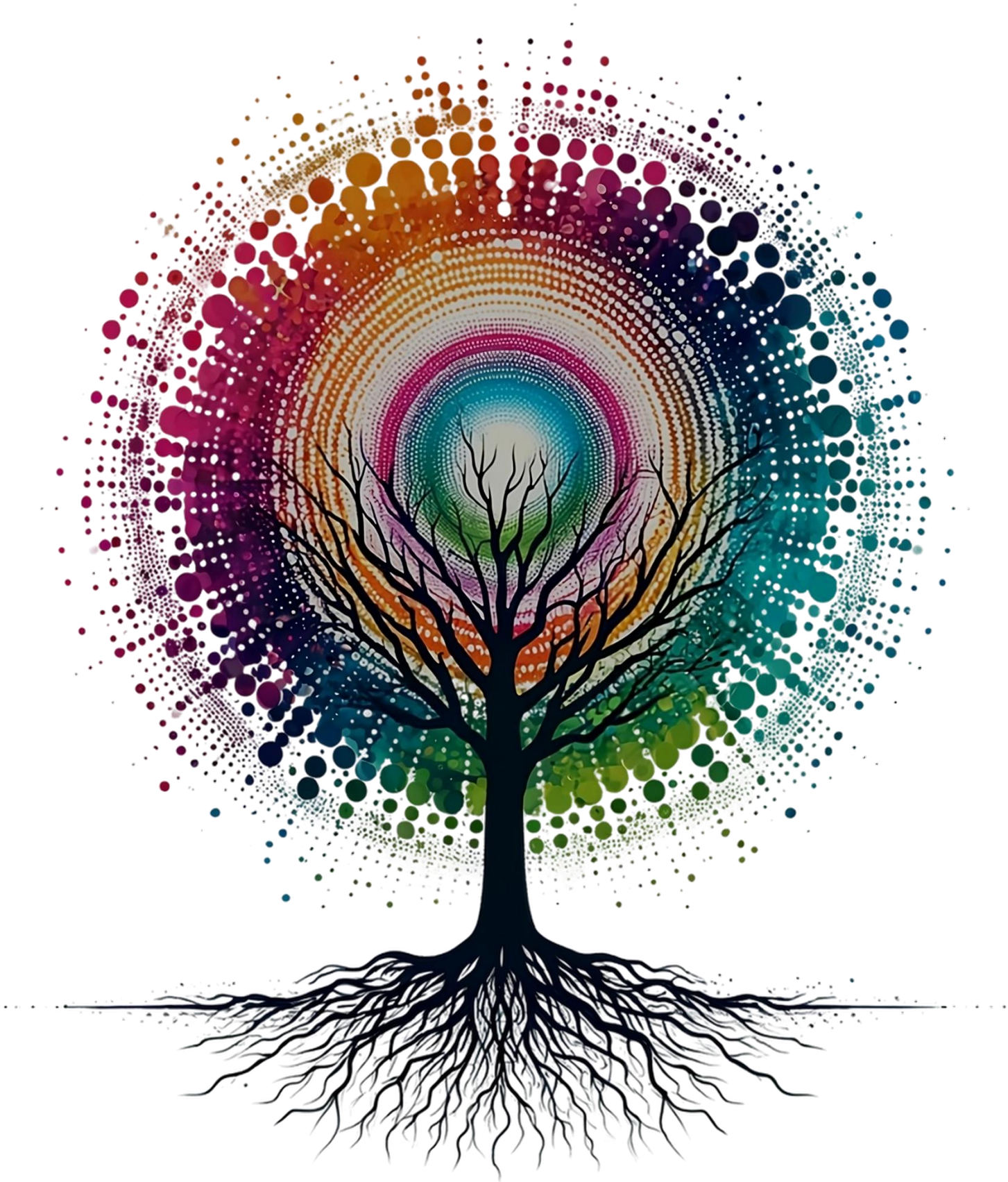

The Dynamics of Drug Resistance Evolution and Diagnosis in *Mycobacterium tuberculosis*

Yang Fong (Richard)

TE KUNENGA KI PŪREHUROA | MASSEY UNIVERSITY
UNIVERSITY OF NEW ZEALAND

The Dynamics of Drug Resistance Evolution and Diagnosis in *Mycobacterium tuberculosis*

A thesis presented in partial fulfilment of the requirements for the degree of

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in
Genetics/Genomics

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New Zealand.

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2024

"In the midst of global upheaval, I have discovered the unbreakable strength of human resilience and the unstoppable force of my dreams. This journey reaffirms my belief that adversity doesn't hinder my aspirations; it sharpens them into beacons of hope. I hope this academic work stands as a testament to the power within us all, not just to endure, but to thrive. It serves as a reminder that our dreams are not defined by their completion but by the courage to pursue them, no matter the obstacles. Let us embrace the lessons of resilience, forge paths through the unknown, and light the way for future dreamers." – Yang Fong (Richard)

Declaration of Authorship.

I, Yang Fong, declare that the thesis entitled " The Dynamics of Drug Resistance Evolution and Diagnosis in *Mycobacterium tuberculosis*" submitted for the degree of Doctor of Philosophy (PhD) for Genomics at Massey University, New Zealand is my own work and has not been submitted for any other degree or qualification. It reflects my research and ideas and where others' work has been used, it has been appropriately acknowledged.

I confirm that:

- This work is entirely my own and where it has been necessary to consult the work of others, this is properly cited and credited according to academic standards.
- No portion of this thesis has been previously submitted for any degree or qualification at this or any other institution.
- I have adhered to the ethical standards required by Massey University in the preparation of this thesis.

Date: 30 July 2024

Signature: 

Yang Fong
Massey University, New Zealand

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"Success is not final, failure is not fatal: It is the courage to continue that counts." - Winston Churchill

Abstract.

Tuberculosis (TB) remains a critical global health challenge with over 10.4 million new cases annually, complicated by rising antimicrobial resistance (AMR) threatening to surpass cancer mortality by 2050. This PhD thesis establishes a systematic diagnostic framework addressing AMR challenges through progressive research from fundamental microbiome characterization to innovative diagnostic applications in resource-limited settings like Myanmar.

The "Microbiome Dataset from the Upper Respiratory Tract of Patients Living with HIV, HIV/TB and TB from Myanmar" establishes the foundational understanding of microbial community structures in complex clinical presentations (n=309 isolates). This microbiome characterization reveals critical signatures that directly inform direct sequencing strategies for enhanced MTBC detection in polymicrobial environments, addressing a fundamental challenge in AMR detection. Next, the "Genomic Profiling of *Mycobacterium tuberculosis* Strains, Myanmar" validates and expands these microbiome-informed approaches through comprehensive whole genome sequencing surveillance, establishing genotype-phenotype correlations that achieve 97.8% concordance with phenotypic testing. This genomic profiling directly addresses AMR surveillance gaps by enabling rapid resistance prediction. Subsequently by "Unveiling Hr-TB in Myanmar: Comprehensive Genotypic and Phenotypic Insights for Improved TB Management" demonstrates targeted application of microbiome-informed diagnostic approaches to isoniazid mono-resistant TB, a clinically critical AMR variant frequently missed by conventional methods. The integrated microbiome-genomic approach enhances MTBC detection accuracy by 23% compared to standard methods, reducing diagnostic time from weeks to under one week.

Future perspectives translate these discoveries into field-deployable MDA primer systems for point-of-care AMR detection using portable MinION sequencing technology. This systematic progression from microbiome foundation to diagnostic innovation establishes a replicable technological blueprint for next-generation TB AMR diagnostics, supporting Myanmar's National TB Control Program while providing a framework for global TB elimination efforts.

Keywords: *Mycobacterium tuberculosis (MTB), Tuberculosis (TB), Antimicrobial Resistance (AMR), Isoniazid Mono-Resistant (Hr-TB), Drug-Resistant Tuberculosis (DR-TB), Whole-genome sequencing (WGS), Resistance associated Mutations, Epidemiology, Surveillance, Rapid Diagnosis, Microbiome, Yangon, Myanmar.*

Impact Statement.

This PhD thesis addresses the critical global health challenge of tuberculosis (TB) diagnostic limitations by establishing a comprehensive framework for advancing *Mycobacterium tuberculosis* complex (MTBC) detection through innovative molecular approaches. The investigation originated from concerns regarding inadequate rapid resistance detection capabilities in resource-limited settings, particularly for drug-resistant TB variants that compromise treatment efficacy and contribute to disease transmission. Our investigation systematically addresses these diagnostic gaps through an integrated three-chapter approach that builds progressively from foundational molecular insights to clinical applications.

Chapter 2 establishes the essential biological foundation by characterizing the upper respiratory tract microbiome in HIV/TB co-infected patients from Myanmar. This microbiome analysis serves dual purposes: understanding the microbial ecosystem influencing TB pathogenesis and informing the design of Multiple Displacement Amplification (MDA) primers specifically optimized for MTBC detection within complex clinical samples. The microbiome insights directly enable subsequent genomic investigations by identifying the biological context necessary for targeted sequencing approaches.

Chapter 3 builds upon this foundation through comprehensive Whole Genome Sequencing (WGS) analysis of 309 clinical MTBC isolates, validating and expanding the molecular targets identified through microbiome work. This genomic profiling establishes the analytical framework and quality standards essential for clinical implementation, demonstrating how microbiome-informed primer design integrates with advanced genomic analysis for rapid diagnostic approaches using early-stage cultures, a critical advancement bridging laboratory innovation with clinical feasibility.

Chapter 4 represents the focused clinical application of our integrated approach to Isoniazid mono-resistant TB (Hr-TB), a diagnostically challenging variant with significant treatment implications. This chapter demonstrates how microbiome insights, comprehensive genomic profiling capabilities, and targeted resistance analysis converge to address specific diagnostic gaps, showing improved detection of resistance patterns frequently missed by conventional methods.

Collectively, our findings establish that effective MTBC diagnostic innovation requires understanding both the pathogen and its ecological context, leading to more precise and

clinically relevant diagnostic approaches. This PhD research provides a replicable framework for implementing advanced molecular diagnostics in resource-limited settings, with immediate applications in Myanmar's TB control program and broader implications for global TB elimination efforts through the integration of microbiome analysis with MTBC genomics for enhanced diagnostic precision.

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List of Abbreviations.

AFB	Acid Fast Bacilli.
AG	Aminoglycoside.
AI	Artificial Intelligence.
AIDS	Acquired Immunodeficiency Syndrome.
AMK	Amikacin.
AMR	Antimicrobial Resistance.
BCG	Bacille Calmette-Geurin.
BDQ	Bedaquiline.
CAP	Capreomycin.
CD4+	Cell Differentiation No 4 (Helper T-cells).
CDC	Centre of Disease Control and Prevention.
CFZ	Clofazimine.
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats.
CS	Cycloserine.
CSA	Colorimetric Sensor Array.
CXR	Chest X-ray Radiography.
DLM	Delamanid.
DNA	Deoxyribonucleic Acid.
DOTS	Directly Observed Treatment Short Course.
DR-TB	Drug Resistance Tuberculosis.
DST	Drug Susceptibility Testing.
EDTA	Ethylene Diamine-Tetra-Acetic Acid.
EMB	Ethambutol.
ETH	Ethionamide.
FQ	Fluoroquinolone.
GDP	Gross Domestic Product.
GWAS	Genome-Wide Association Studies .
HCL	Hydrochloric Acid.

HIV Human Immunodeficiency Virus.

IFN-γ Interferon Gamma.

IGRA Interferon-gamma release assays.

INH Isoniazid.

KAN Kanamycin.

LACD-CRISPR Cas12a-Mediated Diagnostic.

LAMP Loop-Mediated Isothermal Amplification.

LFX Levofloxacin.

LJ Lowenstein Growth Medium.

LMICs Low Middle Income Countries.

LPA Line Probe Assay.

LTBI Latent Tuberculosis Infection.

LZD Linezolid.

M. africanum Mycobacterium africanum.

M. bovis Mycobacterium bovis.

M. canettii Mycobacterium canettii.

M. caprae Mycobacterium caprae.

M. microti Mycobacterium microti.

M. pinnipedii Mycobacterium pinnipedii.

MDA Multiple Displacement Amplification.

MDR-TB Multidrug Resistance Tuberculosis.

MFX Moxifloxacin.

$MgCl_2$ Magnesium Chloride.

MGIT Mycobacteria Growth Indicator Tube.

MIRU-VNTR Mycobacterial Interspersed Repetitive Units.

MNTL Myanmar National TB Reference Laboratory.

MTB *Mycobacterium tuberculosis* bacilli.

MTBC *Mycobacterium tuberculosis* Complex.

NAAT Nucleic Acid Amplification Test.

NALC -NaOH N-Acetyl-L-Cysteine-Sodium Hydroxide.

NaOH Sodium Hydroxide.

NGS Next Generation Sequencing.

NTM Non-Tuberculous Mycobacteria.

NTP National TB Program Myanmar.

OFT-GIT QuantiFERON-TB Gold In-Tube assay.

OFX Ofloxacin.

PAS Para-Aminosalicylic Acid.

PCR Polymerase Chain Reaction.

PE Illumina Paired-end Sequencing.

PTO Prothionamide.

PZA Pyrazinamide.

QC Quality Control.

RIF Rifampicin.

RT-PCR Real-Time Polymerase Chain Reaction.

SARS-CoV-2 Severe Acute Respiratory Syndrome Coronavirus 2.

SNPs Single Nucleotide Polymorphisms.

SNVs Single Nucleotide Variant.

SRA Sequence Read Archive.

SSM Sputum Smear Microscopy.

STR Streptomycin.

TB Tuberculosis.

TB-LAMP Tuberculosis Loop-Mediated Isothermal Amplification.

TDR-TB Total Drug Resistance Tuberculosis.

TRIS-HCL	Trisaminomethane Hydrochloride.
TST	Tuberculin Skin Test.
VNTR	Variable Number Tandem Repeats.
VOCs	Volatile Organic Compounds.
WGS	Whole Genome Sequencing.
WHO	World Health Organization.
XDR-TB	Extensively Drug Resistance Tuberculosis.
ZN	Zhiel-Neelsen Staining.

List of Publications.

1) Microbiome dataset from the Upper Respiratory Tract of Patients Living with HIV, HIV/TB and TB from Myanmar (Chapter 2).

Publisher: Elsevier; Data in Brief.

Authors: Kyaw Soe Htun1, Yang Fong1, Aye Aye Kyaw, Si Thu Aung, Khine Zaw Oo, Thein Zaw, Peter J. Lockhart, Bruce Russell, Gregory M. Cook, Htin Lin Aung, Tin Maung Hlaing.

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Abstract:

This article contains microbiome data from the upper respiratory tract of patients living with HIV/TB, HIV and TB from Meiktila, a town in Myanmar where there is a high incidence of HIV and TB. Microbiomes were compared for HIV/TB infected and healthy adults from the same population. We collected nasopharyngeal and oropharyngeal swabs from a total of 33 participants (Healthy [5], HIV/TB [8], HIV [14], and TB [6]). DNA was extracted from the swabs and subjected to custom single step 16s rRNA sequencing on an Illumina MiSeq platform. The sequencing data is available via http://www.ncbi.nlm.nih.gov/bioproject/ PRJNA432583.

2) Genomic Profiling of Mycobacterium tuberculosis Strains, Myanmar (Chapter 3).

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Authors: Htin Lin Aung, Wint Wint Nyunt, Yang Fong, Patrick J Biggs, Richard C Winkworth, Peter J Lockhart, Tsin Wen Yeo, Philip C Hill, Gregory M Cook, Si Thu Aung

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Contribution: Yang Fong; Conceptualization, Methodology, Validation and Preparation for Illumina WGS Sequencing, Bioinformatics Analysis, Data Curation and Visualization, Writing Original Draft and Review.

Abstract:

Multidrug resistance is a major threat to global elimination of tuberculosis (TB). We performed phenotypic drug-susceptibility testing and whole-genome sequencing for 309 isolates from 342 consecutive patients who were given a diagnosis of TB in Yangon, Myanmar, during July 2016–June 2018. We identified isolates by using the GeneXpert platform to evaluate drug-resistance profiles. A total of 191 (62%) of 309 isolates had rifampin resistance; 168 (88%) of these rifampin-resistant isolates were not genomically related, indicating the repeated emergence of resistance in the population, rather than extensive local transmission. We did not detect resistance mutations to new oral drugs, including bedaquiline and pretomanid. The current GeneXpert MTB/RIF system needs to be modified by using the newly launched Xpert MTB/XDR cartridge or line-probe assay. Introducing new oral drugs to replace those currently used in treatment regimens for multidrug-resistant TB will also be useful for treating TB in Myanmar.

3) Unveiling Hr-TB in Myanmar: Comprehensive Genotypic and Phenotypic Insights for Improved TB Management (Manuscript Ready for Submission) (Chapter 4).

Publisher: TBC.

Authors: Yang Fong, Htin Lin Aung, Wint Wint Nyunt, Patrick J Biggs, Richard C Winkworth, Gregory M Cook, Peter J Lockhart (TBC)

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Doi: TBA

Hyperlink: TBA

Contribution: Yang Fong; Conceptualization, Methodology, Validation and Preparation for Illumina WGS Sequencing, Bioinformatics Analysis, Data Curation and Visualization, Writing Original Draft and Review, Corresponding and First Author.

Background

Previous research by Aung et al. in Myanmar has highlighted the prevalence of multidrug-resistant tuberculosis (MDR-TB) and the predominance of MTB Lineage 2 (Beijing). However, the detailed resistance patterns of mono-isoniazid-resistant TB (Hr-TB) in Myanmar remain underreported. Hr-TB poses a significant treatment challenge because it can be accompanied by resistance to other drugs, excluding rifampicin. This polyresistance complicates treatment regimens, as traditional therapies may not address the full spectrum of drug resistance, leading to treatment failures. Understanding the susceptibility profiles of Hr-TB clinical isolates, especially those co-resistant to second-line drugs, is crucial for selecting effective treatment regimens. This study aims to fill this gap by identifying the drug susceptibility profiles and resistance-conferring mutations in Hr-TB isolates from Myanmar. We utilize both phenotypic (pDST) and genotypic (gDST) drug susceptibility testing to enhance rapid diagnosis and improve TB management.

