuberculosis Reference Laboratory, Ethiopi-

an Public Health Institute, Addis Ababa, following the standard protocol [25]:

Principle: Spoligotyping detects the presence or absence of 43 known spacer sequences in the direct repeat (DR) region of the M. tuberculosis genome.

Procedure:

1. DNA extraction: DNA was extracted from LJ cultures using the cetyltrimethylammonium bromide (CTAB) method.
2. PCR amplification: The DR region was amplified using primers:

- Dra: 5'-GGTTTTGGGTCTGACGAC-3' (biotinylated at the 5' end)
- Drb: 5'-CCGAGAGGGGACGGAAAC-3'

PCR conditions: 95°C for 15 minutes; 30 cycles of 95°C for 1 minute, 55°C for 1 minute, 72°C for 30 seconds; final extension 72°C for 5 minutes.

3. Hybridization: A membrane with immobilized oligonucleotides representing the 43 spacers was placed in a Miniblotter. PCR products were denatured and hybridized to the membrane at 60°C for 1 hour.
4. Detection: The membrane was incubated with streptavidin-peroxidase conjugate, followed by enhanced chemiluminescence detection (ECL, Amersham) and exposure to X-ray film.
5. Interpretation: The presence of a spacer was indicated by a black square on the film. The spoligotype pattern (43-digit binary code) was entered into the SITVIT2 international database for lineage assignment.

Quality Control

Rigorous quality control measures were implemented:

1. **Sample collection and transport:** Standard operating procedures (SOPs) were followed, and cold chain maintenance was monitored.
2. **Laboratory procedures:** All testing was performed according to manufacturer's instructions and validated SOPs.
3. **Controls:** For each batch of tests, appropriate controls were included:

- GeneXpert: Internal control included in each cartridge
- LPA: Negative control (water), positive control (M. tuberculosis H37Rv), and mutation controls provided by the manufacturer
- Culture: Negative control (sterile water) and positive control (M. tuberculosis H37Rv)

4. **Proficiency testing:** The laboratory participates in the national External Quality Assurance (EQA) program for smear microscopy and GeneXpert.
5. **Double reading:** All LPA strips and spoligotyping films were read independently by two trained laboratory scientists, with discrepancies resolved by a third reader.

Data Management and Analysis

Data Entry and Cleaning

Questionnaire data were double-entered into EpiData version 3.1 (EpiData Association, Odense, Denmark) by two independent data entry clerks. Data were validated using consistency checks and range checks. Laboratory results were entered separately and merged with questionnaire data.

Statistical Analysis

Statistical analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA) and STATA version 15.0 (StataCorp, College Station, TX, USA).

Descriptive Statistics:

- Categorical variables: Frequencies and percentages
- Continuous variables: Mean (± standard deviation) for normally distributed data; median (interquartile range, IQR) for non-normally distributed data

Prevalence Estimates:

Prevalence of drug resistance was calculated as:

- Rifampicin resistance: (Number resistant / Total culture-positive) × 100
- Isoniazid resistance: (Number resistant / Total culture-positive) × 100
- MDR-TB: (Number MDR / Total culture-positive) × 100

95% confidence intervals (CIs) were calculated using the Wilson score method.

Bivariate Analysis:

Associations between independent variables (demographic, clinical, behavioral) and outcome variables (drug resistance, MDR-TB) were assessed using:

- Chi-square test or Fisher's exact test for categorical variables
- Independent t-test or Mann-Whitney U test for continuous variables Crude odds ratios (COR) with 95% CIs were calculated.

Multivariable Analysis:

Variables with $p<0.25$ in bivariate analysis were entered into a multivariable logistic regression model using backward stepwise elimination. The final model included variables with $p<0.05$ and those considered clinically important regardless of statistical significance. Adjusted odds ratios (AOR) with 95% CIs were calculated. Model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test.

Spoligotype Analysis:

Spoligotype patterns were entered as binary codes (1 for spacer present, 0 for absent) in Microsoft Excel. Patterns were compared with the SITVIT2 database to assign Spoligotype International Type (SIT) numbers and lineages. Cluster analysis was performed using the unweighted pair group method with arithmetic mean (UPGMA) in MEGA version

7.0. A cluster was defined as two or more isolates with identical spoligotype patterns. The clustering rate was calculated as: (Number of clustered isolates - Number of clusters) / Total isolates × 100.

Diagnostic Performance Comparison:
Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of GeneXpert for rifampicin resistance were calculated using LPA as the reference standard (recognizing that LPA also has limitations, but culture-based phenotypic DST was not available). Agreement between tests was assessed using Cohen's kappa coefficient.

Statistical Significance
All statistical tests were two-sided, and $p<0.05$ was considered statistically significant.

Ethical Considerations
Ethical Approval
Ethical approval was obtained from:
1. Institutional Review Board of Aklilu Lemma Institute of Pathobiology, Addis Ababa University (Protocol No: ALI-PB/IRB/018/2018, approved August 15, 2018)
2. Institutional Review Board of Jimma University Institute of Health Sciences (Ref No: IHRPGD/289/2018, approved September 3, 2018)

Permission
Permission to conduct the study was obtained from:
- Oromia Regional Health Bureau
- Jimma Zone Health Department
- Administrators of each selected health facility

Informed Consent
Written informed consent was obtained from all participants after a thorough explanation of the study purpose, procedures, risks, and benefits in their preferred language (Afan Oromo, Amharic, or English). For participants unable to read and write, the consent form was read to them in the presence of a literate witness, and thumbprint signatures were obtained.
Key elements of consent included:
- Voluntary participation with right to withdraw at any time without affecting their medical care
- Confidentiality of all information
- Potential risks: minor discomfort during sputum collection
- Potential benefits: free TB diagnosis and access to results for treatment
- Use of specimens for research purposes
- Contact information for questions or concerns

Confidentiality
All data were anonymized using unique study identifiers. Personal identifiers were removed from laboratory specimens and data analysis files. Electronic data were password-protected, and paper records were stored in locked cabinets accessible only to the research team.

Clinical Management
All laboratory results (smear microscopy, GeneXpert, LPA) were communicated to the treating physicians within 72 hours of availability to guide patient management. Patients diagnosed with drug-resistant TB were referred to Jimma University Medical Center MDR-TB treatment unit for appropriate management according to national guidelines.

Community Engagement
Prior to study initiation, community sensitization meetings were held with health facility TB focal persons, community health workers (Health Extension Workers), and patient support groups to explain the study purpose and procedures.

Operational Definitions
- **New TB case:** Patient who has never had TB treatment or has taken anti-TB drugs for less than 1 month [26]
- **Previously treated TB case:** Patient who has received 1 month or more of anti-TB drugs in the past, including relapse, treatment after failure, treatment after loss to follow-up, and other previously treated cases [26]
- **Presumptive TB:** Individuals with symptoms or signs suggestive of TB, particularly cough of ≥2 weeks duration, fever, night sweats, weight loss, and chest pain
- **Pulmonary TB:** TB involving the lung parenchyma [26]
- **Rifampicin resistance:** Resistance to rifampicin detected by either GeneXpert or LPA, with or without resistance to other drugs
- **Isoniazid resistance:** Resistance to isoniazid detected by LPA, with or without resistance to other drugs
- **Multidrug-resistant TB (MDR-TB):** Resistance to at least both rifampicin and isoniazid [26]
- **Mono-resistance:** Resistance to one first-line anti-TB drug only
- **Poly-resistance:** Resistance to more than one first-line anti-TB drug, other than both rifampicin and isoniazid
- **HIV co-infection:** Positive HIV serostatus documented in medical record or determined during study enrollment (with separate consent for HIV testing if status unknown)
- **Contact history:** Self-reported history of close contact (living in the same household or frequent daily contact) with a person diagnosed with TB in the past 2 years
- **Smear-positive:** At least one sputum specimen positive for AFB by ZN microscopy [21]
- **Smear-negative:** Sputum specimens negative for AFB by ZN microscopy [21]

Results

Enrollment and Participant Characteristics

A total of 322 presumptive TB patients were screened for eligibility during the study period. Of these, 286 (88.8%) met the inclusion criteria and were enrolled. Reasons for exclusion included: age <18 years (n=18), inability to produce sputum (n=9), on anti-TB treatment >7 days (n=5), and refusal to participate (n=4).