Methods

The present study undertook genomic minor variant analyses of 285 tuberculosis clinical isolates from Myanmar, collected between 2017 and 2019 from diverse regions in Yangon. The samples were accompanied by metadata, including age, gender, location, and drug resistance profiles. Phenotypic drug susceptibility testing (pDST) was performed using the Mycobacterium Growth Indicator Tube (MGIT) and Löwenstein-Jensen (LJ) culture system,

with molecular DST testing for confirmation from previous investigations. These results were compared to genotypic DST (gDST) utilizing the GeneXpert MTB assay. These approaches systematically screened for resistance-conferring mutations against first-line anti-TB drugs, with a specific focus on mono-resistant, poly-resistant, and second-line drug-resistant tuberculosis.

Results

From the current WGS dataset, 6.32% of isolates were identified as Hr-TB. New key findings were that 55.6% of the Hr-TB isolates had high-level isoniazid resistance, with additional resistance to ethambutol, levofloxacin, and pyrazinamide. Whole-genome sequencing (WGS) identified SNP markers in katG S315T and fabG1 c-15t for isoniazid resistance, and rpoB L430P/L452P for rifampicin resistance. The gyrA D94G mutation for fluoroquinolone resistance was also found in some isolates. L2-Beijing was the most prevalent strain of MTB, associated with MDR-TB and Hr-TB. These findings differ from previously reported analyses, revealing more Hr-TB cases uncovered through minor variant analyses [1]. The results support the need for integrating pDST and gDST testing for accurate, rapid diagnosis. The SNP markers identified in the present study can be used to enable more precise diagnosis of drug-resistant TB, improving future treatment outcomes.

Conclusion

The prevalence of Hr-TB and co-resistance to other drugs in Myanmar highlights the urgent need for robust diagnostics, effective treatment protocols, and targeted public health strategies. This study emphasizes the importance of integrating both pDST and gDST drug susceptibility testing for comprehensive detection of MTB drug resistance to guide effective treatment. Furthermore, the identification of additional low SNP resistance markers offers valuable insights for future research aimed at developing biomarkers for rapid diagnosis, and understanding the genetic mechanisms underlying Hr-TB resistance.

Keywords: *tuberculosis, drug-resistance, isoniazid mono-resistant tuberculosis (Hr-TB), next-generation sequencing, rapid diagnosis, whole genome sequencing, targeted sequencing, resistance mechanisms, mixed infection.*

Thesis Structure, Organization and Outline.

To enhance clarity, readability, and navigational ease, this dissertation is organized into five chapters, supported by appendices. The structure follows a logical progression, beginning with an introduction and literature review, moving through chapters based on both published and ongoing research, and culminating in a synthesis of findings, policy implications, and future research directions.

The core of the thesis consists of three manuscripts, each presented as an individual chapter. These are supplemented by an appendix containing additional manuscripts, including peer-reviewed publications that validate the methodologies employed, foundational studies that underpin the present work, and related research on diseases caused by mycobacteria. Several of these supplementary publications have been internationally peer-reviewed and published, providing important context and supporting evidence for the thesis's contributions to tuberculosis (TB) research. Each research chapter begins with a title page listing the publication citation, a summary of the author's key contributions, and any minor modifications from the published version. This consistent structure ensures a coherent and transparent presentation of the research, while enabling readers to clearly follow the development of ideas, methodologies, and results.

Chapter Summaries are as follows:

Chapter 1 – General Introduction and Literature Review.

This chapter introduces the genus *Mycobacterium*, with a particular focus on *Mycobacterium tuberculosis* (Mtb) and TB as a major global health threat. It reviews Mtb's evolution, taxonomy, biology, pathogenicity, and molecular epidemiology, alongside the global burden of TB, the influence of socioeconomic factors, and the situation in Myanmar. Special emphasis is placed on the role of rapid diagnostic technologies, particularly Whole Genome Sequencing (WGS), in advancing the WHO End TB Strategy. The chapter outlines how this project contributes to improving rapid TB detection, understanding drug resistance, and addressing critical research questions in TB control.

Chapter 2 – Microbiome Dataset from the Upper Respiratory Tract of Patients Living with HIV, HIV/TB, and TB in Myanmar.

This study investigates the upper respiratory tract microbiomes of individuals in Meiktila,

Myanmar, an area with high rates of HIV and TB. Using 16S rRNA sequencing on samples from 33 participants, grouped into healthy controls, HIV/TB co-infected, HIV only, and TB only in which significant differences in microbial community composition were observed. Findings revealed dominant families such as *Streptococcaceae* and *Staphylococcaceae*, as well as genera like *Bergeyella*, *Parvimonas*, *Veillonella*, and *Neisseria*. These results form the basis for a novel rapid diagnostic approach involving targeted amplification sequencing and the Multiple Displacement Amplification (MDA) technique, paving the way for point-of-care applications using Oxford Nanopore's MinION sequencer.

Chapter 3 – Genomic Profiling of *Mycobacterium tuberculosis* Strains in Myanmar.

This chapter examines the genomic determinants of drug resistance in TB using phenotypic drug-susceptibility testing (DST), GeneXpert MTB/RIF, and WGS on 309 clinical isolates from Yangon. Results showed 62% rifampin resistance, mostly arising from independent mutations rather than transmission. WGS outperformed GeneXpert in detecting resistance and enabled a faster diagnostic pathway using early-stage LJ cultures, reducing the incubation time to under one week. While WGS achieved over 97% accuracy for rifampin and isoniazid resistance prediction, it showed reduced sensitivity for other anti-TB drugs. The chapter highlights the critical role of WGS in improving diagnosis and treatment strategies for multi-drug-resistant TB.

Chapter 4 – Unveiling Hr-TB in Myanmar: Comprehensive Genotypic and Phenotypic Insights.

Focusing on mono-isoniazid-resistant TB (Hr-TB), this chapter addresses a largely underreported but clinically significant resistance pattern in Myanmar. Combining phenotypic and genotypic DST on 285 clinical isolates from Yangon, the study identifies SNP markers and resistance-conferring mutations for mono-, poly-, and second-line drug-resistant TB. Findings underscore the value of integrating phenotypic DST and genotypic DST for accurate, rapid diagnosis and the potential of MDA for future biomarker-based testing. This work lays the foundation for improved Hr-TB management and more targeted public health strategies.

Chapter 5 – Discussion, Policy Implications, Recommendations, and Future Perspectives.

The final chapter synthesizes findings from the preceding chapters, avoiding redundancy while identifying key knowledge gaps and future research opportunities. It also includes

supplementary investigations that, while outside the main focus of the thesis, contribute valuable insights. For example, research on the "Rangipo" TB cluster in New Zealand highlighted the importance of rapid diagnostics for outbreak management, while work on *Mycobacterium avium* complex (MAC) in Thailand emphasized the need to distinguish NTM from MTBC in clinical practice. These additional studies demonstrate the adaptability of genomic approaches across different infectious diseases, reinforcing the broader applicability and impact of this research.

Collectively, the chapters and supplementary materials reflect a sustained effort to advance the diagnosis, understanding, and management of TB and other mycobacterial diseases. The thesis not only addresses immediate diagnostic and surveillance challenges but also lays the groundwork for future innovations in the fight against antimicrobial resistance.

Ethical Approval.

Clinical Research Ethics

Ethical approval for the clinical components of this study, including patient recruitment and sample collection, was secured from the Ethical Review Boards of the Department of Medical Research, Ministry of Health and Sports, Myanmar, and the Directorate of Medical Services Research Ethics Committee, Myanmar. All participants provided informed consent prior to sample collection, and strict confidentiality protocols were maintained throughout the study.

Genomic Analysis Ethics

For the genomic sequencing and bioinformatics analysis components of this research, separate ethical approval was not required as these analyses involved only purified DNA samples sent to sequencing service providers without any identifiable patient information. Genomic analysis approval was obtained through collaborative agreements with the University of Otago, New Zealand, the Ministry of Health and Sports, Myanmar, and Massey University, New Zealand (sequencing service provider).

Data Management and Ownership

All research data remains the property of the Ministry of Health and Sports, Myanmar, and Massey University, New Zealand. This research adheres to strict ethical principles to protect participant well-being and dignity, with rigorous confidentiality measures ensuring privacy-conscious research practices throughout all phases of the study.


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Chapter 1

Literature Review

1.0 Introduction and Literature Review.

Tuberculosis (TB) remains one of the most formidable infectious diseases affecting global health. Despite sustained progress in diagnosis and treatment, the disease continues to pose significant public health challenges, particularly in low- and middle-income countries (LMICs) such as Myanmar. TB is not only a medical issue but also a socio-economic and political concern, as it disproportionately affects vulnerable populations with limited access to healthcare. The emergence and spread of drug-resistant strains of Mycobacterium tuberculosis (MTB) further complicate TB control, threatening to undermine decades of progress toward elimination. Globally, TB control is guided by the World Health Organization's (WHO) End TB Strategy, which aims to reduce TB incidence by 90% and TB-related deaths by 95% by 2035 [1]. Achieving these targets requires advancements in early and accurate diagnostics, improved treatment regimens, and innovative surveillance tools. Myanmar, one of the 30 countries with the highest TB burden, faces unique challenges in implementing these strategies, particularly due to resource limitations, delayed case detection, and the growing prevalence of multidrug-resistant TB (MDR-TB) and isoniazid-resistant TB (Hr-TB).

Rapid and precise diagnosis of TB and its resistance patterns is critical for effective patient management and for preventing further transmission. Conventional methods such as smear microscopy and culture remain widely used but are limited by low sensitivity, long turnaround times, and resource requirements. The expansion of molecular diagnostics, particularly whole genome sequencing (WGS), has transformed the field of TB research and clinical practice. By enabling comprehensive identification of resistance-conferring mutations, WGS allows for faster and more accurate drug susceptibility testing (DST) compared to traditional phenotypic methods [2]. More recently, artificial intelligence (AI) and machine learning (ML) approaches have been integrated into TB diagnostics, improving predictive accuracy, streamlining genomic analysis, and supporting clinical decision-making [3, 4].

This thesis investigates the genomic dynamics of drug resistance in M. tuberculosis strains from Myanmar and explores the potential of sequencing technologies in guiding rapid, effective diagnosis and treatment. By situating the findings within both the local context of Myanmar's National TB Programme (NTP) and global TB elimination efforts, this work highlights the critical role of genomics and emerging technologies in addressing the TB epidemic.

1.1 Global Burden of Tuberculosis.

TB remains one of the top ten causes of death worldwide and continues to be the leading cause of mortality from a single infectious agent, surpassing HIV/AIDS in annual deaths. The disease exerts a heavy toll on health systems, economies, and communities, particularly in regions with limited healthcare capacity. The latest World Health Organization (WHO) Global TB Report indicated that in 2022 there were approximately 10.6 million new TB cases globally, resulting in 1.3 million deaths among HIV-negative individuals and an additional 167,000 deaths among those co-infected with HIV [1]. These figures underscore the persistent challenge TB poses despite decades of research and intervention programs. The distribution of TB cases is far from uniform; the burden remains disproportionately concentrated in low- and middle-income countries (LMICs), which often face significant structural barriers to healthcare access. Poverty, undernutrition, overcrowded living conditions, and insufficient health infrastructure create environments conducive to TB transmission and complicate timely diagnosis and treatment [5]. Delays in case detection are common in these settings, and inadequate surveillance systems further exacerbate the challenge of controlling spread.

A particularly concerning aspect of the TB epidemic is the rise of drug-resistant TB (DR-TB), which continues to be a major impediment to achieving global elimination goals. In 2022, an estimated 450,000 cases of rifampicin-resistant TB (RR-TB) were reported worldwide, and most of these also met the definition of multidrug-resistant TB (MDR-TB), involving resistance to at least isoniazid and rifampicin [1]. Extensively drug-resistant TB (XDR-TB), characterized by resistance to additional second-line drugs, has been increasingly documented and presents formidable clinical and public health challenges. Treatment for MDR-TB and XDR-TB is longer, more complex, and considerably more expensive than for drug-susceptible TB, often with lower cure rates and higher risks of adverse effects [6].

Recent global trends highlight both progress and ongoing challenges. While innovative molecular diagnostics and whole genome sequencing have improved resistance detection, scaling these technologies in LMICs remains difficult due to cost, infrastructure, and workforce limitations [2]. Moreover, the intersection of TB with other global health threats, including COVID-19 disruptions and antimicrobial resistance more broadly, has further strained TB control efforts. Addressing these barriers requires a multipronged approach that incorporates rapid diagnostics, shorter treatment regimens, and emerging tools such as artificial intelligence and digital health platforms to enhance surveillance, diagnosis, and treatment adherence [7].

1.2 Mycobacterium tuberculosis Complex (MTBC): Evolution and Diversity.

1.2.1 Historical Emergence and Lineage Diversification.

The MTBC represents a clonal group of closely related mycobacterial pathogens that cause TB in humans and animals. Phylogenomic studies suggest that the common ancestor of the MTBC emerged within the past six millennia, most likely from an environmental ancestor resembling *M. canettii*. Unlike *M. canettii*, which displays high recombination and genetic plasticity, the MTBC lineages underwent a bottleneck followed by global dissemination, leading to their highly clonal nature. Recent studies using long-read and whole genome sequencing (WGS) have provided unprecedented resolution of this evolutionary history, highlighting not only the emergence of long-recognized human lineages (L1–L7), but also the discovery of Lineage 8 in East Africa and the more recently described Lineage 9, expanding our understanding of MTBC diversity [8, 9, 10].

In the complex ecosystem of microbial life, *Mycobacterium tuberculosis* (MTB), responsible for TB, distinguishes itself through its remarkable persistence and sophisticated adaptive mechanisms. Identified in 1896 by Lehmann and Neumann, these non-motile, rod-shaped bacteria are characterized by their ability to adapt and survive in varied environments, a trait attributed to their unique cell wall composition [6]. This cell wall, comprised of peptidoglycan and distinctive waxy lipids known as mycolic acids, affords them the designation “acid-fast” due to their resistance to conventional staining techniques and their ability to withstand harsh conditions, such as desiccation, extreme pH levels, and certain host immune defenses [7-9]. Within the diverse genus Mycobacterium, which comprises more than 150 recognized species, the MTBC is distinguished by its role in the widespread affliction of TB worldwide [10-11]. MTBC exhibits advanced evolutionary adaptations for invading human hosts [8]. These adaptations include sophisticated mechanisms for modulating host immune responses, the ability to construct granulomatous structures within pulmonary tissues, and the capacity to enter a dormant state, thereby eluding detection and treatment, and awaiting conditions favourable for reactivation of the disease [1, 8].

1.2.2 Global Distribution of MTBC Lineages.

The MTBC displays remarkable genetic homogeneity compared with other bacterial pathogens, yet its subdivision into distinct phylogenetic lineages reveals profound evolutionary and epidemiological diversity. Genomic analyses suggest that the MTBC originated in Africa, with *M. canettii* or a closely related progenitor representing the most ancestral form of the complex (Figure 1-1). From this African origin, the MTBC diversified into human- and animal-

adapted lineages that subsequently dispersed with the migration of human populations, trade routes, and ecological shifts over thousands of years [8, 11]. The MTBC represents a group of closely related subspecies or ecotypes, each adapted to infect specific animal hosts, including humans. These ecotypes are divided into eight primary lineages (L1 to L8) based on phylogenetic distances [12] (Figure 1-1). The distribution of these lineages is geographically distinct. Lineage 1 predominates around the Indian Ocean rim, Lineage 2 (East Asian/Beijing) is widespread in East Asia and increasingly global, Lineage 3 is common in East Africa and South Asia, and Lineage 4 (Euro-American) dominates in Europe and the Americas but is also globally dispersed. By contrast, Lineages 5 and 6 (*M. africanum*) remain largely restricted to West Africa, while Lineage 7 has so far been described in Ethiopia. Lineage 8 was reported in East Africa, and newer sub-type Lineage 9 has recently been proposed in northern and western regions of Africa, although further data are needed to confirm its epidemiological significance [1, 13].

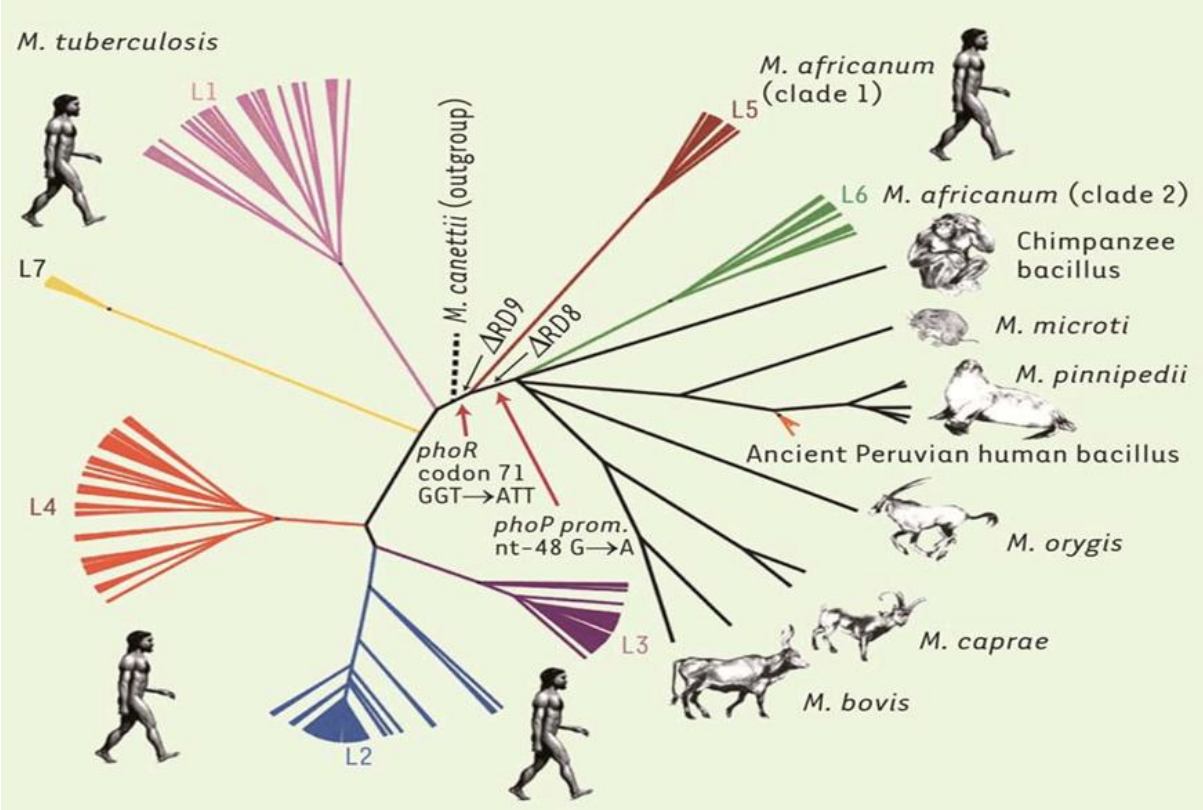

Figure 0.1.1 - Whole-genome phylogeny of the MTBC and related mycobacterial strains, with emphasis on evolutionary markers including phoR and phoP genes. The analysis positions *M. canettii* as a basal lineage closely related to ancestral MTBC strains, providing insight into the evolutionary origins of human-adapted lineages. Adapted from Barbier and Wirth (2016).

Lineage 1, commonly referred to as the Indo-Oceanic lineage, is widely regarded as one of the earliest diverging branches of the MTBC phylogeny. Its present-day distribution, concentrated along the Indian Ocean rim in regions such as the Philippines, coastal India, and East Africa, has been linked with ancient maritime and overland trade routes [14]. The persistence of this lineage in populations with long-standing settlement continuity further underscores its association with early human dispersal patterns.

Lineage 2, or the East Asian/Beijing lineage, has acquired particular epidemiological importance. Originally described in East Asia, especially in northern China, Korea, and parts of Russia, this lineage has subsequently expanded on a global scale. Molecular epidemiological studies indicate that Lineage 2 strains are disproportionately represented among multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB cases [6, 15, 16]. Mechanistic investigations have suggested that the evolutionary success of this lineage may be explained by a higher propensity to accumulate resistance-conferring mutations, combined with compensatory mutations that mitigate the associated fitness costs [17]. The capacity of Lineage 2 to retain transmissibility under conditions of intense antibiotic pressure has made it a central focus of current genomic surveillance strategies [12].

Lineage 3, the East African–Indian lineage, is distributed predominantly across South Asia, the Middle East, and East Africa. Its spread corresponds closely to long-standing trade and migration networks along the Indian Ocean, and it remains an important contributor to the TB burden in high-incidence countries such as India and Pakistan [18]. Lineage 4, the Euro-American lineage, represents the most globally dispersed branch of the MTBC. It is dominant in Europe and the Americas but is also highly prevalent in Africa and parts of Asia. Historical analyses attribute its expansion to both colonial-era population movements and subsequent global migrations [19, 20]. Unlike Lineage 2, Lineage 4 demonstrates a high degree of internal genetic heterogeneity, with multiple sub-lineages that display regional patterns of adaptation [21].

By contrast, Lineages 5 and 6, collectively known as *M. africanum*, are restricted largely to West Africa. These lineages exhibit phenotypic and epidemiological characteristics distinct from MTB, including reduced transmissibility and slower progression to active disease [22, 23]. Despite these differences, *M. africanum* continues to account for a substantial proportion of cases in its endemic regions, highlighting the importance of lineage-specific approaches in TB control. Lineage 7 has, to date, been reported almost exclusively in Ethiopia, where it may represent a relict population maintained in geographic isolation [24]. More recently, Lineage 8

was identified in East Africa and has been interpreted as a basal branch of the MTBC phylogeny [8]. In addition, a proposed Lineage 9 has been described in northern and western Africa, although its epidemiological significance remains under investigation [9]. These discoveries indicate that MTBC diversity is more extensive than previously recognized and that additional under-sampled regions may harbor further undiscovered lineages.

In addition to human-adapted strains, the MTBC includes several animal-adapted members that underscore its zoonotic potential. Among them, *M. bovis* is of greatest public health relevance, responsible for bovine TB and contributing up to 10% of human TB cases in some high-burden regions where pasteurization and animal surveillance are limited [25]. Other species, including *M. caprae, M. microti, M. pinnipedii*, *M. orygis, and M. mungi*, predominantly circulate in animals but occasionally spill over into humans. Documented examples include *M. caprae* in pastoral communities, *M. microti* in rodent-to-human transmission events, and *M. pinnipedii* in zoo staff exposed to infected seals. These cases illustrate the One Health dimension of TB and highlight the need for integrated surveillance that encompasses human, veterinary, and environmental health [26].

Taken together, the global distribution of MTBC lineages illustrates the long-standing co-evolution between this pathogen and human populations. The evolutionary complexity of MTBC, combined with its adaptive immune evasion strategies, makes TB one of the most difficult infectious diseases to eradicate. The emergence of lineages with greater transmissibility or drug resistance threatens to undermine existing control efforts. The stratified geographic patterns observed across lineages are the result of both ancient dispersals and more recent socio-political processes, such as urbanization, migration, and global trade. Importantly, the rise of Lineage 2 as a dominant driver of antimicrobial resistance underlines the need for lineage-informed strategies in surveillance, diagnosis, and treatment within global TB control frameworks [2, 7].

1.3 MTB Pathogenesis: Insights and Perspectives.

TB caused by MTB, remains one of the most persistent infectious diseases, primarily targeting the lungs but also capable of disseminating to extrapulmonary sites such as the kidneys, spine, and central nervous system [1, 5]. The pathogen's ability to establish chronic infection is underpinned by its capacity to evade immune surveillance, manipulate host cellular responses, and persist for years in a latent state. Despite substantial advances in diagnostics, therapeutics, and vaccines, TB continues to cause approximately 10.6 million new cases and 1.3 million deaths annually [1]. The rise of multidrug-resistant (MDR), extensively drug-resistant (XDR),

and reports of totally drug-resistant (TDR) strains further complicates global control efforts, reinforcing the urgent need for novel therapeutic and immunological strategies [6].

MTB thrives in the oxygen-rich environment of the lungs, a niche that has shaped its evolutionary adaptation. Its compact genome of approximately 4.38 Mb, with a high G+C content of around 65%, encodes more than 4,000 genes, including over 190 transcriptional regulators that enable dynamic responses to hostile host conditions such as oxidative stress, hypoxia, and nutrient deprivation [27, 28]. A hallmark of MTB biology is its lipid-rich cell wall, dominated by long-chain mycolic acids, which confers intrinsic resistance to many antimicrobial agents, modulates host immune responses, and facilitates aerosol transmission [29, 30]. Infection begins with the inhalation of aerosolized droplets containing viable bacilli, which are deposited in the alveoli. There, alveolar macrophages engulf the bacteria as part of the host's first line of defense. However, instead of being destroyed, MTB arrests phagosome maturation, avoiding lysosomal fusion, and survives within a modified intracellular niche [31]. This adaptation not only permits bacterial persistence but also subverts macrophages into reservoirs that facilitate long-term infection.

The outcome of infection largely depends on host immunity and manifests in two main scenarios. In primary TB infection, effective immune responses lead to the formation of granulomas, organized aggregates of immune cells that encapsulate MTB and often drive bacilli into dormancy [32]. Over time, granulomas may calcify, leaving radiographic markers of prior infection. Yet these structures are not entirely protective; immunosuppression particularly HIV co-infection which can trigger reactivation from latency [33]. By contrast, in post-primary TB, bacteria disseminate via blood or lymphatic circulation, leading to extrapulmonary disease in organs such as the kidney, bone, and brain [34]. Secondary infections are frequently more severe and are often complicated by prior antibiotic exposure, contributing to the emergence of drug resistance.

At the molecular level, MTB exhibits extraordinary metabolic plasticity that supports persistence within hostile microenvironments. During intracellular survival, MTB shifts from glycolysis to lipid metabolism, preferentially utilizing fatty acids and cholesterol through pathways such as the glyoxylate shunt, enabling survival during nutrient limitation [35, 36]. Enzymes such as *isocitrate lyase* and *methylcitrate synthase* are critical for this adaptation and represent promising drug targets [37]. Similarly, the ability to metabolize lactate and alternative carbon sources within granulomas underscores its adaptability during dormancy [38].

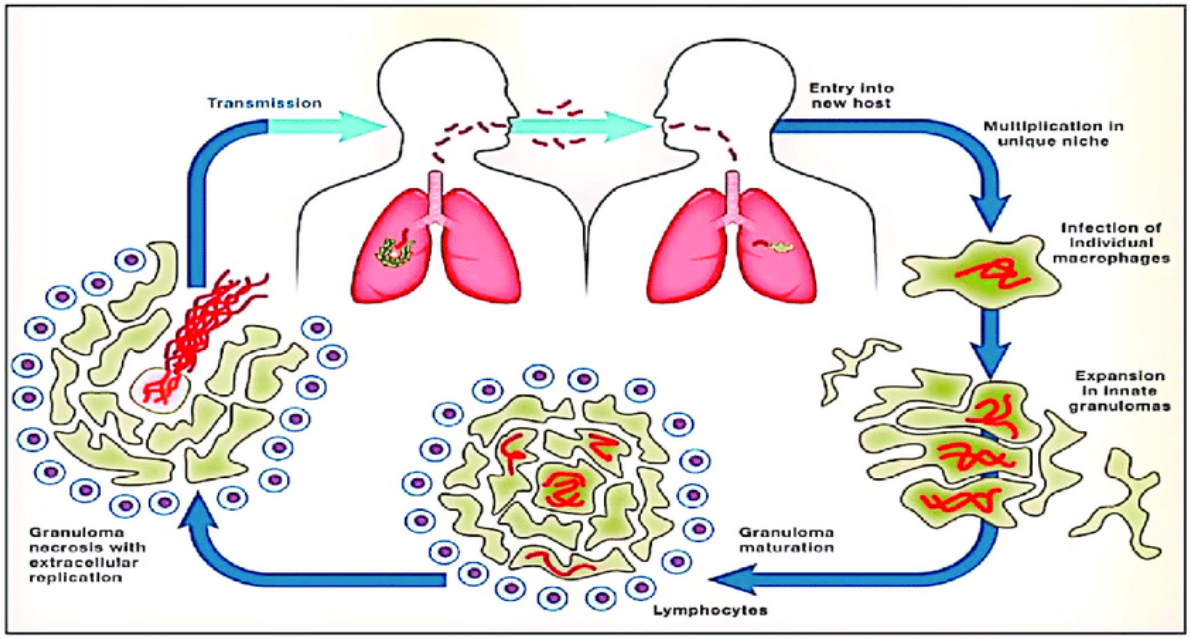

Figure 1.2 - Pathogenesis of *Mycobacterium tuberculosis* in the human host, illustrating entry of bacilli via inhaled droplets, macrophage uptake, granuloma formation, and potential dissemination. Adapted from Cambier et al. (2014).

The interaction between MTB and host immunity is defined by a dynamic arms race. MTB interferes with antigen processing, inhibits major histocompatibility complex (MHC) presentation, and dampens T-cell activation, thereby delaying adaptive immune responses [39, 40]. In turn, the host mobilizes antimicrobial mechanisms such as nitric oxide production and Th1-mediated immunity. Granuloma formation, once considered purely protective, is now understood as a double-edged sword: while containing bacilli, granulomas can also serve as niches of bacterial persistence [32].

The global rise of MDR and XDR strains highlights MTB's capacity to adapt under therapeutic pressure. Resistance is primarily driven by chromosomal mutations that alter drug targets, reduce drug permeability, or upregulate efflux pumps [16]. Notably, Lineage 2 (Beijing) strains have been shown to accumulate drug-resistance mutations more readily, posing a particular challenge for public health control [6, 15]. Understanding the interplay between pathogen evolution, host immune evasion, and drug resistance is therefore central to developing next-generation therapies.

Host-directed therapies (HDTs) and immunomodulators that strengthen the host response rather than directly targeting bacterial pathways [41]. These approaches, alongside the identification of new metabolic and genetic vulnerabilities in MTB, may provide novel avenues to counteract both drug-sensitive and drug-resistant forms of TB. In this context, advances in

immunology, molecular biology, and genomics are reshaping strategies for TB prevention and treatment, offering cautious optimism for reducing the global burden of this ancient yet resilient pathogen.

1.4 Evolving Paradigms in Tuberculosis Management.

MTB presents clinically in two principal forms: latent TB infection (LTBI) and active TB disease. LTBI is characterized by the asymptomatic carriage of MTB, where immune mechanisms sequester bacilli within granulomas, effectively containing bacterial replication and rendering the host non-infectious [42, 43] (Figure 1-3). Despite containment, LTBI retains the potential for reactivation, particularly in immunocompromised hosts, emphasizing the need for preventive interventions. By contrast, active TB manifests with clinical symptoms including persistent cough, chest pain, hemoptysis, fever, and weight loss, reflecting bacterial proliferation and tissue destruction (Figure 1-3). This stage facilitates human-to-human transmission via aerosolized respiratory droplets, positioning active TB as the primary driver of ongoing global spread [1] (Figure 1-3). Diagnostic confirmation typically integrates clinical assessment with radiological imaging and microbiological testing, including sputum smear microscopy, culture, and molecular assays [44].

Antimicrobial resistance (AMR). For LTBI, short-course regimens such as isoniazid–rifapentine for 12 weeks (3HP) or rifampicin monotherapy for 4 months, are now widely recommended as preventive therapy, substantially reducing the risk of reactivation [45, 46] (Table 1-2). In cases of active TB, first-line treatment remains a standardized six-month combination of rifampicin, isoniazid, pyrazinamide, and ethambutol (RIPE), often implemented under the Directly Observed Treatment, Directly Observed Treatment, Short-course (DOTS) framework to promote adherence [46, 47]. DOTS was designed in response to widespread treatment failure and the emergence of drug resistance caused by poor adherence to lengthy regimens [48, 49]. Its framework is built on five essential pillars:

1. **Political and financial commitment –** Sustainable resources for TB programs.
2. **Case detection by quality-assured bacteriology –** Early and accurate diagnosis, initially through sputum smear microscopy.
3. **Standardized treatment regimen with direct observation –** Ensuring patients complete a 6-month regimen (isoniazid, rifampicin, pyrazinamide, ethambutol) under direct supervision.
4. **Effective drug supply and management –** Guaranteeing uninterrupted access to quality-assured medicines.

5. **Monitoring and evaluation system –** Recording patient outcomes to track program effectiveness [50].

The DOTS model revolutionized TB control by significantly improving treatment completion rates and reducing community transmission. Clinical trials and programmatic evaluations demonstrated cure rates exceeding 85% in drug-susceptible TB when DOTS was properly implemented [51, 52]. However, DOTS is not without limitations. Its dependence on direct supervision can impose significant logistical and social burdens, particularly in resource-limited or geographically remote settings. Moreover, the standard six-month regimen has contributed to adherence fatigue, while DOTS alone has proven insufficient to address MDR-TB and XDR-TB, where treatment requires longer, more toxic, and less effective regimens [1, 53].

However, MTB's capacity for adaptive resistance has necessitated the expansion of therapeutic strategies. Mono- and poly-drug resistance require tailored regimens that exclude ineffective drugs, while multidrug-resistant TB (MDR-TB), defined by resistance to rifampicin and isoniazid, demands second-line agents such as fluoroquinolones and injectable drugs, extending treatment duration and increasing toxicity [53, 54] (Table 1-2). Extensively drug-resistant TB (XDR-TB) Totally drug-resistant TB (TDR-TB) highlight the fragility of current regimens, with survival outcomes significantly poorer due to limited treatment options [1, 55] (Table 1-2).

The DOTS strategy remains central to TB control, yet it has increasingly been complemented by precision diagnostic approaches. Drug susceptibility testing (DST) and rapid molecular assays such as GeneXpert MTB/RIF enable earlier identification of resistant cases, while WGS now offers unprecedented resolution in detecting resistance-conferring mutations and mapping transmission networks [56, 57]. WGS facilitates personalized regimens by predicting drug susceptibility profiles with high accuracy, thereby minimizing empirical use of toxic second-line agents and improving patient outcomes.

The integration of genomics into TB management exemplifies a paradigm shift from standardized to individualized care. Beyond diagnosis, WGS and other omics-based approaches contribute to real-time epidemiological surveillance, tracking outbreaks, and monitoring the spread of high-risk resistant clones, particularly those of Lineage 2 (Beijing) that are strongly associated with MDR-TB and XDR-TB [12, 15]. Combined with novel therapeutics such as bedaquiline, delamanid, and pretomanid, these strategies mark a new era of precision medicine in TB control [58].

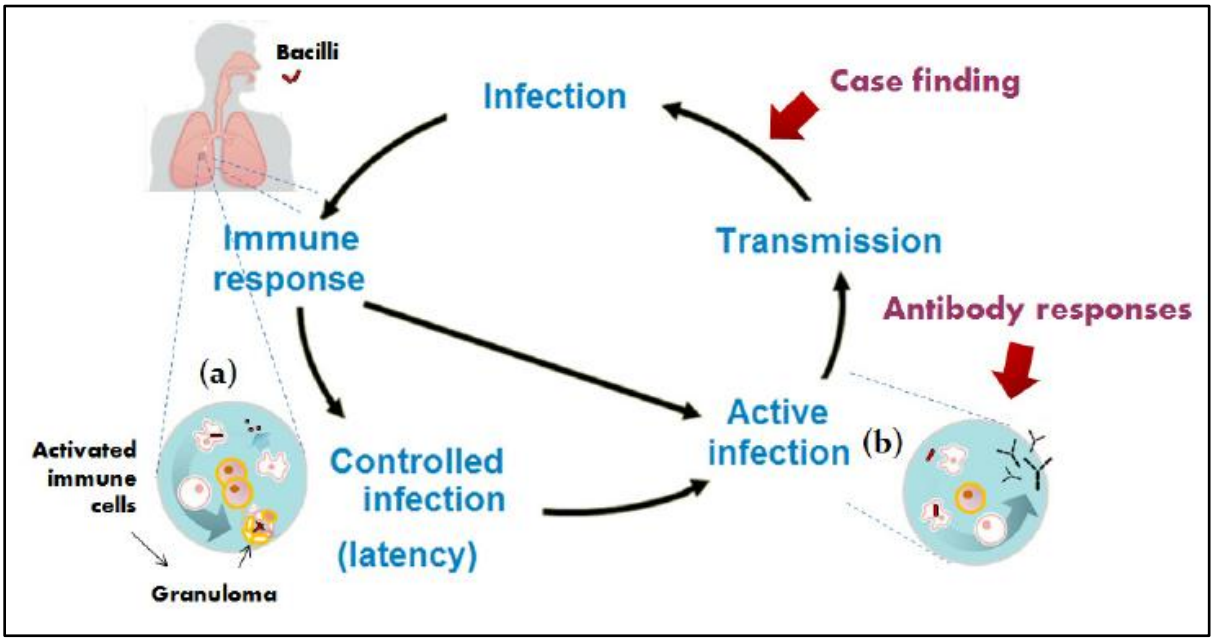

Figure 1-3. Infographic illustrating the distinction between latent and active TB. In LTBI, immune responses contain bacilli within granulomas (Inset a). In active TB, compromised immunity leads to bacillary proliferation, tissue necrosis, and transmissibility (Inset b). Adapted from Miguel et al. (2012).