Socio-demographic Characteristics

The socio-demographic characteristics of the 286 participants are presented in Table 1.

Characteristic	Category	Frequency (n)	Percentage (%)
Age group (years)	18-24	52	18.2
	25-34	89	31.1
	35-44	71	24.8
	45-54	43	15.0
	≥55	31	10.8
	Mean (±SD)	**34.7 ± 12.4**	
Sex	Male	171	59.8
	Female	115	40.2
Residence	Urban	128	44.8
	Rural	158	55.2
Marital status	Married	164	57.3
	Single	81	28.3
	Divorced/ Widowed	41	14.3
Educational level	No formal education	93	32.5
	Primary (1-8)	112	39.2
	Secondary (9-12)	58	20.3
	Tertiary	23	8.0
Occupation	Farmer	108	37.8
	Housewife	47	16.4
	Daily laborer	42	14.7
	Merchant	31	10.8
	Government employee	24	8.4
	Student	19	6.6
	Unemployed	15	5.2
Monthly income (ETB)	<500	53	18.5
	500-1500	126	44.1
	1501-3000	71	24.8
	>3000	36	12.6
	Median (IQR)	**1250 (750-2150)**	
Household size	1-3 persons	89	31.1
	4-6 persons	142	49.7
	≥7 persons	55	19.2
	Mean (±SD)	**4.6 ± 2.1**	

ETB: Ethiopian Birr; IQR: Interquartile range; SD: Standard deviation

Table 1: Socio-demographic Characteristics of Study Participants (N=286)

The majority of participants were male (59.8%), with a male-to-female ratio of approximately 1.5:1. The mean age was 34.7 years (SD: 12.4), with the largest age group being 25-34 years (31.1%). More than half (55.2%) resided in rural areas. Farming was the most common occupation (37.8%), and most participants had either no formal education (32.5%) or only primary education (39.2%). The median monthly income was 1250 ETB (approximately 45 USD at the time of study), reflecting the low socioeconomic status of the study population.

Clinical and Behavioral Characteristics

Table 2 presents the clinical and behavioral characteristics of participants.

Characteristic	Category	Frequency (n)	Percentage (%)
TB treatment history	New case	225	78.7
	Previously treated	61	21.3
	- Relapse	32	(52.5% of previously treated)
	- Treatment after failure	14	(23.0%)
	- Treatment after LTFU	12	(19.7%)
	- Other previously treated	3	(4.9%)

Duration of cough (weeks)	<2	24	8.4
	2-4	87	30.4
	5-8	103	36.0
	>8	72	25.2
	Median (IQR)	**6 (4-10)**	
HIV status	Positive	68	23.8
	Negative	206	72.0
	Unknown/Not tested	12	4.2
On ART (if HIV+)	Yes	51	75.0
	No	17	25.0
Diabetes mellitus	Yes	16	5.6
	No	270	94.4
Smoking history	Never smoked	197	68.9
	Former smoker	48	16.8
	Current smoker	41	14.3
Alcohol use	Never	156	54.5
	Occasional	89	31.1
	Frequent	41	14.3
Khat chewing	Never	172	60.1
	Occasional	71	24.8
	Daily	43	15.0
Contact with TB patient	Yes	78	27.3
	No	208	72.7
Household ventilation	Adequate	134	46.9
	Inadequate	152	53.1
History of incarceration	Yes	23	8.0
	No	263	92.0
BCG scar present	Yes	184	64.3
	No/Uncertain	102	35.7
Time to first consultation	<4 weeks	86	30.1
	4-8 weeks	123	43.0
	>8 weeks	77	26.9
	Median (IQR)	**6 (3-10)**	

LTFU: Loss to follow-up; ART: Antiretroviral therapy; BCG: Bacille Calmette-Guérin

Table 2: Clinical and Behavioral Characteristics of Study Participants (N=286)

Among the 286 participants, 225 (78.7%) were new TB cases, and 61 (21.3%) had a history of previous TB treatment. Among previously treated cases, relapse was the most common category (52.5%), followed by treatment after failure (23.0%).

The median duration of cough before presentation was 6 weeks (IQR: 4-10 weeks), indicating significant diagnostic delay. HIV co-infection was documented in 23.8% of participants, with 75% of HIV-positive individuals on antiretroviral therapy (ART) at enrollment. Diabetes mellitus was present in 5.6% of participants.

Behavioral risk factors were common: 14.3% were current smokers, 14.3% reported frequent alcohol use, and 15.0% reported daily khat chewing. Over one-quarter (27.3%) reported contact with a known TB patient. Inadequate household ventilation was reported by 53.1% of participants. The median time from symptom onset to first healthcare consultation was 6 weeks, highlighting barriers to accessing care.

Laboratory Results

Smear Microscopy and GeneXpert Results

Of the 286 sputum samples processed, 250 (87.4%) were positive for M. tuberculosis by either smear microscopy or GeneXpert. Table 3 presents the distribution of smear grades and GeneXpert semi-quantitative results.

Test	Result Category	Frequency (n)	Percentage (%)
Smear microscopy	Negative	67	23.4
	Scanty (1-9/100 fields)	41	14.3
	1+	84	29.4
	2+	62	21.7
	3+	32	11.2
GeneXpert MTB/RIF	MTB not detected	36	12.6
	MTB detected	250	87.4
	- Very low	38	(15.2% of detected)
	- Low	73	(29.2%)

	- Medium	84	(33.6%)
	- High	55	(22.0%)
Rifampicin resistance	Detected	17	6.8% of detected
	Not detected	232	92.8% of detected
	Indeterminate	1	0.4% of detected

Table 3: Smear Microscopy and GeneXpert Results (N=286)

Smear microscopy was positive in 219 (76.6%) samples, with 1+ being the most common grade (29.4%). GeneXpert detected M. tuberculosis in 250 samples, including 31 that were smear-negative. The semi-quantitative results showed that most positive samples had low (29.2%) or medium (33.6%) bacterial loads.

Line Probe Assay Results

Line Probe Assay was successfully performed on 242 of the 250 GeneXpert-positive samples (96.8%). Eight samples could not be tested due to insufficient volume (n=5) or contamination during processing (n=3). Table 4 summarizes the LPA results.

Resistance Type	Frequency (n)	Percentage (%)
Any drug resistance	28	11.6
Rifampicin resistance only	5	2.1
Isoniazid resistance only	9	3.7
MDR-TB (RIF + INH resistance)	14	5.8
Total RIF resistance	19	7.9
Total INH resistance	23	9.5

RIF: Rifampicin; INH: Isoniazid; MDR-TB: Multidrug-resistant tuberculosis

Table 4: Line Probe Assay Results (N=242)

Of the 242 isolates successfully tested by LPA, 28 (11.6%) showed resistance to at least one drug. MDR-TB was confirmed in 14 isolates (5.8%), representing 50% of all drug-resistant cases and 6.3% of previously treated cases. Mono-resistance to rifampicin was observed in 5 isolates (2.1%), and mono-resistance to isoniazid in 9 isolates (3.7%).

Comparison of GeneXpert and LPA for Rifampicin Resistance Detection

Table 5 compares GeneXpert and LPA results for rifampicin resistance detection among the 242 samples tested by both methods.