Classification	Drug Resistance Profile	Recommended Diagnostic Method	Typical Treatment Regimen	Estimated Cure Rate	Key References
Mono-resistant TB	Resistant to one first-line drug (INH, RIF, PZA, or EMB)	Sputum smear, culture, drug susceptibility testing (DST), and whole genome sequencing (WGS) where available	Modified first-line regimen excluding the resistant drug	85–95%	WHO (2020); Caminero et al. (2011)
Poly-resistant TB	Resistant to ≥2 first-line drugs, but not both INH and RIF	Smear, culture, DST, WGS	Tailored first-line regimen with substitutions	80–90%	WHO (2020); Caminero et al. (2011)
MDR-TB	Resistant to at least INH and RIF	Rapid molecular testing (e.g., Xpert MTB/RIF), culture-based	Longer regimen (fluoroquinolones, second-line oral agents ± injectables)	60–75%	Dheda et al. (2017); WHO (2023)

		DST, WGS at specialised labs			
XDR-TB	MDR-TB plus resistance to ≥1 fluoroquinolone and ≥1 injectable second-line drug (AM, KM, or CM)	Molecular DST, WGS in reference laboratories	Highly individualised regimens incorporating new agents (e.g., bedaquiline, pretomanid, linezolid)	40–55%	Conradie et al. (2020); WHO (2023)
TDR-TB	Resistant to all available first- and second-line drugs	WGS and phenotypic DST in highly specialised laboratories	Experimental or compassionate-use regimens, often palliative	<40%	Udwadia et al. (2012); WHO (2023)

Table 1-2. Classification of drug-resistant TB, diagnostic modalities, and associated treatment regimens. RIF: Rifampicin; INH: Isoniazid; AM: Amikacin; KM: Kanamycin; CM: Capreomycin; OFX: Ofloxacin. Cure rates are estimates and may vary depending on adherence, comorbidities, and specific resistance profiles.

Despite these advances, formidable challenges remain. Prolonged treatment duration, high pill burden, adverse drug effects, and socioeconomic barriers undermine adherence, particularly in low- and middle-income countries where TB burden is highest. Furthermore, equitable access to molecular diagnostics and new therapies is constrained by cost and infrastructure requirements [1, 44]. Overcoming these barriers will require integrated approaches that combine biomedical innovation with strengthened health systems, patient-centred care models, and global political commitment to TB elimination targets.

In sum, evolving paradigms in TB management reflect the dynamic interplay between scientific progress and the pathogen's adaptive potential. Preventive therapy for LTBI, shorter and safer regimens for active TB, and genomics-informed precision care represent critical steps forward. Yet the continued rise of drug resistance and inequitable access to innovations underscore that TB management remains one of the greatest global health challenges of the 21st century.

1.5 Poverty, Health Inequalities, and Tuberculosis.

TB remains one of the most prominent infectious diseases shaped by social and economic inequalities. Although the causative pathogen, MTB, is biologically ubiquitous, the persistence of TB as a global health challenge is inseparable from poverty and broader social determinants of health [59, 60]. The unequal burden of TB across populations reflects not only biological susceptibility but also deep structural inequities that determine exposure, access to care, and treatment outcomes. According to the *World Health Organization (WHO) Global TB Report 2023*, 10.6 million new TB cases and 1.3 million deaths were recorded in 2022, with the overwhelming majority occurring in low- and middle-income countries (LMICs), particularly those with fragile healthcare infrastructures and widespread poverty [1].

Health inequalities are evident in the distribution of TB across socio-economic gradients. Individuals living in overcrowded housing, with limited ventilation and poor sanitation, are disproportionately exposed to MTB transmission [59]. Malnutrition, itself a marker of poverty, not only increases susceptibility to infection but also accelerates progression from latent TB infection (LTBI) to active disease by weakening host immunity [60]. These vulnerabilities intersect with other inequities, including HIV status, diabetes prevalence, and occupational exposure in precarious work settings, amplifying the risk of disease and complicating treatment adherence [5]. Such factors demonstrate that TB is not evenly distributed across societies but rather concentrates among socially and economically disadvantaged groups.

The unequal distribution of TB is further illustrated in the WHO high-burden country list, which identifies India, Indonesia, China, the Philippines, and Pakistan as accounting for more than half of global TB cases [1]. These countries are marked by large population densities, persistent poverty, and substantial health disparities that exacerbate TB transmission and hinder effective control. Table 1-3 provides a comparative overview of TB incidence, mortality, and drug-resistant cases across the ten highest-burden countries, underscoring the close relationship between economic disadvantage and TB burden. In 2023, the ten countries with the highest TB burden are as follows:

Rank	Country	GDP (PPP Billions)	TB Infection Cases (Thousands)	MDR-TB Cases (Thousands)	XDR-TB Cases (Hundreds)	Deaths (Thousands)
1	India	3,460	2,700	270	30	480
2	Indonesia	1,320	750	120	15	180
3	China	17,700	710	110	10	160

4	Philippines	1,070	640	100	8	110
5	Pakistan	1,270	620	90	7	110
6	Bangladesh	840	520	70	5	86
7	Nigeria	540	470	60	4	100
8	Democratic Republic of the Congo	180	380	50	3	78
9	South Africa	350	360	40	2	66
10	Myanmar	340	340	30	1	62

Table 1-1 - This table shows key data for the ten countries most affected by TB in 2023. It includes information on each country's population size, GDP income, total TB cases, cases of MDR-TB and XDR-TB, and TB-related deaths, based on data from WHO, 2023 Global TB report [1].

Healthcare access is another domain where inequalities profoundly shape TB outcomes. In many LMICs, delays in diagnosis are common due to under-resourced health systems, limited laboratory capacity, and geographic or financial barriers to care [61]. Even where diagnosis occurs, adherence to lengthy treatment regimens is often undermined by the direct and indirect costs of care, stigma, and loss of income during treatment [62]. These structural barriers fuel the cycle of poverty and disease, as untreated or inadequately treated TB not only worsens individual health outcomes but also perpetuates community transmission. Stigma and discrimination add an additional layer of inequality, as individuals in marginalized communities may avoid seeking care altogether, resulting in late presentation, severe disease, and greater risk of transmission [63].

The COVID-19 pandemic starkly illuminated these vulnerabilities, exacerbating health inequalities in TB care (Figure 1-4). LMICs, already burdened with high TB incidence, experienced major disruptions in diagnosis and treatment as resources were diverted toward pandemic response [64] (Figure 1-4). Lockdowns and health service interruptions disproportionately affected poor and marginalized populations, leading to increased treatment interruptions, rising drug resistance, and setbacks in achieving global TB control targets [65]. Similarly, conflict, forced migration, and rising global cost of living have disproportionately impacted TB care in already vulnerable populations, reflecting the compounding effect of structural inequalities on disease outcomes.

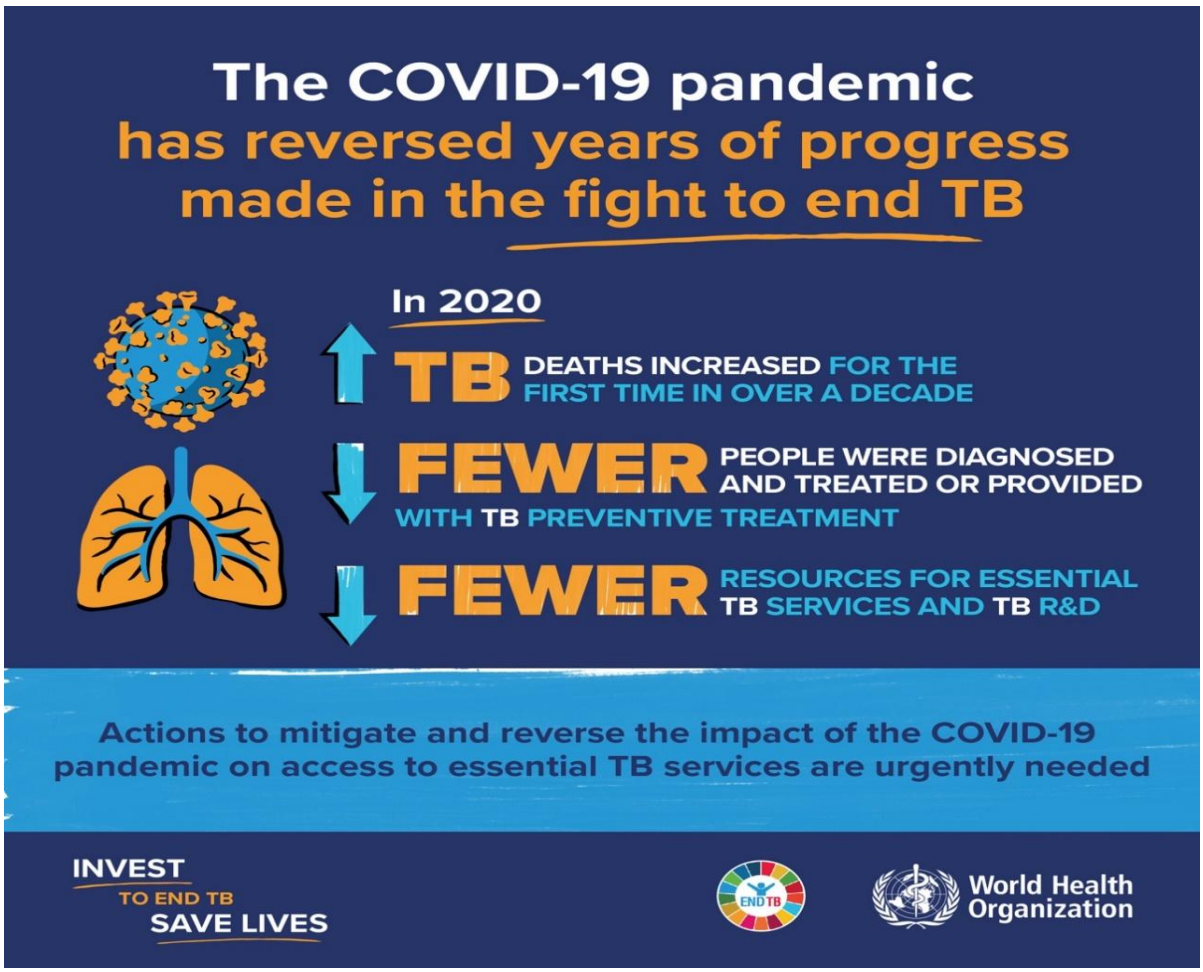

Figure 1-1 - The WHO unveiled a comprehensive global TB brochure, strategically designed to accentuate the profound implications of the COVID-19 pandemic on essential TB services.

Addressing TB therefore requires more than biomedical interventions. The *WHO End TB Strategy* explicitly recognizes the importance of tackling social determinants, calling for integrated approaches that combine diagnostics and treatment with poverty reduction, social protection, and universal health coverage [66] (Figure 1-5). Evidence demonstrates that interventions such as cash transfers, nutritional support, and education improve adherence and reduce transmission by addressing underlying inequalities [67]. This shift towards a social determinant framework highlights TB as not merely a biomedical problem but a disease of inequity, deeply embedded in structures of poverty and marginalization.

In conclusion, TB continues to exemplify how health inequalities drive infectious disease outcomes. Its persistence reflects the interplay of poverty, malnutrition, inadequate housing, fragile health systems, and stigma, compounded by global crises such as COVID-19 and displacement. Efforts to eliminate TB must therefore adopt a holistic approach that integrates biomedical advances with structural interventions to address the social and economic

conditions enabling its transmission. Without addressing these inequalities, global ambitions to end TB by 2035 remain unattainable.

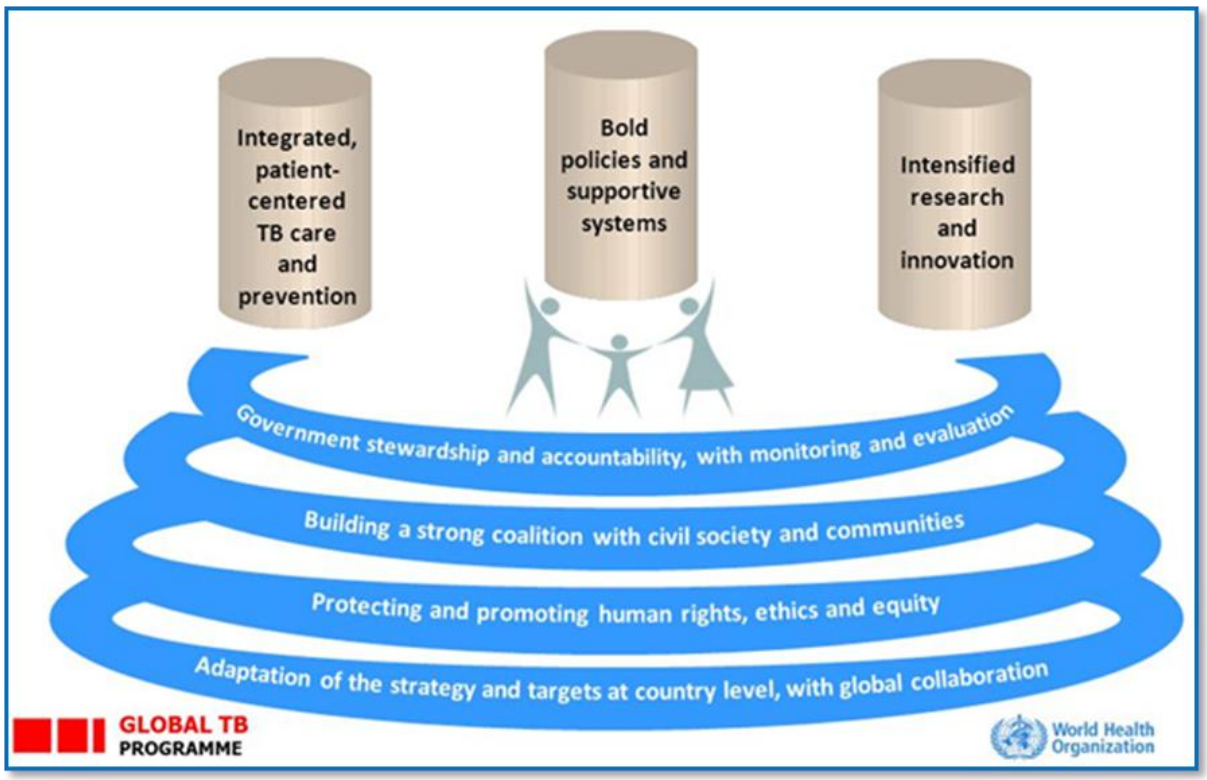

Figure 1-2 - The WHO global end TB strategy programme focusing on three pillars and 4 principles to aiming to eradicate TB worldwide in near future [1].

1.6 Complexity of Tuberculosis in Myanmar.

Myanmar remains one of the highest tuberculosis (TB) burden countries in Southeast Asia, with an incidence rate of 338 cases per 100,000 population in 2022 [1]. This positions the country among the 30 highest TB-burden countries globally, with approximately 180,000 new TB cases reported annually, including more than 40,000 in children [1, 68]. Beyond sheer prevalence, Myanmar exemplifies the nature of TB, where the disease intersects with other infectious diseases such as HIV/AIDS, malaria, dengue and the wider challenge of antimicrobial resistance (AMR), while being compounded by socio-economic inequality, civil conflict, and weak health infrastructure.

HIV/AIDS has been one of the most significant co-epidemics. TB is the leading cause of mortality among people living with HIV, and coinfection accelerates progression to active disease [69]. In Myanmar, HIV prevalence remains concentrated among key populations such as people who inject drugs and sex workers, creating pockets of high TB–HIV coinfection. Immunosuppression associated with HIV reduces host defences against Mycobacterium TB,

while TB amplifies HIV replication, worsening patient outcomes [1]. Despite efforts by the National TB Programme (NTP) to integrate HIV and TB services, coverage remains uneven, particularly in rural and conflict-affected regions where displaced populations face heightened vulnerability [68].

The presence of malaria, particularly artemisinin-resistant *Plasmodium falciparum* in the Greater Mekong Subregion, adds further complexity [70]. Coinfection with malaria and TB is under-studied, yet evidence from comparable settings demonstrates increased morbidity and diagnostic difficulties [71]. Malaria's seasonal outbreaks disproportionately affect forested and rural communities, overlapping with areas where TB detection and treatment are already limited. Similarly, dengue fever, a leading vector-borne disease in Myanmar further complicates diagnosis due to overlapping symptoms such as fever, weight loss, and fatigue, often resulting in delays in TB confirmation and treatment initiation [72]. Equally critical is Myanmar's role as a regional hotspot for antimicrobial resistance (AMR). MDR-TB and XDR-TB cases are rising, with more than 9,000 MDR-TB cases reported annually [1]. Resistance arises from treatment interruptions, poor-quality drugs, and disrupted health services due to ongoing civil conflict. Beyond TB, Myanmar has also documented artemisinin resistance in malaria and emerging HIV drug resistance, reinforcing its status as a regional AMR hotspot [73]. Patients with HIV–TB coinfection are especially vulnerable to developing resistance due to polypharmacy and immunosuppression [74].

These biological and clinical challenges are magnified by civil war, political instability, and health inequalities. Ethnic minority regions face the dual burden of conflict and poor healthcare access, leading to under-diagnosis and delayed treatment [75]. The COVID-19 pandemic further diverted critical resources, causing steep declines in TB detection and contributing to increased drug resistance [65]. Health inequalities between urban and rural populations remain stark: urban centres such as Yangon serve as hotspots for TB transmission due to overcrowding and poverty, while remote ethnic states face extreme shortages in laboratory capacity and trained personnel [76].