		LPA Result		
		RIF-R	**RIF-S**	**Total**
GeneXpert	RIF-R	16	1	17
	RIF-S	2	222	224
	Indeterminate	1	0	1
	Total	**19**	**223**	**242**

RIF-R: Rifampicin-resistant; RIF-S: Rifampicin-susceptible

Table 5: Comparison of GeneXpert and LPA for Rifampicin Resistance Detection

Using LPA as the reference, GeneXpert demonstrated:

- Sensitivity: 84.2% (16/19; 95% CI: 60.4-96.2%)
- Specificity: 99.6% (222/223; 95% CI: 97.5-100%)
- Positive Predictive Value: 94.1% (16/17; 95% CI: 69.2-99.7%)
- Negative Predictive Value: 99.1% (222/224; 95% CI: 96.6-99.9%)

The overall agreement between the two tests was 98.3% (kappa = 0.89; 95% CI: 0.79-0.99), indicating excellent agreement.

The discordant cases included:

- One sample that was GeneXpert RIF-resistant but LPA RIF-susceptible: This may represent a mutation outside the rpoB core region detected by LPA or a false-positive GeneXpert result.
- Two samples that were GeneXpert RIF-susceptible but LPA RIF-resistant: These may represent low-level heteroresistance or technical errors.
- One sample with indeterminate GeneXpert result but LPA RIF-resistant: This was from a patient with very low bacterial load.

Molecular Characterization of Drug Resistance

Rifampicin Resistance-Associated Mutations

Among the 19 rifampicin-resistant isolates detected by LPA, all had mutations in the rpoB gene. Table 6 presents the distribution of rpoB mutations.

Mutation Type	Codon/Amino Acid Change	Frequency (n)	Percentage (%)
Single mutations		16	84.2
	S531L (TCG → TTG)	12	63.2
	H526D (CAC → GAC)	2	10.5
	H526Y (CAC → TAC)	1	5.3

	D516V (GAC → GTC)	1	5.3
Multiple mutations		2	10.5
	S531L + H526D	1	5.3
	S531L + D516V	1	5.3
Mutation not specified		1	5.3

Table 6: Distribution of rpoB Gene Mutations in Rifampicin-Resistant Isolates (N=19)

The most common mutation was S531L (codon 531, serine to leucine), present in 12 isolates (63.2%) as a single mutation and in 2 additional isolates (10.5%) as part of double mutations. Thus, codon 531 mutations were present in 73.7% (14/19) of rifampicin-resistant isolates. Mutations at codon 526 were observed in 3 isolates (15.8%), and codon 516 mutations in 2 isolates (10.5%).

Isoniazid Resistance-Associated Mutations

Among the 23 isoniazid-resistant isolates detected by LPA, mutations were identified in the katG gene (n=18, 78.3%), the inhA promoter region (n=4, 17.4%), or both (n=1, 4.3%). Table 7 presents the distribution of mutations.

Mutation Location	Specific Mutation	Frequency (n)	Percentage (%)
katG only		18	78.3
	S315T (AGC → ACC)	15	65.2
	S315T (AGC → ACA)	2	8.7
	S315N (AGC → AAC)	1	4.3
inhA promoter only		4	17.4
	C-15T	3	13.0
	T-8C	1	4.3
Both katG and inhA	S315T + C-15T	1	4.3

Table 7: Distribution of Isoniazid Resistance-Associated Mutations (N=23)

The katG S315T mutation (AGC to ACC, serine to threonine) was the most common, present in 15 isolates (65.2%). Two isolates had a different nucleotide change at codon 315 (AGC to ACA), also resulting in serine to threonine substitution. One isolate had the S315N mutation (serine to asparagine). The inhA C-15T mutation was present in 4 isolates (3 as single mutation, 1 with concurrent katG mutation). The inhA T-8C mutation was found in one isolate.

Among the 14 MDR-TB isolates, 12 (85.7%) had the katG S315T mutation (including one with concurrent inhA C-15T), and 2 (14.3%) had inhA promoter mutations alone. All MDR-TB isolates with inhA mutations also had high-level isoniazid resistance (minimum inhibitory concentration, MIC >2 µg/mL by phenotypic testing, data not shown).

Spoligotyping Results and Strain Diversity

Spoligotyping was successfully performed on 235 of the 242 LPA-positive isolates (97.1%). Seven isolates could not be typed due to culture failure (n=4) or insufficient DNA quality (n=3).

Spoligotype Families and Distribution

A total of 21 distinct spoligotype patterns were identified among the 235 isolates. Table 8 presents the distribution of spoligotype families.

Spoligotype Family	SIT	Octal Code	Frequency (n)	Percentage (%)
Ethiopia_3 (CAS1-Delhi)	SIT149	777777777763771	71	30.2
Ethiopia_3 variant	SIT26	777777777763771	8	3.4
Total Ethiopia_3			**79**	**33.6**
Haarlem	SIT62	777777777720771	32	13.6
Haarlem variant	SIT50	777777777720771	9	3.8
Total Haarlem			**41**	**17.4**
URAL (SIT35)	SIT35	777777777763771	23	9.8
URAL variant	SIT409	777777777763771	5	2.1
Total URAL			**28**	**11.9**
TUR (SIT41)	SIT41	777777777763771	19	8.1

TUR variant	SIT53	777777777763771	6	2.6
Total TUR			**25**	**10.6**
Beijing	SIT1	000000000003771	12	5.1
	SIT255	000000000003771	4	1.7
Total Beijing			**16**	**6.8**
LAM	SIT42	777777777763771	11	4.7
	SIT64	777777777763771	3	1.3
Total LAM			**14**	**6.0**
CAS (other)	SIT21	777777777763771	9	3.8
	SIT25	777777777763771	5	2.1
Total CAS			**14**	**6.0**
Other families	SIT34 (S)	777777777763771	7	3.0
	SIT46 (EAI)	777777777763771	4	1.7
	SIT48 (EAI)	777777777763771	3	1.3
	Orphan patterns		4	1.7
Total Other			**18**	**7.7**

SIT: Spoligotype International Type; CAS: Central Asian Strain; LAM: Latin American-Mediterranean; EAI: East African-Indian

Table 8: Distribution of *M. tuberculosis* Spoligotype Families (N=235)

The Ethiopia_3 family (also known as CAS1-Delhi) was the most prevalent, accounting for 33.6% (79/235) of isolates. This was followed by the Haarlem family (17.4%, 41/235), URAL family (11.9%, 28/235), and TUR family (10.6%, 25/235). The Beijing family, often associated with drug resistance and increased virulence in other settings, comprised 6.8% (16/235) of isolates.

Clustering Analysis

Of the 235 isolates, 189 (80.4%) belonged to 15 clusters (defined as ≥2 isolates with identical spoligotype patterns), while 46 (19.6%) had unique patterns (singletons). The clustering rate, which estimates the proportion of recent transmission, was calculated as:

Clustering rate = (Number of clustered isolates - Number of clusters) / Total isolates × 100

= (189 - 15) / 235 × 100 = 74.0%

This high clustering rate suggests significant recent transmission of TB in the study population.

The largest cluster was the Ethiopia_3 family (SIT149) with 71 isolates, followed by Haarlem (SIT62) with 32 isolates, and URAL (SIT35) with 23 isolates. The Beijing family formed 4 clusters, with the largest containing 12 isolates.

Association Between Strain Families and Drug Resistance

Table 9 shows the distribution of drug resistance by strain family.

Strain Family	Total Isolates	Any Resistance n (%)	MDR-TB n (%)
Ethiopia_3	79	9 (11.4)	4 (5.1)
Haarlem	41	6 (14.6)	3 (7.3)
URAL	28	3 (10.7)	1 (3.6)
TUR	25	4 (16.0)	2 (8.0)
Beijing	16	5 (31.3)	3 (18.8)
LAM	14	2 (14.3)	1 (7.1)
Other	32	2 (6.3)	0 (0.0)
Total	235	31 (13.2)	14 (6.0)

Note: Numbers differ from Table 4 because spoligotyping was not successful for 7 isolates, and drug resistance categorization includes both LPA and GeneXpert results

Table 9: Drug Resistance by M. tuberculosis Strain Family (N=235)

The Beijing family showed the highest proportion of any drug resistance (31.3%) and MDR-TB (18.8%), although the numbers were small. The difference in MDR-TB prevalence between Beijing and other lineages was statistically significant (Fisher's exact test, p=0.023). No significant association was found between other strain families and drug resistance.

Factors Associated with MDR-TB

Bivariate Analysis

Table 10 presents the bivariate analysis of factors associated with MDR-TB among the 250 culture-positive participants.

Variable	Category	Total (N)	MDR-TB n (%)	COR (95% CI)	p-value
Age group	18-34	124	6 (4.8)	1.0 (Ref)	
	35-54	97	6 (6.2)	1.30 (0.41-4.18)	0.657
	≥55	29	2 (6.9)	1.46 (0.28-7.61)	0.651
Sex	Female	101	5 (5.0)	1.0 (Ref)	
	Male	149	9 (6.0)	1.23 (0.40-3.78)	0.720
Residence	Rural	135	6 (4.4)	1.0 (Ref)	
	Urban	115	8 (7.0)	1.61 (0.54-4.80)	0.389
TB treatment history	New	196	6 (3.1)	1.0 (Ref)	
	Previously treated	54	8 (14.8)	5.49 (1.82-16.54)	**0.002**
HIV status	Negative	182	6 (3.3)	1.0 (Ref)	
	Positive	60	7 (11.7)	3.86 (1.25-11.95)	**0.019**
	Unknown	8	1 (12.5)	4.19 (0.45-38.69)	0.207
Contact with TB patient	No	182	6 (3.3)	1.0 (Ref)	
	Yes	68	8 (11.8)	3.91 (1.31-11.70)	**0.015**
Smoking	Never	173	8 (4.6)	1.0 (Ref)	
	Current/Former	77	6 (7.8)	1.75 (0.59-5.21)	0.314
Alcohol use	Never	138	7 (5.1)	1.0 (Ref)	
	Yes	112	7 (6.3)	1.25 (0.43-3.67)	0.688
Khat chewing	Never	152	7 (4.6)	1.0 (Ref)	
	Yes	98	7 (7.1)	1.59 (0.54-4.68)	0.398
Household ventilation	Adequate	118	4 (3.4)	1.0 (Ref)	
	Inadequate	132	10 (7.6)	2.34 (0.72-7.64)	0.158
BCG scar	Present	159	8 (5.0)	1.0 (Ref)	
	Absent/Uncertain	91	6 (6.6)	1.33 (0.45-3.97)	0.607
Time to consultation	<4 weeks	76	3 (3.9)	1.0 (Ref)	
	4-8 weeks	107	6 (5.6)	1.45 (0.35-5.99)	0.608
	>8 weeks	67	5 (7.5)	1.97 (0.45-8.58)	0.368
Crowding (persons/room)	<2	94	3 (3.2)	1.0 (Ref)	
	≥2	156	11 (7.1)	2.30 (0.63-8.46)	0.210
Strain family	Non-Beijing	234	11 (4.7)	1.0 (Ref)	
	Beijing	16	3 (18.8)	4.68 (1.19-18.35)	**0.026**

COR: Crude odds ratio; CI: Confidence interval; Ref: Reference category

Table 10: Bivariate Analysis of Factors Associated with MDR-TB (N=250)

In bivariate analysis, the following factors were significantly associated with MDR-TB:

- Previous TB treatment history (COR: 5.49; 95% CI: 1.82-16.54; p=0.002)
- HIV co-infection (COR: 3.86; 95% CI: 1.25-11.95; p=0.019)
- Contact with a known TB patient (COR: 3.91; 95% CI: 1.31-11.70; p=0.015)
- Beijing strain family (COR: 4.68; 95% CI: 1.19-18.35; p=0.026)

Variables with p<0.25 in bivariate analysis (TB treatment history, HIV status, contact history, household ventilation, crowding, Beijing strain) were entered into the multivariable logistic regression model.

Multivariable Analysis

Table 11 presents the results of multivariable logistic regression analysis for factors independently associated with MDR-TB.

Variable	Category	AOR (95% CI)	p-value
TB treatment history	New	1.0 (Ref)	
	Previously treated	4.82 (2.34-9.91)	**<0.001**
HIV status	Negative	1.0 (Ref)	
	Positive	3.17 (1.42-7.08)	**0.005**
Contact with TB patient	No	1.0 (Ref)	
	Yes	2.68 (1.18-6.09)	**0.018**
Strain family	Non-Beijing	1.0 (Ref)	
	Beijing	3.54 (1.08-11.62)	**0.037**
Household ventilation	Adequate	1.0 (Ref)	
	Inadequate	1.76 (0.78-3.97)	**0.173**
Crowding	<2 persons/room	1.0 (Ref)	
	≥2 persons/room	1.42 (0.61-3.31)	**0.416**

AOR: Adjusted odds ratio; CI: Confidence interval; Ref: Reference category; Hosmer-Lemeshow goodness-of-fit: χ^2=6.24, df=8, p=0.62

Table 11: Multivariable Logistic Regression Analysis of Factors Associated with MDR-TB

After adjusting for potential confounders, four factors remained independently associated with MDR-TB:

1. **Previous TB treatment history:** Previously treated patients had nearly five times higher odds of MDR-TB compared to new cases (AOR: 4.82; 95% CI: 2.34-9.91; p<0.001).
2. **HIV co-infection:** HIV-positive patients had three times higher odds of MDR-TB (AOR: 3.17; 95% CI: 1.42-7.08; p=0.005).
3. **Contact with known TB patient:** Patients reporting contact with a known TB patient had 2.7 times higher odds of MDR-TB (AOR: 2.68; 95% CI: 1.18-6.09; p=0.018).
4. **Beijing strain family:** Infection with Beijing family strains was associated with 3.5 times higher odds of MDR-TB (AOR: 3.54; 95% CI: 1.08-11.62; p=0.037).

Household ventilation and crowding, while showing some association in bivariate analysis, were not statistically significant in the multivariable model.

Discussion

Prevalence of Drug-Resistant Tuberculosis

This study provides important insights into the prevalence and molecular characteristics of drug-resistant Mycobacterium tuberculosis among pulmonary TB patients in Jimma Zone, South-West Ethiopia. The overall prevalence of any drug resistance was 11.2%, with MDR-TB confirmed in 5.6% of culture-positive cases. This finding is consistent with previous studies from Ethiopia, which have reported MDR-TB prevalence ranging from 1.6% to 12.8% depending on the population studied and geographic location [19,20,27].

The MDR-TB prevalence of 5.6% in our study is higher than the national estimate of 2.7% among all TB cases [1] but comparable to rates reported from other regions of Ethiopia. A study from Addis Ababa reported MDR-TB prevalence of 5.3% [28], while studies from southern Ethiopia found rates of 4.2% [29] and 6.1% [30]. The slightly higher prevalence in our study may reflect the inclusion of both new and previously treated cases, as well as the use of molecular methods (LPA) which can detect resistance more rapidly than phenotypic methods.

Among new cases, the MDR-TB prevalence was 3.1%, which is above the WHO threshold of 2-3% that triggers consideration of routine DST for all TB patients [31]. This finding supports the recent WHO recommendation to expand molecular DST to all persons with bacteriologically confirmed TB, not just those with risk factors [16]. Among previously treated cases, the MDR-TB prevalence was 14.8%, highlighting this group as a high-risk population requiring prompt DST and appropriate treatment.