Table 1-4 presents comparative epidemiological data, illustrating the scale of Myanmar's TB epidemic. The prevalence of TB in Myanmar surpasses global norms, particularly among HIV-positive populations. Case detection rates, while improving, remain uneven and fragile in the face of conflict and weak financing. Notably, Myanmar allocates one of the lowest proportions of GDP to healthcare in the world, which contributes to systemic fragility and persistent health inequalities [77].

Source	Pop (m)	Prevalence (per 100,000)	Incidence (per 100,000)	Incidence (HIV-positive)	Mortality Rate (excl. TB/HIV)	Case Detection Rate	Reference
Global TB Report 2021	54.6	525	308	26	33	78%	[1]
Myanmar (NTP) 2021 Report	54.4	525	167,000 (95% CI: 112,000-234,000)	14,000 (95% CI: 9,400-20,000)	31	92%	[81]
Lancet Regional Health SEA Report 2021	54.5	525	180,000	20,000	30	81%	[83]

Table 1-2 - Epidemiological data from Myanmar reveal TB prevalence surpassing global norms, especially among HIV-infected individuals, highlighting a significant health burden and ongoing challenge.

Despite these formidable challenges, Myanmar's TB response has demonstrated elements of resilience. Since 2007, the NTP has consistently achieved treatment success rates exceeding 85%, largely due to the implementation of the DOTS strategy, which remains a cornerstone of TB control globally [1]. More recently, innovative measures such as the scaling up of molecular diagnostics, including Xpert MTB/RIF, and the gradual introduction of WGS for resistance surveillance have marked important advances in Myanmar's capacity to monitor and respond to drug-resistant TB [80]. Nevertheless, the effectiveness of these initiatives continues to be limited by uneven geographical coverage, disruptions caused by ongoing conflict and political instability, and the chronic underfunding of the national health sector. Myanmar's TB epidemic therefore exemplifies the complex interplay of infectious diseases with broader structural determinants, where TB converges with HIV/AIDS, malaria, dengue, and the rising threat of AMR. This layered crisis cannot be addressed through biomedical interventions alone. Progress requires not only the development and expansion of effective diagnostics and treatment regimens but also systemic investments in health equity, conflict-sensitive service delivery, and integrated strategies that address multiple diseases simultaneously.

It is within this context that this PhD thesis seeks to contribute to bridging existing gaps, with a particular focus on rapid diagnosis as a pathway to strengthen TB control in Myanmar. By investigating the role of emerging diagnostic technologies including WGS, point-of-care molecular platforms, and novel biomarker-based tools, this research will evaluate their potential to accelerate case detection, enable earlier treatment initiation, and improve resistance surveillance. A central premise of this thesis is that enhancing rapid diagnostic capacity can mitigate the delays that currently drive transmission, reduce the burden of undetected drug-resistant TB, and support a more equitable and effective health response in fragile and resource-constrained settings such as Myanmar.

1.7 Advancements in Detecting Tuberculosis: Evolution and Future Directions.

The advancement of diagnostic techniques for TB is a critical component in the comprehensive management and therapeutic intervention of this complex disease. TB manifests in a spectrum of clinical forms, encompassing latent infections that remain immunologically contained as well as active disease states associated with symptomatic progression and high transmission potential. The challenge becomes further complicated by the emergence of multidrug-resistant and extensively drug-resistant strains, which necessitate precise, rapid, and context-appropriate diagnostic modalities [5]. Central to global TB control efforts is the accurate detection of active disease, yet according to the WHO's Global TB Report (2023), nearly forty-one percent of all active cases evade diagnosis each year, leaving approximately 4.3 million individuals untreated [1]. Such undetected individuals often function as silent reservoirs and potential "super-spreaders," perpetuating community transmission and undermining global eradication efforts. This diagnostic gap highlights the inadequacy of existing methods and underscores the imperative for innovation in diagnostic science.

The historical trajectory of TB diagnostics reflects a gradual evolution from rudimentary to highly sophisticated platforms. Tuberculin skin test (TST) Chest X-ray (CXR) represented the initial pillars of TB diagnosis (Table 1-5). While TST could indicate prior exposure to Mycobacterium TB complex (MTBC), it could not reliably differentiate between latent and active disease, nor was it specific in populations vaccinated with Bacille Calmette–GuéBacille Calmette-Guérin (BCG). Similarly, CXR provided valuable visual insights into pulmonary pathology but lacked specificity, often misclassifying non-tuberculous lung abnormalities as TB, especially in HIV-co-infected individuals with atypical presentations [1, 81].

Sputum smear microscopy, which became the cornerstone of diagnosis in high-burden countries due to its affordability and simplicity, significantly advanced TB control in the twentieth century (Table 1-5). However, its sensitivity is low in paucibacillary cases, in children, and in individuals co-infected with HIV. Culture-based methods, often heralded as the "gold standard" due to their superior sensitivity and capacity for drug susceptibility testing, are labor-intensive, time-consuming, often requiring weeks and highly dependent on high-level biosafety infrastructure that is absent in many low- and middle-income countries [82] (Table 1-5).

Nucleic acid amplification technologies (NAATs) in the early 2000s marked a paradigm shift in TB diagnostics. The GeneXpert MTB/RIF assay and its subsequent iteration, Xpert Ultra, allow the simultaneous detection of MTBC DNA and rifampicin resistance within two hours (Table 1-5). Polymerase chain reaction (PCR) amplification of TB-specific sequences, coupled with molecular probes that detect mutations in the rpoB gene. Their high sensitivity and specificity revolutionized TB detection and provided critical tools for early identification of drug resistance, although their reliance on stable electricity, expensive cartridges, and laboratory infrastructure restricts universal deployment [83]. Line probe assays (LPAs) expanded the resistance detection landscape by targeting multiple genes associated with first- and second-line drug resistance. At the same time, loop-mediated isothermal amplification (TB-LAMP) emerged as a more cost-effective and accessible method. TB-LAMP uses four to six primers to amplify MTB DNA at a constant temperature, producing results in under an hour, with detection confirmed via turbidity or fluorescence. Unlike PCR, LAMP does not require thermocyclers, making it particularly advantageous for peripheral or resource-constrained laboratories [1, 84].

Recent years have seen the advent of CRISPR-Cas–based diagnostic systems, which represent a major frontier in rapid molecular detection (Table 1-5). These systems, including CRISPR-Cas12a and Cas13-based assays, utilize programmable guide RNAs to recognize MTBC-specific sequences. Upon target recognition, collateral cleavage activity is induced, cutting fluorescently labeled reporter molecules to generate a visible signal. When paired with isothermal pre-amplification such as LAMP or recombinase polymerase amplification, CRISPR assays have achieved extremely high sensitivity, in some cases down to a few DNA copies per microliter, outperforming conventional NAATs in paucibacillary disease [85]. These systems, due to their portability, low cost, and minimal infrastructure requirements, are particularly promising for conflict-affected and fragile healthcare systems, such as those in Myanmar.

Beyond sputum-dependent methods, the search for non-invasive biomarkers has become increasingly important, especially in children, HIV-positive individuals, and those with extrapulmonary TB. Lipoarabinomannan (LAM), which is released from the MTB cell wall, Volatile organic compounds (VOCs) detected in exhaled breath, urine, or saliva (Table 1-5). Portable colorimetric sensor arrays and electronic-nose devices are currently under evaluation to analyze these signatures, offering rapid, non-invasive diagnostics that bypass the logistical challenges of sputum collection [78, 79, 86]. Biosensors integrated with smartphone applications extend these opportunities further. By using a phone's camera and processing power, test results can be digitized, analyzed, and coupled with artificial intelligence algorithms that refine diagnostic thresholds, incorporate patient risk factors, and generate clinical decision outputs at the point of care [7].

Genomic technologies have also fundamentally reshaped TB diagnosis and surveillance. Whole genome sequencing (WGS) Next-generation sequencing (NGS) provides comprehensive profiles of drug resistance, strain lineage, and transmission dynamics [2, 87] (Table 1-5). While short-read sequencing has become the standard in many high-income laboratories, the recent introduction of long-read platforms, Oxford Nanopore Technologies (ONT) and Pacific Biosciences (PacBio), has significantly advanced the field. Unlike short-read sequencing, which requires computationally intensive assembly of fragmented reads, long-read platforms generate near-complete genome coverage in a single run. This allows precise detection of resistance-associated mutations, structural variants, and mobile genetic elements. ONT's portable MinION sequencer has been piloted in high-burden, low-resource settings for real-time genomic surveillance, demonstrating its potential for outbreak tracing and rapid drug resistance detection without dependence on centralized laboratory infrastructure [88, 89].

In parallel, digital droplet PCR (ddPCR) has emerged as a powerful tool for the detection of low-frequency mutations and the quantification of bacterial load (Table 1-5). Unlike traditional qPCR, which measures bulk amplification curves, ddPCR partitions the sample into thousands of droplets, each functioning as an independent micro-reaction. This enables absolute quantification of DNA molecules and detection of rare resistance alleles at frequencies below one percent, which may be missed by other methods. ddPCR has proven particularly effective in diagnosing paucibacillary TB, monitoring treatment response, and detecting early emergence of drug resistance [90]. Additional molecular amplification methods are expanding the diagnostic toolkit. Multiple Displacement Amplification (MDA), which employs the high-fidelity Phi29 DNA polymerase, can amplify entire genomes from minimal DNA inputs (Table

1-5). This is especially valuable in cases of low bacillary load, such as extrapulmonary TB or pediatric infections. Coupling MDA with long-read sequencing platforms enables complete drug resistance profiling directly from clinical samples, bypassing the delays of culture and dramatically reducing time-to-diagnosis [91].

More recently, Artificial intelligence (AI) and machine learning (ML) now play pivotal roles in the TB diagnostic ecosystem. AI-driven algorithms have shown accuracy comparable to expert radiologists in interpreting chest X-rays, facilitating automated triage in high-burden countries [7]. Beyond imaging, ML models trained on large-scale genomic datasets can interpret complex or previously uncharacterized mutations, enhance resistance prediction and reducing diagnostic uncertainty [3]. AI systems are also embedded within sequencing workflows to automate base calling, error correction, and resistance annotation, reducing dependency on specialized bioinformatics expertise in low-resource contexts [4].

Comparative overview of TB diagnostic methods

Diagnostic Method	Mechanism of Detection	Time to Result	Sensitivity/Specificity*	Infrastructure Requirement	Key Advantages	Key Limitations	Suitability in LMICs
Tuberculin Skin Test (TST)	Delayed-type hypersensitivity reaction to purified protein derivative (PPD)	48–72 hrs	Moderate / Low (cross-reactivity with BCG, NTM)	Minimal (clinical setting)	Simple, inexpensive	Cannot differentiate latent vs active TB; poor specificity in BCG-vaccinated populations	Moderate (useful for latent TB screening, but limited in endemic regions)
Chest X-ray (CXR)	Radiological visualization of lung pathology	Same day	Variable; non-specific	Radiology facilities, trained readers	Non-invasive, rapid	Poor specificity, misclassification common	Limited (triage tool where radiology available)
Sputum Smear Microscopy	Ziehl–Neelsen or fluorescent staining of acid-fast bacilli	Same day	Low (esp. in HIV, children)	Basic lab, microscope	Widely available, cheap	Poor sensitivity; cannot detect resistance	High (still widely used despite limitations)
Culture (solid/liquid)	Growth of MTB on Lowenstein–Jensen or MGIT media	2–8 weeks	High / High	Biosafety level 3 labs, infrastructure	Gold standard; full DST possible	Very slow; costly; infrastructure heavy	Low (not widely scalable in LMICs)
GeneXpert MTB/RIF / Ultra	Real-time PCR with molecular beacon probes (rpoB gene)	2 hrs	High / High	Cartridge-based platform, electricity	Simultaneous TB + rifampicin resistance detection	High cost; cartridge dependence	Moderate–High (scaled in Myanmar but inequitable access)
Line Probe Assays (LPA)	Reverse hybridization of amplified DNA to oligonucleotide probes	1–2 days	High / High	Molecular lab capacity	Detects MDR and XDR resistance mutations	Requires technical expertise	Moderate (referral labs only)
TB-LAMP	Loop-mediated isothermal amplification (DNA)	<1 hr	High for smear-positive; lower for smear-negative	Minimal (heating block, basic training)	Simple, rapid, WHO-endorsed for decentralized use	Reduced sensitivity in paucibacillary cases	High (suitable for rural Myanmar)

CRISPR-Cas assays	Guide RNA + Cas protein collateral cleavage of reporter	30–60 min	Very high (near single-copy detection)	Low (portable)	Ultra-sensitive, rapid, low cost	Early stage, limited large-scale validation	High potential (especially in conflict zones)
Whole Genome Sequencing (Illumina)	Short-read sequencing of entire MTB genome	1–2 weeks	Very high / High	High-end sequencing labs, bioinformatics	Comprehensive resistance & transmission profiling	Costly, infrastructure intensive	Low (requires central labs)
Long-read sequencing (Nanopore/PacBio)	Direct sequencing with long reads (10–100 kb)	1–3 days (real-time possible with ONT)	Very high / High	Portable (Nanopore), bioinformatics	Detects structural variants, rapid resistance profiling, portable (ONT)	Higher error rates (improving), costs	Moderate–High (Nanopore pilots feasible in Myanmar)
Digital Droplet PCR (ddPCR)	Partitioning PCR into droplets, absolute quantification of DNA	<1 day	Very high sensitivity	Molecular platform	Detects rare variants, low bacillary load	Higher cost, technical expertise	Moderate (research & referral labs)
Multiple Displacement Amplification (MDA)	Isothermal whole genome amplification via Phi29 DNA polymerase	<1 day	High (from very low DNA input)	Molecular lab	Enables sequencing from paucibacillary samples	Risk of amplification bias	Moderate (paired with sequencing)
Biosensors & Smartphone Apps	Colorimetric/electronic detection of LAM or VOCs, linked to mobile apps & AI	<1 hr	Variable, improving	Minimal (portable device + smartphone)	Low cost, scalable, AI-enabled predictive modelling	Early stage, standardization lacking	High potential (ideal for community use in Myanmar)

*Values vary by study design, patient population, and implementation setting.

Table 1-5. Comparative overview of tuberculosis diagnostic methods, highlighting performance characteristics, infrastructure needs, and suitability for implementation in low- and middle-income countries (LMICs) such as Myanmar.

The convergence of these technologies signals the rise of a new diagnostic paradigm. Nanopore sequencing, ddPCR, LAMP, MDA, CRISPR-Cas diagnostics, and AI-enhanced smartphone biosensors collectively form an integrated diagnostic ecosystem capable of bridging diagnostic divides (Table 1-5). Importantly, these tools not only improve accuracy and timeliness but also decentralize diagnostics, extending advanced detection to marginalized populations, refugees, and communities in conflict zones where laboratory infrastructure is often absent. The synergy between molecular innovations and digital platforms has the potential to close the diagnostic gap that currently leaves forty percent of cases undetected worldwide [1].

Looking ahead, CRISPR-Cas systems hold promise not only as diagnostic platforms but also as future therapeutic tools. Their programmable nucleic acid recognition offers unprecedented specificity, and their integration with amplification technologies makes them adaptable for both laboratory and field use. This convergence of molecular precision and digital intelligence presents not only a scientific breakthrough but also a profound public health opportunity: to tailor treatments rapidly, guide precision therapy, and address structural inequities in TB care. This PhD thesis situates Myanmar as a case study for how such technologies, when adapted to fragile and inequitable health systems, can bridge diagnostic gaps, enhance treatment precision, and accelerate progress toward the WHO's End TB Strategy.

1.8 Research Aims and Objectives.

This research investigates the complex relationship between MTB, its evolving drug-resistant strains, and their disproportionate impact on vulnerable populations. Myanmar, as one of the highest TB-burden countries in Southeast Asia, provides a critical case study where TB intersects with poverty, conflict, malnutrition, HIV/AIDS, malaria, and weak health systems. These structural and social determinants create significant health inequalities, shaping not only disease incidence and mortality but also access to diagnostics, treatment, and preventive care. By integrating genomic, molecular, and microbiome-based approaches, this research aims to generate new insights into TB pathogenesis, drug resistance, and diagnostic innovation that can inform equitable public health interventions in resource-limited and conflict-affected settings.

The specific objectives are:

1. To characterize the genetic diversity of MTB strains in Myanmar and globally using WGS, with a focus on lineage-specific adaptations, virulence factors, and resistance-conferring mutations that drive disparities in disease outcomes.

2. To evaluate the utility of advanced diagnostic platforms, with a primary focus on WGS and the application of MDA to enrich DNA from early-stage MGIT cultures (as early as five days compared to the conventional six weeks), representing a significant breakthrough in reducing diagnostic timelines.
3. To investigate the oral microbiome in TB, HIV, and TB/HIV co-infected populations, assessing its diagnostic potential through metagenomic sequencing and exploring how co-infections influence host-pathogen interactions in socio-economically disadvantaged groups.
4. To conduct comparative genomic analyses of drug-resistant TB strains, identifying mechanisms of antimicrobial resistance and informing treatment strategies that can improve clinical outcomes and reduce inequities in access to effective therapies.
5. To develop evidence-based recommendations for TB control and elimination, emphasizing equitable access to rapid diagnostics, conflict-sensitive health delivery systems, and integration of cross-disease approaches that address the broader structural determinants of health in Myanmar and similar high-burden regions.

Through these aims, this research contributes to advancing precision medicine in TB while simultaneously addressing the inequities that perpetuate its burden. By situating TB within the broader framework of health inequalities, this thesis aligns with the World Health Organization's *End TB Strategy* and the United Nations' Sustainable Development Goals (SDGs), with the overarching vision of reducing diagnostic gaps, enhancing treatment accessibility, and moving towards a more equitable global TB response.

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Chapter 2

Microbiome Dataset from the Upper Respiratory Tract of Patients Living with HIV, HIV/TB and TB from Myanmar

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Massey University Statement of Contribution (DRC 16) Declaration Form (Chapter 2).

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Abstract.

This article contains microbiome data from the upper respiratory tract of patients living with HIV/TB, HIV and TB from Meiktila, a town in Myanmar where there is a high incidence of HIV and TB. Microbiomes were compared for HIV/TB infected and healthy adults from the same population. We collected nasopharyngeal and oropharyngeal swabs from a total of 33 participants (Healthy (5), HIV/TB (8), HIV (14), and TB (6)). DNA was extracted from the swabs and subjected to custom single step 16s rRNA sequencing on an Illumina MiSeq platform. The sequencing data is available via http://www.ncbi.nlm.nih.gov/bioproject/ PRJNA432583.