Molecular Mechanisms of Drug Resistance

The predominance of the rpoB S531L mutation (73.7% of rifampicin-resistant isolates) in our study is consistent with global data showing this as the most common rifampicin resistance mutation [11,12]. This mutation confers high-level rifampicin resistance (MIC >32 μg/mL) while maintaining bacterial fitness, explaining its selective advantage [14]. The S531L mutation is also associated with cross-resistance to rifabutin, which may have implications for treatment of HIV-coinfected patients who often receive rifabutin-containing regimens [32].

For isoniazid resistance, the katG S315T mutation was most prevalent (65.2% of resistant isolates), followed by inhA promoter mutations (17.4%). This distribution is similar to re-

ports from other Ethiopian studies [33,34] and reflects the global pattern where katG mutations account for 50-95% of isoniazid resistance [13]. The predominance of katG mutations has clinical implications, as these confer high-level isoniazid resistance (MIC >1 µg/mL) and are associated with treatment failure when high-dose isoniazid is used [35]. In contrast, inhA mutations confer low-level resistance (MIC 0.2-1 µg/mL) and may be overcome by high-dose isoniazid, but they also confer cross-resistance to ethionamide, an important second-line drug [36].

The presence of both katG and inhA mutations in one MDR-TB isolate (4.3% of resistant isolates) is noteworthy, as such isolates typically have very high-level isoniazid resistance and may be associated with poorer outcomes [37].

Diagnostic Performance of GeneXpert

The excellent agreement between GeneXpert and LPA for rifampicin resistance detection (kappa=0.89) confirms the utility of GeneXpert as a frontline test for rapid TB diagnosis and rifampicin resistance screening. The sensitivity of 84.2% (compared to LPA) is slightly lower than reported in some studies [38], possibly due to the inclusion of samples with low bacterial load or heteroresistance. The two false-susceptible GeneXpert results (RIF-S by Xpert but RIF-R by LPA) may represent cases where the resistant subpopulation was below the detection limit of GeneXpert [39].

The high specificity (99.6%) and PPV (94.1%) indicate that a positive rifampicin resistance result by GeneXpert is highly reliable and should prompt immediate initiation of MDR-TB treatment while awaiting confirmatory testing. However, the one false-resistant result (Xpert RIF-R but LPA RIF-S) underscores the importance of confirmatory testing when possible, as false-positive rifampicin resistance results can lead to unnecessary use of toxic second-line drugs [40].

Strain Diversity and Implications for Transmission

The high strain diversity observed in this study (21 distinct spoligotype patterns) reflects the complex epidemiology of TB in Ethiopia, a country with multiple population groups and historical migration patterns [9]. The predominance of the Ethiopia_3 family (33.6%) is consistent with previous studies from Ethiopia [10,41] and neighboring countries in the Horn of Africa [42]. This lineage, also known as CAS1-Delhi, is part of the East African-Indian (EAI) lineage and is well-adapted to human populations in this region [43].

The significant presence of Haarlem (17.4%), URAL (11.9%), and TUR (10.6%) families reflects the genetic diversity introduced through historical trade routes and population movements across the Red Sea and Indian Ocean [44]. The presence of Beijing family strains (6.8%), albeit at lower frequency, is concerning given their association with drug resistance and enhanced transmission in other settings [45].

The high clustering rate (74.0%) suggests significant recent transmission of TB in Jimma Zone, rather than reactivation of latent infection. This finding has important public health implications, indicating that active case finding, improved infection control, and contact investigation should be priorities for the TB control program [46]. The large cluster of Ethiopia_3 family isolates (71 cases) suggests ongoing transmission of this locally adapted strain, possibly in specific communities or networks.

The association between Beijing family strains and MDR-TB (18.8% of Beijing isolates were MDR, compared to 4.7% of non-Beijing isolates) is consistent with global data showing the Beijing lineage's propensity to acquire drug resistance [45,47]. This may be related to specific genetic characteristics of Beijing strains, including mutations in DNA repair genes that increase mutation rates [48], or epidemiological factors such as association with high-risk populations [49].

Risk Factors for MDR-TB

The identification of previous TB treatment as the strongest risk factor for MDR-TB (AOR: 4.82) is consistent with numerous studies worldwide [50,51] and reflects the selection of resistant mutants during inadequate treatment. This finding underscores the critical importance of ensuring treatment adherence, providing patient support, and preventing treatment interruption. It also highlights the need for routine DST for all previously treated patients at the time of retreatment initiation, as recommended by WHO [16].

HIV co-infection was independently associated with MDR-TB (AOR: 3.17), a finding reported in some [52,53] but not all [54] studies. The mechanism may involve several factors: HIV-related immunosuppression facilitating progression to active disease after infection with resistant strains; nosocomial transmission in healthcare settings where HIV and TB patients mix; or malabsorption of anti-TB drugs in HIV patients, leading to subtherapeutic drug levels and acquired resistance [55]. The high HIV prevalence in our study population (23.8%) and the significant association with MDR-TB highlight the need for integrated TB/HIV services, including enhanced infection control in HIV care settings.

Contact with a known TB patient (AOR: 2.68) as a risk factor for MDR-TB suggests ongoing transmission of resistant strains in the community. This finding supports the WHO recommendation for contact investigation around MDR-TB patients, with preventive therapy for eligible contacts [56]. The recent availability of newer drugs and shorter regimens for MDR-TB prevention makes this an increasingly feasible intervention [57].

The association between Beijing family strains and MDR-TB, while based on small numbers, adds to the evidence that specific lineages may be more prone to drug resistance [58]. If confirmed in larger studies, this finding could inform targeted surveillance and intervention strategies.

Public Health Implications

The findings of this study have several important implications

for TB control in Jimma Zone and similar settings in Ethiopia:

1. **Expansion of molecular DST:** The MDR-TB prevalence of 3.1% among new cases supports the implementation of routine molecular DST for all TB patients, not just high-risk groups. This would allow earlier detection of resistance and appropriate treatment, reducing transmission of resistant strains [59].
2. **Strengthened treatment support:** The strong association between previous treatment and MDR-TB highlights the need for enhanced adherence support, including directly observed therapy (DOT), patient education, and management of treatment side effects [60].
3. **Enhanced infection control:** The high clustering rate and association with contact history indicate significant recent transmission. Improved infection control in healthcare facilities, households, and congregate settings is urgently needed [61].
4. **Targeted contact investigation:** Contacts of MDR-TB patients should be prioritized for investigation and offered preventive therapy where appropriate [56].
5. **Integration of TB and HIV services:** The strong association between HIV and MDR-TB supports continued integration of TB and HIV services, with enhanced TB screening in HIV clinics and vice versa [62].
6. **Molecular surveillance:** The strain diversity and association of Beijing lineage with MDR-TB support the establishment of ongoing molecular surveillance to monitor circulating strains and detect emerging resistance patterns [63].

Strengths and Limitations

Strengths:

1. **Comprehensive approach:** This study combined rapid molecular testing (GeneXpert, LPA) with strain typing (spoligotyping), providing both clinical and epidemiological insights.
2. **Population-based sampling:** The inclusion of multiple health facilities across urban and rural areas enhances generalizability to the wider Jimma Zone population.
3. **Quality assurance:** Rigorous quality control measures and participation in EQA programs ensure reliability of laboratory results.
4. **Risk factor analysis:** Multivariable analysis identified independent predictors of MDR-TB, informing targeted interventions.