Specifications table

Subject area	*Biology*
More specific subject area	*Microbiome, Infectious diseases*
Type of data	*Figure*
How data was acquired	*Culture-independent Illumina massively parallel sequencing of 16S rRNA genes using the Illumina sequencing-by-synthesis method on the MiSeq platform.*
Data format	*16S rRNA QIIME profiles*
Experimental factors	*Bacterial genomic DNA was extracted and used as a template to amplify the V3-V4 region of the 16S rRNA gene. The amplicons were barcoded, pooled, and sequenced using a paired-end protocol (Illumina).*
Experimental features	*Illumina massively parallel sequencing of the 16S rRNA gene libraries and OTU assignment analysis.*
Data source location	*Meiktila, Myanmar (Latitude: 20.8778 Longitude: 95.8584)*
Data accessibility	*Data is within this article and available via http://www.ncbi.nlm.nih.gov/ bioproject/ PRJNA432583*
Related research article	*Please add author names, title and publication details/status of the most relevant research article here, if available*

Table 2-1 - Specifications table with brief description on the experimental design for this publication.

2.1 Value of the data.

- These data are the first microbiome data reported from Myanmar. They provide insights into the microbial dynamics of infected individuals from a small population in a country where there is a high prevalence of TB, drug-resistant TB and TB/HIV co-infection.

- Exploration of these data may contribute further insight into the prevention and treatment of bacterial infections in the respiratory tract of HIV, TB and HIV/TB patients, including innovative use of probiotic

2.2. Data.

16S rRNA profiles of infected adults were compared with those of healthy adults from the same population and small town in Myanmar (Figure 2-1) shows the assignment of sequenced reads to operational taxonomic units and the bacterial community composition of the upper respiratory tract of TB, HIV and HIV/TB patients and healthy individuals.

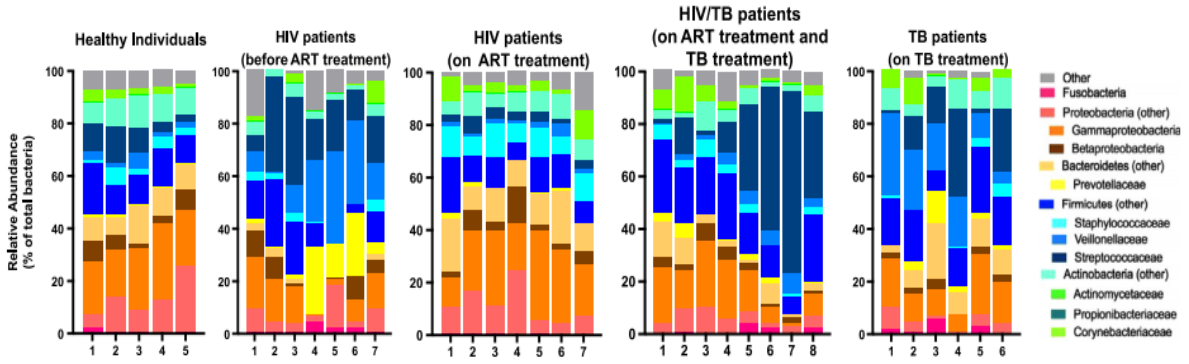

Figure 2-1 - Relative abundance of operational taxonomic units (% OTU) indicating bacterial community composition of the upper respiratory tract of TB, HIV and HIV/TB patients and healthy individuals from Myanmar. The antiretroviral therapy (ART) treatment regimen contained Azidothymidine, Lamivudine and Efavirenz. The TB treatment regimen consisted of Isoniazid, Rifampicin, Streptomycin, Ethambutol and Pyrazinamide.

2.3 Experimental design, materials, and methods.

We collected nasopharyngeal and oropharyngeal swabs from a total of 33 participants (Healthy (5), HIV/TB (8), HIV (14), and TB (6)) living in Meiktila, a small town in Myanmar with a high incidence of HIV and TB. The swabs were collected by trained surveillance officers using internationally accepted Standard Operating Procedures (SOPs) as outlined in the Specimen Collection Guidelines from the CDC [1]. As per the Myanmar national AIDS programme's guidelines for clinical management of HIV Infection in Myanmar, HIV patients are on the antiretroviral (ART) treatment regimen containing azidothymidine, lamivudine and efavirenz and HIV/TB patients are on the ART regimen as well as the TB regimen containing isoniazid, rifampicin, streptomycin, ethambutol, and pyrazinamide [2]. Genomic DNA was extracted less than 24 h post-collection from each swab using the QIAamp DNA Microbiome Kit (Qiagen) as per the manufacturer's instructions. DNAs were then pooled and subjected to 16s rRNA sequencing.

For high-throughput 16s rRNA sequencing, a custom single-step dual-index PCR approach

including the enrichment of the 16S rRNA V3 and V4 hypervariable regions was performed as described previously [3]. The development of each 16s rRNA V3-V4 forward and reverse primers consisted of Illumina adapters 29-nt forward sequence and 24-nt reverse sequence, an 8-nt index sequence, a 10-nt pad sequence, a 2-nt linker, and the gene-specific primer. The amplified products were then purified and normalized using SequelPrep™ normalization plate (ThermoFisher Scientific: Waltham, MA, USA), following the recommendations of the manufacturer. After normalization, 5 ml of each eluate was pooled together and quantified on a Qubit 3.0 Fluorometer (Life Technologies: Carlsbad, CA, USA) and Agilent Bioanalyzer High Sensitivity (HS) chip for visualization of 16S rRNA V3-V4 PCR band (600–650 bp) before dilution to 10 nM and 2 nM libraries. The prepared library was then sequenced as per manufacturer's protocol on a MiSeq for 500 sequencing cycles (2 250 PE) using version 2 chemistry and custom designed sequencing primers (for read 1, read 2 and index read) that targeted the 16S V3V4 region.

Image analysis, base-calling, raw data quality assessment and demultiplexing were processed on the MiSeq instrument via MiSeq Reporter version 2.6. Sequence trimming and analysis was conducted using Trimmomatic (v0.36) with a default parameter phred score of 33 [4]. Next the reads were merged into a single overlapping contig using FLASH, a paired-end assembler tool to improve assessment of bacterial diversity with overlapping reads of 100 bp before being processed via QIIME [5-6]. For microbial profiling based on operational taxonomic unit (OTU) analysis, the UCHIME/UCLUST algorithm with "Greengenes" reference database was used to detect and remove any potential chimeric recombinant sequences from the generated data adjusted to 97% cutoff sequence similarity identity from the taxonomic classification of each read [7-8]. Next, to assess whether microbial communities were significantly different UNIFRAC was used to estimate the overall phylogenetic distance between microbial communities from the generated OTU table [9].

The most abundant reads assigned to OTUs at phylum and family levels in HIV, TB, HIV/TB patients and healthy individuals are shown in Fig 2-1. We observed decreased abundance of bacteria belonging to the family '*Streptococcaceae*' and increased abundance of "*Staphylococcaceae*" in the upper respiratory tract of HIV patients on the ART regimen compared to HIV treatment naïve patients. However, the microbiota profile of HIV patients that are on the ART and co-infected with TB was similar to that of HIV treatment naïve patients except there was a lower abundance of "*Veillonellaceae*" in HIV/TB patients. The

abundance of "*Veillonellaceae*" was higher in the TB patients on the TB treatment compared to the healthy individuals.

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2.5 Transparency document and Supporting Information.

Transparency data associated with this article can be found in the online version at https://doi.org/10.1016/10.1016/j.dib.2018.10.003.

2.6 Supplementary Data.

Additional Information (Custom One-step PCR Amplification)

341 Forward Primer Assembly

P5 Adapter: AATGATACGGCGACCACCGAGATCTACAC
Pad Sequence: TCGTCGGCAG (10nt)
Linker Sequence: AG (2nt)
Barcode: NNNNNNNN (Illumina Nextera XT Barcoding, 8nt)
341 Forward Primer: CCTACGGGNGGCWGCAG

Full Sequence: 5'-
AATGATACGGCGACCACCGAGATCTACACTCGTCGGCAGAGNNNNNNNNCCTAC
GGGNGGCWGCAG-3'

785 Reverse Primer Assembly

P7 Adapter: CAAGCAGAAGACGGCATACGAGAT
Pad Sequence: TCGTCGGCAG (10nt)
Linker Sequence: AG (2nt)
Barcode: NNNNNNNN (Illumina Nextera XT Barcoding, 8nt)
785 Reverse Primer: GACTACHVGGGTATCTAATCC

Full Sequence: 5'-
CAAGCAGAAGACGGCATACGAGATTCGTCGGCAGAGNNNNNNNNGACTACHVG
GGTATCTAATCC-3'

The integration of the 341 Forward and 785 Reverse Primer Assemblies in PCR, targeting the V3V4 region, significantly enhances the study of oral microbes. These assemblies, equipped with a Pad Sequence and a Linker Sequence, improve PCR effectiveness and reduce by-products, while the unique barcode from the Illumina Nextera XT system allows for the simultaneous sequencing of multiple samples. This method streamlines and economizes the process of analyzing oral microbial communities, offering crucial insights into oral health and disease with increased efficiency and cost-effectiveness.

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Chapter 3

Genomic Profiling of Mycobacterium tuberculosis Strains, Myanmar

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Abstract

Multidrug resistance is a major threat to global elimination of tuberculosis (TB). We performed phenotypic drug-susceptibility testing and whole-genome sequencing for 309 isolates from 342 consecutive patients who were given a diagnosis of TB in Yangon, Myanmar, during July 2016–June 2018. We identified isolates by using the GeneXpert platform to evaluate drug-resistance profiles. A total of 191 (62%) of 309 isolates had rifampin resistance; 168 (88%) of these rifampin-resistant isolates were not genomically related, indicating the repeated emergence of resistance in the population, rather than extensive local transmission. We did not detect resistance mutations to new oral drugs, including bedaquiline and pretomanid. The current GeneXpert MTB/RIF system needs to be modified by using the newly launched Xpert MTB/XDR cartridge or line-probe assay. Introducing new oral drugs to replace those currently used in treatment regimens for multidrug-resistant TB will also be useful for treating TB in Myanmar.

Keywords: Myanmar; *Mycobacterium tuberculosis*; antimicrobial resistance; bacteria; expanded drug resistance testing; genomic profiling; new oral regimens; respiratory infections; strains; tuberculosis; tuberculosis and other mycobacteria.

3.1 Introduction.

Tuberculosis (TB) is the infectious disease that causes the most deaths worldwide (≈5,000/day) [1]. Of major concern is the increasing prevalence of drug resistance worldwide [1]. There are different forms of TB drug resistance: pre–multidrug-resistant TB (pre–MDR TB, resistant to 1 of 2 first-line drugs: isoniazid or rifampin); multidrug-resistant TB (MDR TB, resistant to 2 first-line drugs: isoniazid and rifampin); pre–extensively drug-resistant (pre-XDR, resistant to either fluoroquinolones or injectable drugs in addition to MDR); and extensively drug-resistant TB (XDR TB, resistant to fluoroquinolones and injectable drugs in addition to MDR) [1]. An estimated 0.5 million cases of MDR TB were reported in patients worldwide during 2018, but only one third had access to effective treatment, resulting in 56% of patients being successfully treated [1]. In addition, an estimated 6% of diagnosed case of MDR TB cases are actually cases of XDR TB [1].

Myanmar is recognized by the World Health Organization (WHO) as having high burdens of TB (338 cases/100,000 population), MDR TB (21 cases/100,000 population), and co-infections of TB and HIV (29 cases/100,000 population) [1]. A nationwide drug-resistant TB survey was conducted during 2012–2013 by the Myanmar National Tuberculosis Programme (NTP) to identify the drug susceptibility profile for first-line drugs (phenotypic drug susceptibility testing for second-line drugs was established during 2016) [2]. This survey identified MDR TB among 5% of new cases and 27.1% of previously treated cases, and the Yangon region was identified as a hotspot for drug-resistant TB [3]. Having an estimated population of 8 million persons, Yangon is the most populous city in Myanmar. All patients with suspected pulmonary TB are referred to a TB diagnostic center run by the NTP for testing by using the GeneXpert platform (https://www.cepheid.com). Routine, phenotypic drug-susceptibility testing (DST) of first-line or second-line drugs is rarely performed for new patients, and currently testing is based solely on the Xpert MTB/RIF (M. tuberculosis/rifampin) assay. Therefore, clinical decisions reflect the detection of rifampin resistance and national therapeutic guidelines on the basis of WHO recommendations.

Technological advances in next-generation, whole-genome sequencing (WGS) and downstream bioinformatic analyses now enable comprehensive detection of drug resistance and provide an alternative to existing approaches [3-5]. Such sequence-based, drug-resistant profiles have high concordance with phenotypic DST [3,4]. In addition, phylogenetic analyses of sequence data can be used to identify transmission patterns in the absence of epidemiologic

data, which is often lacking in high-burden settings such as Myanmar [3,6]. We combined clinical, genomic, and phenotypic drug-resistance data to provide insights into drug resistance and transmission patterns in Yangon. In this study, we used WGS analyses of 309 M. tuberculosis isolates to determine how the increasing burden of MDR TB has been driven in Yangon.

3.2 Methods.

3.2.1 Study Design and Participants.

This population-based, cross-sectional study included consenting participants >15 years of age who had GeneXpert-confirmed positive pulmonary TB at 3 major NTP TB diagnostic centers (Aung San, Latha, and North Oakkalapa) in Yangon during July 2016–June 2018. We aimed to recruit 250 patients consecutively given a diagnosis of infection with rifampin-resistant (RR) M. tuberculosis and 200 patients infected with rifampin-susceptible (RS) M. tuberculosis. Recruitment numbers at each facility reflected the relative numbers of patients given a diagnosis during the previous year. Patients were eligible to be included in the study if they had lived in Yangon at the time of registration, had a TB-positive confirmation by GeneXpert, and provided written informed consent. Patients were excluded if their residential address was outside Yangon at the time of registration or they did not provide informed consent. We obtained a brief clinical report for each patient (basic demographics, residential address, history of TB treatment, HIV status, and random blood glucose testing results for diabetes mellitus). The Institutional Review Boards of the Department of Medical Research, Ministry of Health and Sports of Myanmar, and the Human Health Ethics Review Committee of the University of Otago (Dunedin, New Zealand) approved this study.

3.2.2 Laboratory Procedures.

We collected all clinical sputum samples at the time of diagnosis and before commencement of treatment. We sent samples to the National Tuberculosis Reference Laboratory in Yangon for DST. Testing for resistance to isoniazid, rifampin, ethambutol, streptomycin, para-aminosalicylic acid, ethionamide, D-cycloserine, fluoroquinolones (ofloxacin, levofloxacin, capreomycin), and aminoglycosides (amikacin, kanamycin) was performed by using the proportion method on Löwenstein–Jensen culture medium (https://apps.who.int/iris/bitstream/handle/10665/83807/WHO_CDS_TB_2001.288_eng.pdf). We determined resistance to new and repurposed drugs (i.e., pyrazinamide, bedaquiline,

pretomanid, delamanid, linezolid, and clofazimine) on the basis of genomic markers know to be associated with resistance [5,7,8]. Clinicians were provided with the WGS and accompanying phenotypic DST data as soon as it was available, and clinical decisions were made entirely at their discretion.

We extracted genomic DNA from cultures of single sputum specimens by using MoBio Microbial DNA Isolation Kits (https://www.qiagen.com) and sequenced DNA by using Illumina MiSeq (https://www.illumina.com) as described [9,10]. All sequencing data from this study were deposited into the National Center for Biotechnology Information Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra; accession no. PRJNA638161).

3.2.3 Analysis.

We performed genomic mapping by using Burrow-Wheeler Aligner-maximum exact matches (version 7.17-r1188; https://bio-bwa.sourceforge.net) and the M. tuberculosis reference genome H37Rv (GenBank accession no. NC_000962.3). Mapping used a custom M. tuberculosis masking browser extensible data file to exclude highly repetitive GC-rich conserved domains. We used SAMtools and BCFtools utilities version 1.9 to call single-nucleotide polymorphisms (SNPs) [11]. M. tuberculosis TB-Profiler version 2.8.2 (https://github.com) was used to predict resistance to 17 drugs on the basis of genotyping of gene targets and classification to phylogenetic lineages by using SNP barcodes [7,8]. Maximum-likelihood phylogenetic analyses were conducted by using RaxML, as implemented in the Gubbins pipeline version 2.3.4 [12]. We used the online platform iTOL version 5.5 for annotation and management of phylogenetic trees [13]. Isolates were considered closely related (genomically linked) if the pairwise distance between them was <12 SNPs [5]. Statistical analyses were performed by using GraphPad Prism version 8.0 (https://www.graphpad.com) and the $\chi 2$ test. A p value <0.05 was considered statistically significant.

3.3 Results.

Over the recruitment period, 342 patients (194 with RR and 148 with RS M. tuberculosis) participated in the study; 33 case-patients were excluded because of laboratory contamination or failed sputum culture and DNA extraction. Of the final 309 GeneXpert-positive included participants, 200 (65%) were male (Table 3.1), 118 were RS and 191 were

RR, and all had phenotypic DST successfully completed. RR was strongly associated with a history of TB treatment (p<0.0001) (Table 3.1).

Characteristic	Resistant, n = 191	Susceptible, n = 118	p value
Sex			
M	120	80	0.39
F	71	38	
Treatment history			
Retreatment	108	31	<0.0001
New	83	87	
District			
North	64	74	<0.0001
South	7	3	
East	75	31	
West	45	10	
Age, y			
10–19	7	4	0.90
20–39	102	59	
40–59	67	44	
>60	15	11	
HIV			
Positive	6	1	0.28
Negative	200	102	
Random blood glucose, mg/dL			
≥200	13	8	0.64
<200	193	95	
Laboratory testing			
Lineage 2	164	37	<0.0001
Other	27	81	

*Xpert MTB/RIF (*M. tuberculosis*/rifampin), Cepheid (https://www.cepheid.com).

Table 3-1 - Characteristics of patients who were infected with rifampin-susceptible and rifampin-resistant *Mycobacterium tuberculosis* strains that were identified by using Xpert MTB/RIF assay, Myanmar*.

We compared the results of the GeneXpert, phenotypic DST, and genomic analyses to further evaluate drug resistance (Figure 3-1). Of 118 cases diagnosed as RS by using GeneXpert, 16 (14%) were identified as isoniazid resistant on the basis of genomic analyses (Figure 3-1); resistance was conferred either by a mutation in the katG gene (S315T; 12 [75%] of 16) or in the promoter region of the inhA gene (c-15t; 4 [25%] of 16) (Table 3-2; Figure 3-1). All 16 cases were phenotypically confirmed as isoniazid resistant (Table 3-2).

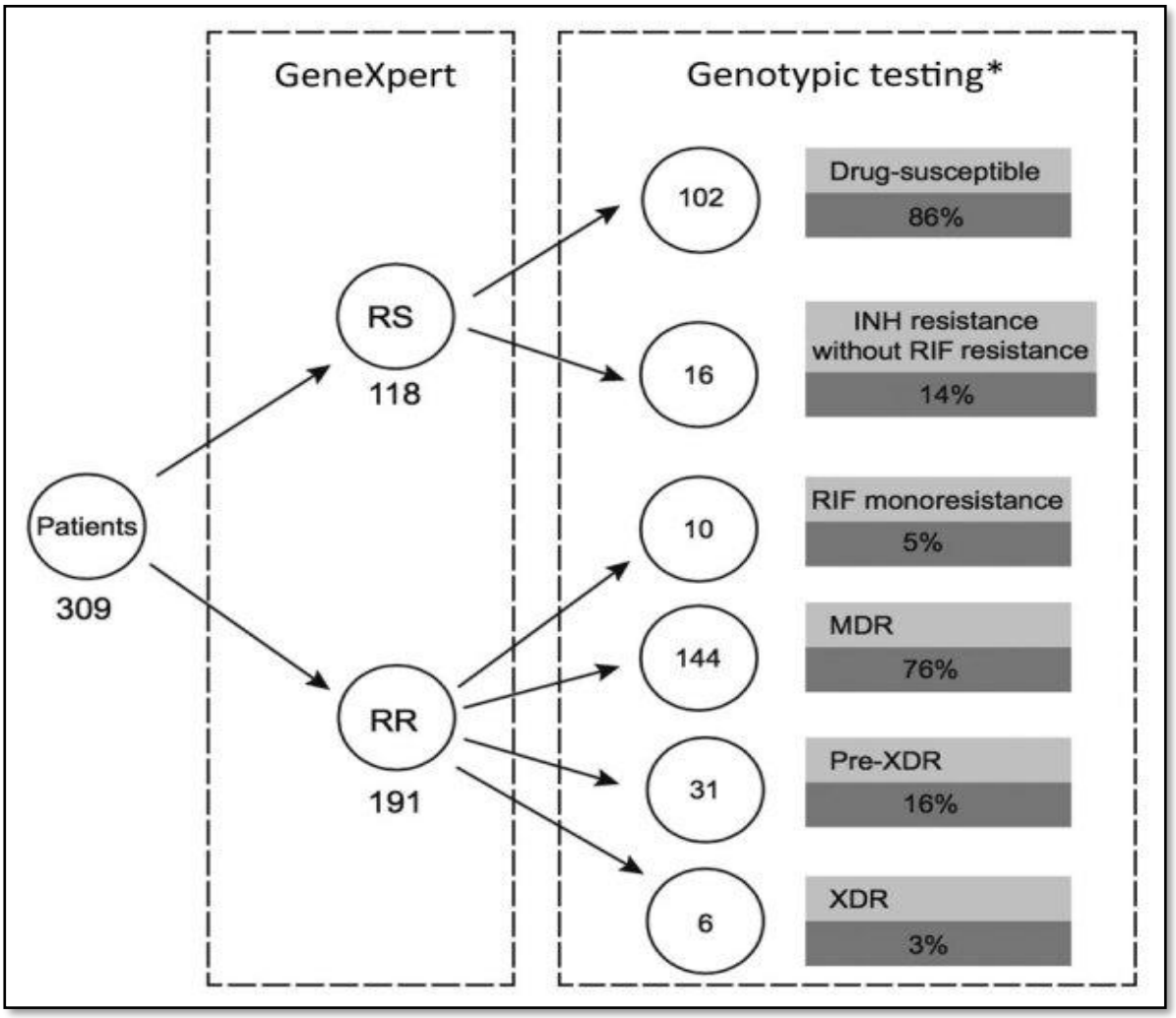

Figure 3-1 - Genomic profiling of *Mycobacterium tuberculosis* strains, Myanmar, comparing discriminatory power offered by GeneXpert (Cepheid, https://www.cepheid.com) and additional genotypic testing, such as line-probe assay or whole-genome sequencing. RIF resistance and sensitivity were determined by using the Xpert MTB/RIF assay (Cepheid). INH, isoniazid; MDR, multidrug resistant; RIF, rifampin; RR, rifampin resistant; RS rifampin sensitive; XDR, extensively drug resistant. *Resistance profile confirmed by phenotypic testing.

	Performance of genome-based† drug resistance profile prediction with respect to phenotypic drug-susceptibility testing					
	Mutation		No mutation			
Drug	Sensitive	Resistant	Sensitive	Resistant	Sensitivity, %	Specificity, %
Isonizaid	0	196	113	0	100.0	100.0
Rifampin	0	191	118	0	100.0	100.0
Ethambutol	34	80	155	40	70.2	79.5
Streptomycin	26	140	80	63	84.3	55.9
Ofloxacin/levofloxacin/capreomycin	13	31	263	2	70.4	99.2
Amikacin/kanamycin	0	6	303	0	100.0	100.0
Para-aminosalicylic acid	15	0	294	0	NA	100.0
Ethionamide	4	28	274	3	87.5	98.9
D-cycloserine	0	0	309	0	NA	100.0

*NA, not applicable. †Whole-genome sequencing–based prediction.

Table 3-2 - Comparison of phenotypic drug susceptibility testing and genomic resistance mutation results for *Mycobacterium tuberculosis* strains, Myanmar*.

All 191 RR isolates identified by GeneXpert were phenotypically resistant; the S450L mutation in the rpoB gene was the dominant mutation (137 [72%] of 191 (Table 3-2; Figure 3-2). WGS further identified that 10 (5%) were only rifampin resistant (pre-MDR), 144 (75%) were MDR, 31 (16%) were pre-XDR, and 6 (3%) were XDR; results were confirmed by phenotypic DST (Figure 3-1). All pre-XDR isolates harbored mutations in the gyrA gene, and D94G was most prevalent (12 [39%] of 31), followed by A90V (8 [26%] of 31) (Table 3-2; Figure 3-2). Resistance to aminoglycoside injectable drugs (XDR) was predominantly associated with rrs A1401G (4 [6 [67%] of 6) and G1484T (2 [33%] of 6) mutations. Mutations in the embB gene (M306V; 42 [53%] of 80), the M306I mutant (34 [43%] of 80), and mutations in the rpsl gene (K43R; 126 [90%] of 140) were present in all ethambutol-resistant and streptomycin-resistant isolates, resulting in a sensitivity of 70.2% and a specificity of 79.5% for ethambutol and a sensitivity of 84.3% and a specificity of 55.9% for streptomycin (Table 3-2; Figure 3-2). Known mutations conferring resistance to new and repurposed drugs, such as bedaquiline, delamanid, pretomanid, linezolid and clofizamine, were not identified by WGS in the 207 drug-resistant isolates (26 pre-MDR, 144 MDR, 31 pre-XDR, 6 XDR).

Using specific SNP barcodes, we classified the M. tuberculosis isolates as either lineage 1 (73, 24%), lineage 2 (201, 65%), lineage 3 (16, 5%) or lineage 4 (19, 6%) (Figure 3-3; Appendix Table 1). Most isolates were identified as belonging to sublineage 2.2.1, Beijing

strain (Appendix Table 1). Isolates linked to TB lineage 2 were more commonly drug resistant than those belonging to other lineages (175 [85%] of 207 vs. 32 [15%] of 207; p<0.0001). In contrast, the other lineages were more commonly associated with drug susceptibility (76 [75%] of 102 vs. 26 [25%] of 102; p<0.001) (Table 3-1; Appendix Table 2). Drug-resistant isolates were also more commonly found in the East and West districts of Yangon (124 [60%] of 207; p<0.0001) (Table 3-1; Appendix Table 2) and to be associated with patients who had previously received treatment (112 [54%] of 207; p<0.0001) (Appendix Table 2). A total of 181 [87%] of 207 isolates were genomically unlinked on the basis of a standard pairwise distance threshold. The remaining 26 (13%) of 207 drug-resistant isolates formed 9 potential transmission chains (Figure 3-4).

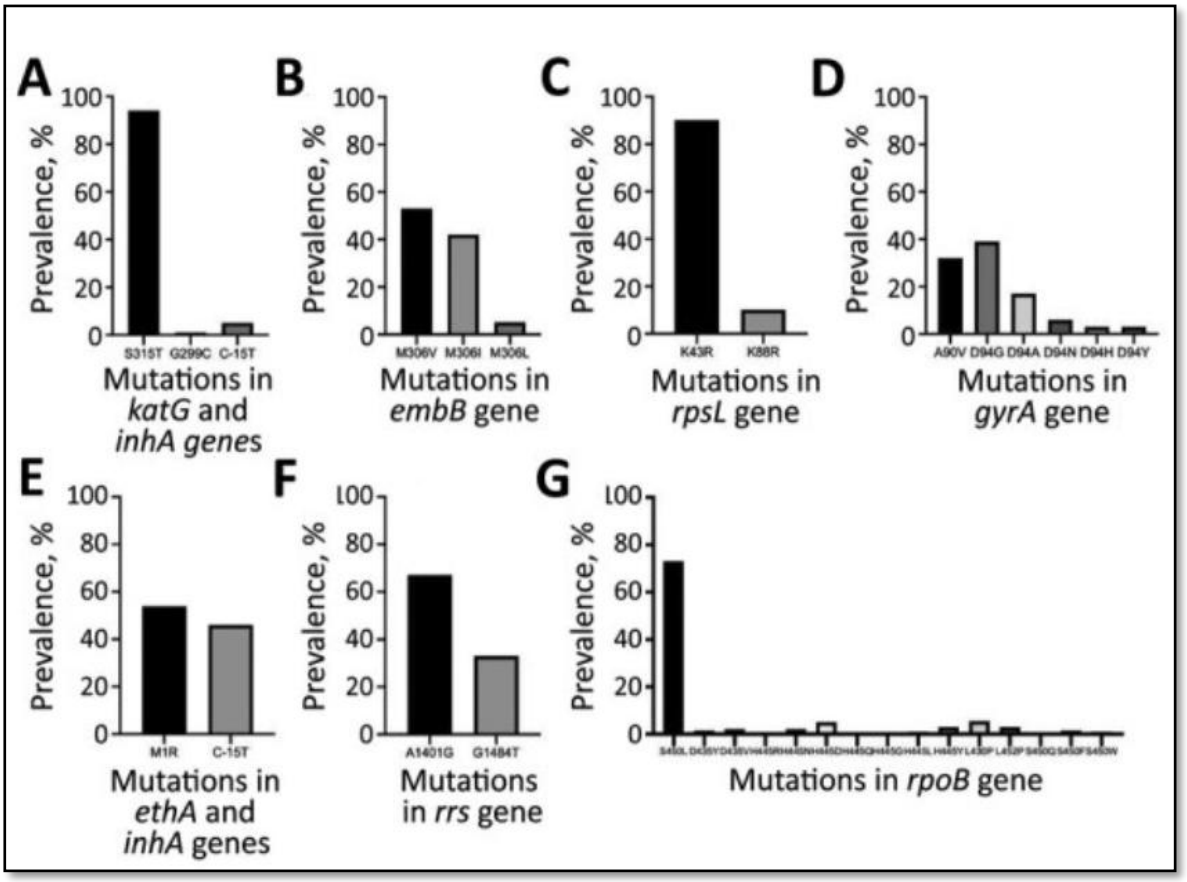

Figure 3-2 - Prevalence of resistance-conferring mutations in genes of phenotypically resistant isolates of *Mycobacterium tuberculosis* strains, Myanmar. A) katG and inhA; B) embB; C) rpsL; D) gyrA; E) ethA and inhA; F) rrs; G) rpoB.

Cases within most of these groups were located within the same districts (Figure 3-4), and each group contained a combination of new and previously treated TB patients. In 6 groups, all isolates had the same resistance profile; the remaining 3 (i.e., groups 5, 6, and 8) groups,

had different resistance profiles. In group 6, an XDR isolate appears to have developed from an isoniazid resistant (pre-MDR) isolate (Figure 3-4).

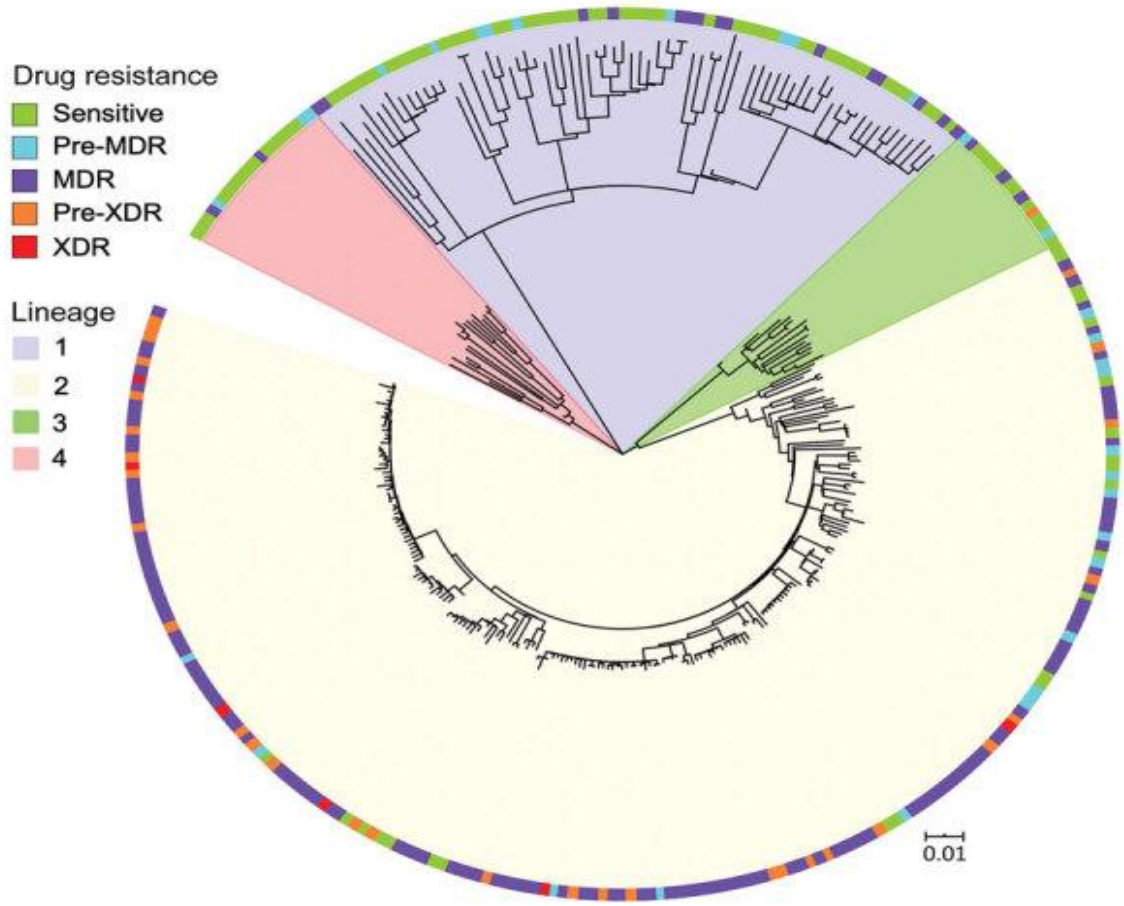

Figure 3-3 - Maximum-likelihood tree based on whole-genome analysis of 309 *Mycobacterium tuberculosis* strains from Myanmar. Lineages and drug resistance status of isolates are shown. MDR indicates multidrug-resistant to 2 first-line drugs (isoniazid and rifampin); pre-MDR, resistant to 1 of 2 first-line drugs (isoniazid or rifampicin); pre-XDR, resistant to fluoroquinolones or injectable drugs in addition to MDR; XDR, resistant to fluoroquinolones and injectable drugs, in addition to MDR. Scale bar indicates nucleotide substitutions per site. MDR, multidrug resistant; XDR, extensively drug resistant.