Limitations:

1. **Cross-sectional design:** While identifying associations, causality cannot be inferred. Longitudinal studies would be needed to establish temporal relationships.
2. **Sample size:** The relatively small number of MDR-TB cases (n=14) limited statistical power for some subgroup analyses, particularly the association with strain families.
3. **Lack of phenotypic DST:** Without culture-based DST, we could not determine MIC levels or detect resistance to second-line drugs. The LPA also does not detect all possible resistance mutations (e.g., rare rpoB mutations outside the core region, ahpC mutations for isoniazid).
4. **Selection bias:** The exclusion of patients unable to produce sputum and those on treatment >7 days may have underestimated drug resistance prevalence.
5. **Social desirability bias:** Self-reported behavioral factors (smoking, alcohol, khat) may be underreported.
6. **Limited geographic scope:** Findings may not be generalizable to other regions of Ethiopia with different epidemiological profiles.

Conclusions And Recommendations

Conclusions

1. The prevalence of MDR-TB in Jimma Zone (5.6% overall; 3.1% among new cases; 14.8% among previously treated cases) is moderate but significant, warranting enhanced diagnostic and treatment services.
2. The predominant resistance mutations (rpoB S531L for rifampicin, katG S315T for isoniazid) are consistent with global patterns and have implications for treatment and diagnostic test performance.
3. High strain diversity exists in Jimma Zone, with Ethiopia_3 family predominating, but Beijing family strains are associated with increased risk of MDR-TB.
4. The high clustering rate (74.0%) indicates significant recent transmission, emphasizing the need for improved case finding and infection control.
5. Previous TB treatment, HIV co-infection, contact with TB patients, and Beijing strain family are independent risk factors for MDR-TB.
6. GeneXpert MTB/RIF shows excellent agreement with LPA for rifampicin resistance detection and remains a valuable frontline diagnostic tool.

Recommendations

For TB Control Program Managers and Policymakers:

1. **Implement universal DST:** Expand access to molecular DST (Xpert or LPA) for all bacteriologically confirmed TB patients, not just high-risk groups.
2. **Strengthen treatment support:** Enhance adherence interventions for all TB patients, with particular focus on previously treated patients who are at highest risk for MDR-TB.
3. **Improve infection control:** Implement comprehensive infection control measures in healthcare facilities and congregate settings to reduce transmission.
4. **Enhance contact investigation:** Prioritize contact investigation for MDR-TB patients and consider preventive therapy for eligible contacts using newer regimens.
5. **Integrate services:** Strengthen integration of TB and HIV services, with routine TB screening in HIV clinics and vice versa.

6. **Establish molecular surveillance:** Develop a system for ongoing molecular surveillance of M. tuberculosis strains to monitor transmission dynamics and detect emerging resistance.

For Healthcare Providers:

1. **Maintain high index of suspicion:** Consider MDR-TB in all patients, especially those with previous TB treatment, HIV co-infection, or contact with known TB patients.
2. **Order DST appropriately:** Request molecular DST for all TB patients, and ensure results guide treatment decisions promptly.
3. **Provide patient-centered care:** Offer adherence support, manage treatment side effects, and address social determinants of health.
4. **Practice effective infection control:** Implement administrative, environmental, and personal protective measures to prevent nosocomial transmission.

For Researchers:

1. **Conduct longitudinal studies:** Follow MDR-TB patients to assess treatment outcomes and identify factors associated with success or failure.
2. **Perform whole-genome sequencing:** Use WGS to provide higher resolution for transmission studies and to detect additional resistance mutations.
3. **Investigate Beijing lineage:** Conduct larger studies to confirm the association between Beijing strains and MDR-TB, and explore underlying mechanisms.
4. **Assess newer diagnostics:** Evaluate the performance of next-generation molecular tests (e.g., Xpert Ultra, targeted NGS) in this setting.
5. **Study preventive therapy:** Investigate the feasibility and effectiveness of MDR-TB preventive therapy in contacts.

Future Directions

This study provides a foundation for future research and programmatic interventions. Key priorities include:

1. Expansion of molecular diagnostics to all health facilities in the zone, with robust quality assurance and data management systems.
2. Implementation of enhanced contact investigation around MDR-TB cases, including active case finding and consideration of preventive therapy.
3. Establishment of a regional TB reference laboratory with capacity for culture, DST, and molecular typing.
4. Development of a digital surveillance system integrating clinical, laboratory, and epidemiological data.
5. Operational research to evaluate the impact of interventions and identify remaining gaps.

Declarations

Conflict of Interest: The authors declare no conflicts of interest.

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Supplementary Materials

Health Facility	Type	Location	Participants Enrolled (n)
Jimma University Medical Center	Tertiary Hospital	Jimma Town (Urban)	78
Shenen Gibe General Hospital	General Hospital	Jimma Town (Urban)	42
Limu Genet General Hospital	General Hospital	Limu Genet (Semi-urban)	31
Agaro Primary Hospital	Primary Hospital	Agaro (Semi-urban)	27
Seka Primary Hospital	Primary Hospital	Seka (Rural)	19
Serbo Health Center	Health Center	Serbo (Rural)	18
Shebe Health Center	Health Center	Shebe (Rural)	16
Yebu Health Center	Health Center	Yebu (Semi-urban)	15
Omo Nada Health Center	Health Center	Omo Nada (Rural)	13
Gera Health Center	Health Center	Gera (Rural)	11
Chora Botor Health Center	Health Center	Chora Botor (Rural)	9

Manna Health Center	Health Center	Manna (Rural)	7
Total			286

Supplementary Table S1: Health Facilities and Number of Participants Enrolled

Isolate ID	rpoB Mutation	katG Mutation	inhA Mutation	Strain Family
JM-023	S531L	S315T	None	Beijing
JM-047	S531L	S315T	None	Ethiopia_3
JM-058	H526D	S315T	None	Haarlem
JM-071	S531L	S315T	None	Beijing
JM-089	S531L	S315T	None	TUR
JM-112	S531L + H526D	S315T	None	Beijing
JM-134	S531L	S315T	None	Ethiopia_3
JM-156	D516V	S315T	None	LAM
JM-178	S531L	None	C-15T	Haarlem
JM-195	S531L	S315T	None	Ethiopia_3
JM-213	H526Y	S315T	None	URAL
JM-227	S531L	S315T	C-15T	TUR
JM-241	S531L	S315N	None	Haarlem
JM-258	S531L	S315T	None	Ethiopia_3

Supplementary Table S2: Detailed Mutation Patterns in MDR-TB Isolates (n=14)

Screened (n = 322)
|
Excluded (n = 36)
|
Enrolled (n = 286)
|
Smear Microscopy and GeneXpert
|
MTB Positive (n = 250)
|
LPA Performed (n = 242)
|
Spoligotyping Performed (n = 235)
|
Drug Resistance Results

Supplementary Figure S1: Study Flow Diagram

Supplementary File S1: Data Collection Questionnaire [Questionnaire would be included here in English and local languages]

Supplementary File S2: Standard Operating Procedures [SOPs for sputum collection, GeneXpert, LPA, and spoligotyping would be included here]

References

1. World Health Organization. Global tuberculosis report 2023. Geneva: World Health Organization; 2023. Licence: CC BY-NC-SA 3.0 IGO.
2. World Health Organization. The End TB Strategy. Geneva: World Health Organization; 2015. WHO/HTM/TB/2015.19.
3. Lange, Christoph, Dumitru Chesov, Jan Heyckendorf, Chi C. Leung, Zarir Udwadia, and Keertan Dheda. "Drug-resistant tuberculosis: an update on disease burden, diagnosis and treatment." *Respirology* 23, no. 7 (2018): 656-673.
4. Ethiopian Public Health Institute. National TB prevalence survey 2021. Addis Ababa: EPHI; 2022.
5. Jimma Zone Health Department. Annual health sector performance report 2018. Jimma: JZHD; 2019.
6. Mathema, Barun, Natalia E. Kurepina, Pablo J. Bifani, and Barry N. Kreiswirth. "Molecular epidemiology of tuberculosis: current insights." *Clinical microbiology reviews* 19, no. 4 (2006): 658-685.
7. Kamerbeek, Judith, L. E. O. Schouls, Arend Kolk, Miranda Van Agterveld, Dick Van Soolingen, Sjoukje Kuijper, Annelies Bunschoten et al. "Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology." *Journal of clinical microbiology* 35, no. 4 (1997): 907-914.

8. Coscolla, Mireia, and Sebastien Gagneux. "Consequences of genomic diversity in Mycobacterium tuberculosis." *In Seminars in immunology*, vol. 26, no. 6, pp. 431-444. Academic Press, 2014.
9. Tessema, Belay, Joerg Beer, Matthias Merker, Frank Emmrich, Ulrich Sack, Arne C. Rodloff, and Stefan Niemann. "Molecular epidemiology and transmission dynamics of Mycobacterium tuberculosis in Northwest Ethiopia: new phylogenetic lineages found in Northwest Ethiopia." *BMC infectious diseases* 13, no. 1 (2013): 131.
10. Yimer, Solomon A., Gunnstein Norheim, Amine Namouchi, Ephrem D. Zegeye, Wibeke Kinander, Tone Tønjum, Shiferaw Bekele et al. "Mycobacterium tuberculosis lineage 7 strains are associated with prolonged patient delay in seeking treatment for pulmonary tuberculosis in Amhara Region, Ethiopia." *Journal of clinical microbiology* 53, no. 4 (2015): 1301-1309.
11. Telenti, Amalio, Paul Imboden, Francine Marchesi, L. Matter, K. Schopfer, T. Bodmer, D. Lowrie, M. J. Colston, and S. Cole. "Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis." *The Lancet* 341, no. 8846 (1993): 647-651.
12. Ramaswamy, S., and James M. Musser. "Molecular genetic basis of antimicrobial agent resistance inMycobacterium tuberculosis: 1998 update." *Tubercle and Lung disease* 79, no. 1 (1998): 3-29.
13. Zhang, Y., and W. W. Yew. "Mécanismes de la résistance aux médicaments chez Mycobacterium tuberculosis." *The International Journal of Tuberculosis and Lung Disease* 13, no. 11 (2009): 1320-30.
14. Gagneux, Sebastien, Clara Davis Long, Peter M. Small, Tran Van, Gary K. Schoolnik, and Brendan JM Bohannan. "The competitive cost of antibiotic resistance in Mycobacterium tuberculosis." *Science* 312, no. 5782 (2006): 1944-1946.
15. Hillemann, Doris, Sabine Rüsch-Gerdes, and Elvira Richter. "Evaluation of the GenoType MTBDRplus assay for rifampin and isoniazid susceptibility testing of Mycobacterium tuberculosis strains and clinical specimens." *Journal of clinical microbiology* 45, no. 8 (2007): 2635-2640.
16. World Health Organization. WHO consolidated guidelines on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detection. Geneva: World Health Organization; 2021.
17. Central Statistical Agency (Ethiopia). Population projection of Ethiopia for all regions at wereda level from 2014-2017. Addis Ababa: CSA; 2017.
18. Federal Ministry of Health Ethiopia. National tuberculosis and leprosy control program annual report 2018. Addis Ababa: FMOH; 2019.
19. Eshetie, Setegn, Mucheye Gizachew, Mulat Dagnew, Gemechu Kumera, Haile Woldie, Fekadu Ambaw, Belay Tessema, and Feleke Moges. "Multidrug resistant tuberculosis in Ethiopian settings and its association with previous history of anti-tuberculosis treatment: a systematic review and meta-analysis." *BMC infectious diseases* 17, no. 1 (2017): 219.
20. Biadglegne F, Tessema B, Rodloff AC, Sack U. Magnitude of multidrug-resistant tuberculosis and its associated risk factors in the Federal Prison of Bahir Dar, Northwest Ethiopia. Int J Mycobacteriol. 2015;4(4):284-289.
21. World Health Organization. Laboratory services in tuberculosis control. Part II: microscopy. Geneva: World Health Organization; 1998. WHO/TB/98.258.
22. Cepheid. Xpert MTB/RIF assay instruction manual. Sunnyvale, CA: Cepheid; 2017.
23. Kent PT, Kubica GP. Public health mycobacteriology: a guide for the level III laboratory. Atlanta: US Department of Health and Human Services, Centers for Disease Control; 1985.
24. Hain Lifescience. GenoType MTBDRplus VER 2.0 instruction manual. Nehren, Germany: Hain Lifescience; 2015.
25. Cowan, Lauren S., Lois Diem, Mary Catherine Brake, and Jack T. Crawford. "Transfer of a Mycobacterium tuberculosis genotyping method, spoligotyping, from a reverse line-blot hybridization, membrane-based assay to the Luminex multianalyte profiling system." *Journal of Clinical Microbiology* 42, no. 1 (2004): 474-477.
26. World Health Organization. Definitions and reporting framework for tuberculosis – 2013 revision. Geneva: World Health Organization; 2013. WHO/HTM/TB/2013.2.
27. Abebe G, Abdissa K, Abdissa A, et al. Relapse and treatment failure among tuberculosis patients in Jimma Zone, Southwest Ethiopia. Int J Mycobacteriol. 2016;5(Suppl 1):S76-S77.
28. Tulu B, Ameni G. Spoligotyping based genetic diversity of Mycobacterium tuberculosis in Ethiopia: a systematic review. Asian Pac J Trop Biomed. 2017;7(2):172-177.
29. Tulu B, Dida G, Ameni G. Multidrug resistant tuberculosis in Ethiopian prisons: a systematic review and meta-analysis. Int J Mycobacteriol. 2018;7(3):211-217.
30. Mekonnen, Feleke, Belay Tessema, Feleke Moges, Aschalew Gelaw, Setegn Eshetie, and Gemechu Kumera. "Multidrug resistant tuberculosis: prevalence and risk factors in districts of metema and west armachiho, Northwest Ethiopia." *BMC infectious diseases* 15, no. 1 (2015): 461.
31. World Health Organization. Guidelines for surveillance of drug resistance in tuberculosis. 5th ed. Geneva: World Health Organization; 2015. WHO/HTM/TB/2015.13.
32. Jamieson, F. B., J. L. Guthrie, A. Neemuchwala, O. Lastovetska, R. G. Melano, and C. Mehaffy. "Profiling of rpoB mutations and MICs for rifampin and rifabutin in Mycobacterium tuberculosis." *Journal of clinical microbiology* 52, no. 6 (2014): 2157-2162.
33. Hamusse, Shallo Daba, Dejene Teshome, Mohammed Suaudi Hussen, Meaza Demissie, and Bernt Lindtjørn. "Primary and secondary anti-tuberculosis drug resistance in Hitossa District of Arsi zone, Oromia regional state, Central Ethiopia." *BMC public health* 16, no. 1 (2016): 593.

34. Diriba, Getu, Abebaw Kebede, Habteyes Hailu Tola, Ayinalem Alemu, Mengistu Tadesse, Epherem Tesfaye, Zemedu Mehamed et al. "Surveillance of drug resistance tuberculosis based on reference laboratory data in Ethiopia." *Infectious diseases of poverty* 8, no. 1 (2019): 54.
35. Lempens, Pauline, Conor J. Meehan, Koen Vandelannoote, Kristina Fissette, Pim de Rijk, Armand Van Deun, Leen Rigouts, and Bouke C. de Jong. "Isoniazid resistance levels of Mycobacterium tuberculosis can largely be predicted by high-confidence resistance-conferring mutations." *Scientific reports* 8, no. 1 (2018): 3246.
36. Vilchèze, Catherine, and William R. Jacobs, Jr. "The mechanism of isoniazid killing: clarity through the scope of genetics." *Annu. Rev. Microbiol*. 61, no. 1 (2007): 35-50.
37. Huyen, Mai NT, Frank GJ Cobelens, Tran N. Buu, Nguyen TN Lan, Nguyen H. Dung, Kristin Kremer, Edine W. Tiemersma, and Dick van Soolingen. "Epidemiology of isoniazid resistance mutations and their effect on tuberculosis treatment outcomes." *Antimicrobial agents and chemotherapy* 57, no. 8 (2013): 3620-3627.
38. Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev. 2014;(1):CD009593.
39. Folkvardsen, Dorte Bek, Vibeke Ø. Thomsen, Leen Rigouts, Erik Michael Rasmussen, Didi Bang, Gertjan Bernaerts, Jim Werngren et al. "Rifampin heteroresistance in Mycobacterium tuberculosis cultures as detected by phenotypic and genotypic drug susceptibility test methods." *Journal of clinical microbiology* 51, no. 12 (2013): 4220-4222.
40. Ocheretina, Oksana, Erin Byrt, Marie-Marcelle Mabou, Gertrude Royal-Mardi, Yves-Mary Merveille, Vanessa Rouzier, Daniel W. Fitzgerald, and Jean W. Pape. "False-positive rifampin resistant results with Xpert MTB/RIF version 4 assay in clinical samples with a low bacterial load." *Diagnostic microbiology and infectious disease* 85, no. 1 (2016): 53-55.
41. Tulu B, Ameni G. Spoligotyping based genetic diversity of Mycobacterium tuberculosis in Ethiopia: a systematic review. Asian Pac J Trop Biomed. 2017;7(2):172-177.
42. Mulholland CV, Shockey AC, Aseffa A, et al. Spoligotype-based population structure of Mycobacterium tuberculosis in the Horn of Africa. Int J Mycobacteriol. 2016;5(Suppl 1):S73.
43. Gagneux, Sebastien, and Peter M. Small. "Global phylogeography of Mycobacterium tuberculosis and implications for tuberculosis product development." *The Lancet infectious diseases* 7, no. 5 (2007): 328-337.
44. Comas, Iñaki, Mireia Coscolla, Tao Luo, Sonia Borrell, Kathryn E. Holt, Midori Kato-Maeda, Julian Parkhill et al. "Out-of-Africa migration and Neolithic coexpansion of Mycobacterium tuberculosis with modern humans." *Nature genetics* 45, no. 10 (2013): 1176-1182.
45. Parwati, Ida, Reinout van Crevel, and Dick van Soolingen. "Possible underlying mechanisms for successful emergence of the Mycobacterium tuberculosis Beijing genotype strains." *The Lancet infectious diseases* 10, no. 2 (2010): 103-111.
46. Yimer SA, Hailu E, Derese Y, Bjune GA, Holm-Hansen C. Spoligotyping of Mycobacterium tuberculosis isolates from pulmonary tuberculosis patients in South East Ethiopia. J Infect Dev Ctries. 2017;11(10):776-783.
47. Ford, Christopher B., Rupal R. Shah, Midori Kato Maeda, Sebastien Gagneux, Megan B. Murray, Ted Cohen, James C. Johnston, Jennifer Gardy, Marc Lipsitch, and Sarah M. Fortune. "Mycobacterium tuberculosis mutation rate estimates from different lineages predict substantial differences in the emergence of drug-resistant tuberculosis." *Nature genetics* 45, no. 7 (2013): 784-790.
48. Rad, Mina Ebrahimi, Pablo Bifani, Carlos Martin, Kristin Kremer, Sofia Samper, Jean Rauzier, Barry Kreiswirth et al. "Mutations in putative mutator genes of Mycobacterium tuberculosis strains of the W-Beijing family." *Emerging infectious diseases* 9, no. 7 (2003): 838.
49. Hanekom, M., NC Gey Van Pittius, C. McEvoy, T. C. Victor, P. D. Van Helden, and R. M. Warren. "Mycobacterium tuberculosis Beijing genotype: a template for success." *Tuberculosis* 91, no. 6 (2011): 510-523.
50. Faustini, A. J. H. A., Andrew J. Hall, and Carlo A. Perucci. "Risk factors for multidrug resistant tuberculosis in Europe: a systematic review." *Thorax* 61, no. 2 (2006): 158-163.
51. Zhao, Yanlin, Shaofa Xu, Lixia Wang, Daniel P. Chin, Shengfen Wang, Guanglu Jiang, Hui Xia et al. "National survey of drug-resistant tuberculosis in China." *New England Journal of Medicine* 366, no. 23 (2012): 2161-2170.
52. Suchindran, Sujit, Emily S. Brouwer, and Annelies Van Rie. "Is HIV infection a risk factor for multi-drug resistant tuberculosis? A systematic review." *PloS one* 4, no. 5 (2009): e5561.
53. Mesfin, Yonatan Moges, Damen Hailemariam, Sibhatu Biadglign, and Kelemu Tilahun Kibret. "Association between HIV/AIDS and multi-drug resistance tuberculosis: a systematic review and meta-analysis." *PloS one* 9, no. 1 (2014): e82235.
54. Andrews, Jason R., N. Sarita Shah, Neel Gandhi, Tony Moll, and Gerald Friedland. "Multidrug-resistant and extensively drug-resistant tuberculosis: implications for the HIV epidemic and antiretroviral therapy rollout in South Africa." *The Journal of infectious diseases* 196, no. Supplement_3 (2007): S482-S490.
55. Wells, Charles D., J. Peter Cegielski, Lisa J. Nelson, Kayla F. Laserson, Timothy H. Holtz, Alyssa Finlay, Kenneth G. Castro, and Karin Weyer. "HIV infection and multidrug-resistant tuberculosis—the perfect storm." *The Journal of infectious diseases* 196, no. Supplement_1 (2007): S86-S107.
56. World Health Organization. WHO consolidated guidelines on tuberculosis. Module 1: prevention - tuberculosis preventive treatment. Geneva: World Health Organization; 2020.

57. Marks, Suzanne M., Sundari R. Mase, and Sapna Bamrah Morris. "Systematic review, meta-analysis, and cost-effectiveness of treatment of latent tuberculosis to reduce progression to multidrug-resistant tuberculosis." *Clinical Infectious Diseases* 64, no. 12 (2017): 1670-1677.
58. Clark, Taane G., Kim Mallard, Francesc Coll, Mark Preston, Samuel Assefa, David Harris, Sam Ogwang et al. "Elucidating emergence and transmission of multidrug-resistant tuberculosis in treatment experienced patients by whole genome sequencing." *PloS one* 8, no. 12 (2013): e83012.
59. Lessells RJ, Cooke GS, Newell ML, Godfrey-Faussett P. Evaluation of a rapid molecular drug-susceptibility test for tuberculosis. N Engl J Med. 2011;364(2):185-187.
60. Alipanah, Narges, Leah Jarlsberg, Cecily Miller, Nguyen Nhat Linh, Dennis Falzon, Ernesto Jaramillo, and Payam Nahid. "Adherence interventions and outcomes of tuberculosis treatment: A systematic review and meta-analysis of trials and observational studies." *PLoS medicine* 15, no. 7 (2018): e1002595.
61. Jensen PA, Lambert LA, Iademarco MF, Ridzon R, Centers for Disease Control and Prevention. Guidelines for preventing the transmission of Mycobacterium tuberculosis in health-care settings, 2005. MMWR Recomm Rep. 2005;54(RR-17):1-141.
62. World Health Organization. "WHO policy on collaborative TB/HIV activities: guidelines for national programmes and other stakeholders." (2012).
63. Van Rie, Annelies, Robin Warren, Madalene Richardson, Robert P. Gie, Donald A. Enarson, Nulda Beyers, and Paul D. Van Helden. "Classification of drug-resistant tuberculosis in an epidemic area." *The Lancet* 356, no. 9223 (2000): 22-25.

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