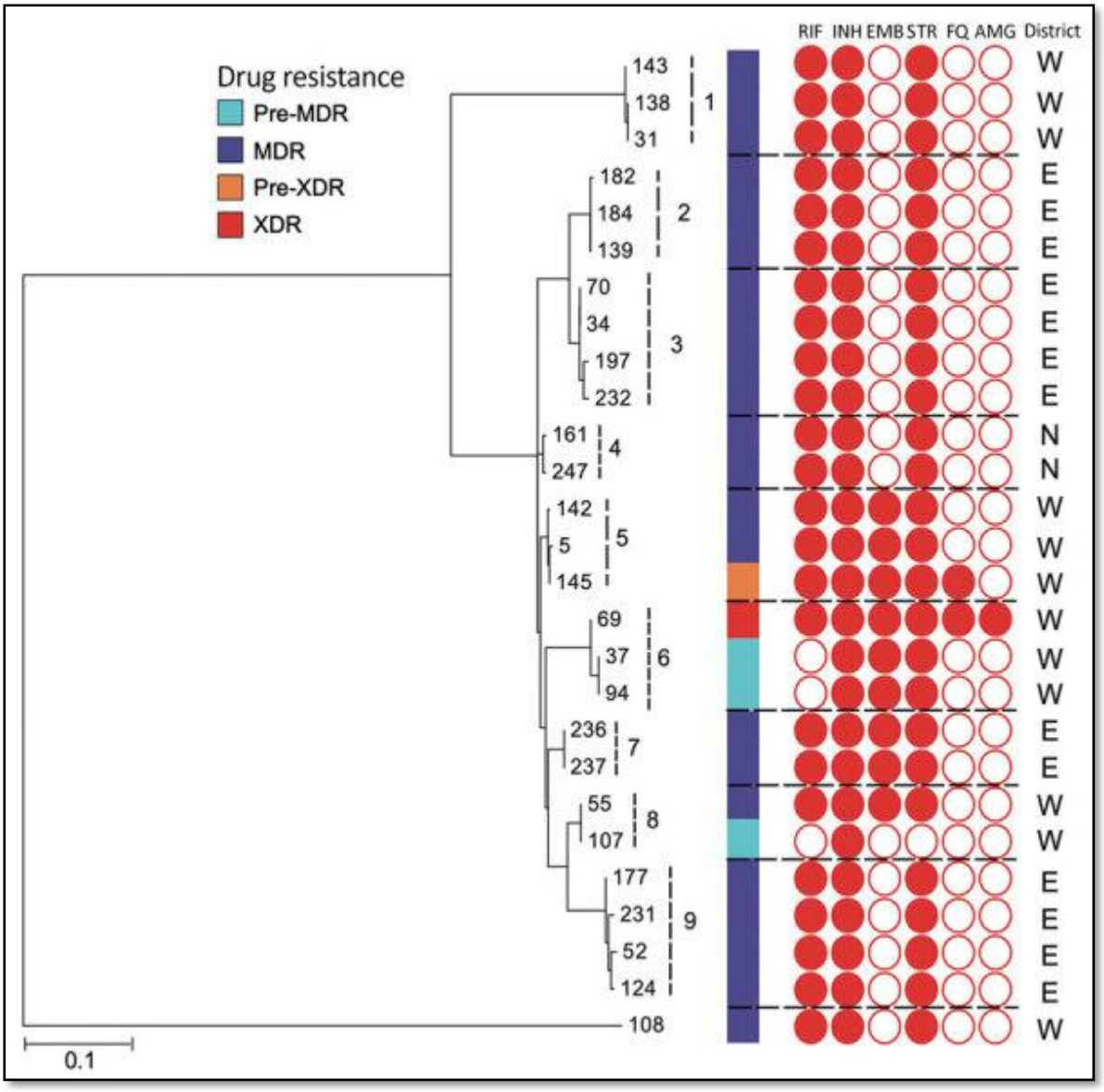

Figure 3-4 - Maximum-likelihood tree of *Mycobacterium tuberculosis* strains, Myanmar, within 9 clusters and their drug resistance profiles. Dotted lines indicate boundaries of individual clusters. An outgroup (#108) differs by >100 single-nucleotide polymorphisms from the strains within 9 clusters. N, E, and W indicate the North, East, and West Districts of Yangon, respectively. MDR, resistant to 2 first-line drugs (isoniazid and rifampin); pre-MDR, resistant to 1 of 2 first-line drugs (isoniazid or rifampicin); pre-XDR, resistant to fluoroquinolones or injectable drugs in addition to MDR; XDR, resistant to fluoroquinolones and injectable drugs, in addition to MDR. AMG, aminoglycosides; ETH, ethambutol; FQ, fluoroquinolones; INH, isoniazid; RIF, rifampin; STR, streptomycin. Scale bar indicates nucleotide substitutions per site.

3.4 Discussion.

This WGS study from Myanmar provides new insights into the landscape of drug-resistant TB in the country's largest city. A large proportion of isolates with high-level drug resistance, including pre-XDR and XDR, were identified. However, there was no resistance to new and repurposed drugs, such as bedaquiline, pretomanid, delamanid, and linezolid. Most drug-resistant cases were associated with previous treatment, and few were clearly associated with community transmission. These findings suggest an additional diagnostic tool, such as the Xpert MTB/XDR cartridge or line-probe assay (LPA), in addition to Xpert MTB/RIF, and new oral regimens, including bedaquiline and pretomanind, are needed for effective surveillance and treatment/management of MDR TB in Mynamar. Further studies are also required to investigate apparent cases of independent emergence and community transmission of MDR TB in Yangon.

Consistent with previous reports on lineage 2 from neighboring countries, this study identified a strong association between lineage 2 M. tuberculosis and drug resistance [14–17]. There was strong agreement between WGS (presence of resistance-conferring mutations) and phenotypic DST to isoniazid and rifampin in this study [18]. These findings indicate quality assurance in the TB laboratory diagnostic service provided by the Myanmar National Tuberculosis Reference Laboratory.

The Xpert MTB/RIF assay has been effective in the simultaneous detection of TB and resistance to rifampin. Because it can provide a diagnosis for a patient within 2 hours, GeneXpert is critical in TB control in high-burden settings. One of the limitations of current cartridges for Xpert is that resistance to isoniazid is assumed when rifampin resistance is detected. This approach captures a large portion of drug-resistant TB cases during diagnosis. However, for a few case-patients, which includes patients who have isoniazid resistance without concurrent rifampin resistance (14% in this study and 9.4% in the recently reported multicountry study), treatment with a first-line regimen can contribute to the emergence of further drug resistance [19].

We previously reported that a patient with undiagnosed isoniazid resistance without concurrent rifampin resistance received a first-line treatment regimen that resulted in development of MDR TB [20]. This finding highlights the limitations and real-world consequences of basing treatment decisions solely on results of the GeneXpert MTB/RIF

system in a high-burden setting, where hundreds of cases are reported daily. This limitation is a serious impediment to controlling the spread of more extensive drug resistance [21]. For example, although Xpert MTB/RIF can correctly diagnose RR MDR cases, it cannot detect pre-XDR and XDR cases. As identified in this study, 20% of rifampin-resistant cases identified by GeneXpert were pre-XDR (17%) and XDR (3%) cases, suggesting that ≈1 of 5 patients received limited treatment on the basis of treatment guidelines at the time of the study.

Most case-patients (including pre-XDR and XDR patients) in this study had drug-resistant isolates that were not closely related (genomically unlinked), which is suggestive of independent emergence of drug resistance because of limited diagnosis or treatment, as well as patient noncompliance. This finding is in contrast to previous studies from other high burden settings, such as China and South Africa, which showed a high proportion of drug-resistant cases that were genomically linked, suggesting community transmission [15, 21–23].

Although it is possible that we simply did not have a high enough sampling fraction of all drug-resistant cases in the population under study, a high number of unclustered drug-resistant cases could be caused by differences in population density; the North, East, and West sections of Yangoon are in an urban industrial setting. These districts have a considerable factory-based workforce and thus draw in highly mobile migrant populations (internal migration), including members from neighboring states and regions, for employment [24]. This finding enables a continuous flow of persons from outside Yangon, which could be independently introducing infections into the region. In addition, their status as migrants means they might have limited access to healthcare services, which is a barrier to rapid diagnosis and appropriate treatment for TB, underscoring the effect of migration on the TB burden in cities in Myanmar, particularly Yangon [20, 25].

In addition to internal migration, cross-border migration has occurred in recent years, such as ≈6 million persons from fellow Greater Mekong Subregion (GMS) countries Cambodia, Laos, Thailand, and Vietnam. Therefore, the Myanmar NTP is collaborating with nongovernmental organizations and NTPs from other GMS countries to reduce the TB burden among Myanmar migrants. Further WGS studies outside Yangon and along these GMS borders are required to provide an insight into the transmission patterns of MDR TB in migrants. Coupling this collaboration with TB-related health education and increase access to care could ultimately reduce the TB burden among migrants.

Further studies are also required to clarify the limitations and roles of both public and private healthcare providers in current treatment pathways for TB in Yangon, which might be contributing to the high rates of MDR TB. In our study, WGS showed a chain of infection, leading to the progression of pre-MDR cases toward XDR and subsequent transmission events, highlighting the need for effective diagnosis. This finding has implications for public health policies and also shows the need for local data to drive effective intervention.

Our study has major implications for clinical practice in Myanmar. First, effective treatment for MDR TB cases requires identification of the high proportion of pre-XDR and XDR TB, which cannot be achieved by current Xpert MTB/RIF testing [27, 28]. The drug resistance–conferring mutations reported in this study can be detected by first-line and second-line LPA, such as GenoType MTBDRplus and MTBDRsl (Hain Lifescience GmbH, https://www.hain-lifescience.de), or the recently launched Xpert MTB/XDR [28, 29]. These platforms can provide clinicians with an expanded drug-susceptibility report without the need for culturing and WGS. Recently, the Myanmar National Tuberculosis Programme diagnostic algorithm has been updated to extend first-line LPA for patients with a history of previous treatment. Second, several new or repurposed drugs (i.e., bedaquiline, delamanid linezolid, and pretomanid) are drugs already available in Myanmar. The apparent absence of preexisting mutations that confer resistance to these drugs justifies their introduction into treatment regimens for drug-resistant TB in Myanmar, as per WHO recommendations [30–32].

Our study has limitations that could lead to overestimation and underestimation of the true magnitude of the MDR epidemic and might not reflect the national situation. First, a large cohort of MDR TB, pre-XDR TB, and XDR TB cases was identified. The study region in Yangon is known to be a high-burden setting compared with other regions of Myanmar, accounting for ≈50% of all national cases [26]. The 3 diagnostic centers in this study are also the major drug-resistant TB treatment centers in Yangon. Therefore, it is likely that the landscape of infections is not representative of all Myanmar. Another limitation is that the study timeframe and size make it unlikely that we captured a full spectrum of MDR TB strains in the population and, as noted earlier, we might have missed identification of some transmission links, thus overestimating the proportion of resistant isolates that are independent.

Our study has major implications for clinical practice in Myanmar. First, effective treatment for MDR TB cases requires identification of the high proportion of pre-XDR and XDR TB, which cannot be achieved by current Xpert MTB/RIF testing [27, 28]. The drug resistance–conferring mutations reported in this study can be detected by first-line and second-line LPA, such as GenoType MTBDRplus and MTBDRsl (Hain Lifescience GmbH, https://www.hain-lifescience.de), or the recently launched Xpert MTB/XDR [28, 29]. These platforms can provide clinicians with an expanded drug-susceptibility report without the need for culturing and WGS. Recently, the Myanmar National Tuberculosis Programme diagnostic algorithm has been updated to extend first-line LPA for patients with a history of previous treatment. Second, several new or repurposed drugs (i.e., bedaquiline, delamanid linezolid, and pretomanid) are drugs already available in Myanmar. The apparent absence of preexisting mutations that confer resistance to these drugs justifies their introduction into treatment regimens for drug-resistant TB in Myanmar, as per WHO recommendations [30-32].

Our study is useful for public health officials in designing interventions for an evidence-based approach for early detection of cases (active case finding) with optimized diagnosis and treatment. Introducing additional diagnostic methods, such as routine LPA or Xpert MTB/XDR in tandem with Xpert MTB/RIF, and treatment regimens with new oral drugs would further assist in controlling and containing MDR TB in Myanmar. In addition, this study underscores the need for local data, rather than being based on general information from similar studies that have different healthcare delivery systems to drive public health policies for effective intervention.

3.5 Acknowledgments.

We thank Aung San, Latha, North Oakkalapa, and the National Tuberculosis Reference Laboratory in Yangon for providing assistance. This study was supported by the New Zealand Health Research Council through the e-ASIA funding scheme. H.L.A. is a Health Research Council Sir Charles Hercus Health Research Fellow.

3.6 Biography.

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3.7 Supplementary Data.

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Genomic Profiling of *Mycobacterium tuberculosis* Strains, Myanmar

Appendix

Appendix Table 1. Frequency distribution of major and sublineages of *Mycobacterium tuberculosis* isolates, Myamnar

Major lineage	Sublineage	No. isolates	Total, no. (%)
Lineage 1	1.1.1	7	73 (24)
	1.1.2	21	
	1.1.3	31	
	1.2.1	10	
	1.2.2	4	
Lineage 2	2.2.1	195	201 (65)
	2.2.2	6	
Lineage 3	3	8	16 (5)
	3.1.2	8	
Lineage 4	4.1	8	19 (6)
	4.3	1	
	4.4	1	
	4.5	5	
	4.7	1	
	4.8	3	
Total			309 (100)

Appendix Table 2. Sociodemographic characteristics of patients in this study and phenotypic DST results, Myamnar*

Characteristic	Sensitive, n = 102	Drug resistance, n = 207				p value
		Pre-MDR, n = 26	MDR, n = 144	Pre-XDR, n = 31	XDR, n = 6	
Sex						
M	69	19	91	15	6	0.08
F	33	7	53	16	0	
Treatment history						
Retreatment	27	9	80	19	4	<0.0001
New	75	17	64	12	2	
District						
North	63	16	52	6	1	<0.0001
East	28	6	58	13	1	
South	2	2	5	0	1	
West	9	2	29	12	3	
Lineage						
Lineage 1	51	7	15	0	0	<0.0001
Lineage 2	26	15	124	30	6	
Lineage 3	11	1	3	1	0	
Lineage 4	14	3	2	0	0	
Genomically linked						
Clustered	8	3	21	1	1	
Unclustered	94	23	123	30	5	
Age, y						
10–19	3	1	5	1	1	0.7
20–39	52	12	73	20	4	
40–59	37	12	54	7	1	
>60	10	1	12	3	0	

*pre-MDR, pre–multidrug-resistant (resistant to 1 of 2 first-line drugs: isoniazid [INH] or rifampin [RIF]); MDE, multidrug-resistant (resistant to 2 first-line drugs: INZ and RIF); pre-XDR, pre-extensively drug-resistant (resistant to either fluoroquinolones or injectable drugs in addition to MDR); XDR, extensively drug-resistant (resistant to fluoroquinolones and injectable drugs in addition to MDR).

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Chapter 4

Enhanced Drug Resistance Detection in Myanmar: Comprehensive Characterization of Isoniazid-Monoresistant Tuberculosis Through Integrated Genomic and Phenotypic Analysis

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DRC 16

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Enhanced Drug Resistance Detection in Myanmar: Comprehensive Characterization of Isoniazid-Monoresistant Tuberculosis Through Integrated Genomic and Phenotypic Analysis

Highlights

- Isoniazid-monoresistant tuberculosis (Hr-TB) is a significant and common form of drug-resistant TB in Yangon, Myanmar.
- The recent and rapid clonal expansion of the *Mycobacterium Tuberculosis* (MTB) L2-Beijing lineage indicates a strong epidemiological link to the increased prevalence of Hr-TB in this region.
- Specific mutations have been identified, providing valuable markers for the development of future rapid diagnostic tools.
- The integration of phenotypic and genotypic drug susceptibility testing (DST) is essential for effective TB management.

Background: Building upon our previous genomic profiling work [1], this study addresses a critical gap in understanding isoniazid-monoresistant tuberculosis (Hr-TB) in Myanmar. While our earlier research identified the predominance of rifampicin-resistant strains and the Beijing lineage, Hr-TB patterns remained underexplored. This study aims to characterize Hr-TB through comprehensive drug susceptibility profiling and resistance mechanism analysis.

Methods: We conducted a cross-sectional study analyzing 285 tuberculosis clinical isolates from Myanmar (2017-2019) using both phenotypic (pDST) and genotypic (gDST) drug susceptibility testing, including minor variant analysis of samples with low mycobacterial burden.

Results: Hr-TB was identified in 6.32% of isolates, with 55.6% showing high-level isoniazid resistance and additional resistance to ethambutol, levofloxacin, and pyrazinamide. Key resistance mutations included katG S315T and fabG1 c-15t for isoniazid resistance. The L2-Beijing lineage predominated, consistent with our previous findings.

Conclusion: The prevalence of Hr-TB with co-resistance patterns necessitates integrated pDST and gDST approaches for comprehensive TB management in Myanmar. These findings expand upon our previous genomic profiling work and provide crucial insights for diagnostic and treatment strategies.

Keywords: tuberculosis, drug-resistance, isoniazid mono-resistant tuberculosis, next-generation sequencing, rapid diagnosis, whole genome sequencing, targeted sequencing, resistance mechanisms, mixed infection.

4.1 Introduction.

Tuberculosis (TB) remains a leading cause of global mortality, with the World Health Organization (WHO) estimating over 10 million TB cases annually [2]. The escalating challenge of antimicrobial resistance (AMR) in *Mycobacterium tuberculosis* (MTB), particularly against frontline treatments like rifampicin and isoniazid, poses significant obstacles to global TB elimination efforts [3]. Our previous genomic profiling study in Myanmar revealed extensive drug resistance patterns and the predominance of the Beijing lineage, establishing a foundation for understanding TB resistance mechanisms in this region [1].

Building upon these foundational findings, isoniazid-monoresistant TB (Hr-TB) represents a particularly prevalent and challenging form of drug resistance [4]. Recent systematic reviews indicate that Hr-TB affects approximately 1.4 million cases globally [4], with 79% classified as Hr-TB alone [5]. The clinical significance of Hr-TB extends beyond single-drug resistance, as these strains frequently develop polyresistance to other first-line and second-line drugs [6,7]. Contemporary research demonstrates that Hr-TB treatment requires careful consideration [8] of resistance patterns to optimize outcomes [8].

The diagnostic landscape for drug-resistant TB has evolved significantly with advances in molecular diagnostics [9]. Next-generation sequencing approaches now enable comprehensive resistance profiling directly from clinical samples [10,11]. Recent developments in targeted sequencing have improved the detection of drug-resistant TB, particularly for culture-free diagnostics [12]. These technological advances complement traditional phenotypic testing methods, offering rapid and accurate resistance characterization [13]. Current WHO recommendations for Hr-TB treatment emphasize the importance of tailored regimens that address specific resistance patterns [14]. The integration of genomic and phenotypic approaches has become essential for effective TB management, particularly in high-burden settings [15]. Recent clinical trials have demonstrated the efficacy of shorter, all-oral regimens [5] for certain drug-resistant TB cases [16], highlighting the importance of precise resistance characterization.

Our previous investigation by Aung et al. identified repeated emergence of rifampicin resistance through acquired mutations rather than extensive local transmission in Myanmar [1]. However, Hr-TB patterns and minor variant analysis were not extensively characterized in that study. This investigation addresses this gap by employing sensitive analytical approaches, including analysis of samples with low mycobacterial DNA concentration and quality, to

provide comprehensive Hr-TB characterization. The economic burden of drug-resistant TB remains substantial globally [6], necessitating cost-effective diagnostic and treatment strategies. Recent evidence suggests that rapid molecular diagnostics can significantly improve patient outcomes and reduce transmission [17]. The burden of TB among vulnerable populations continues to pose significant challenges [15], requiring targeted public health interventions.

This study aims to expand upon our previous genomic profiling work by characterizing Hr-TB isolates from Myanmar through comprehensive drug susceptibility testing and resistance mechanism analysis. We utilize an integrated approach combining phenotypic and genotypic methods to enhance rapid diagnosis and inform treatment strategies, contributing to improved TB management in Myanmar and similar high-burden settings.

4.2 Methods.

4.2.1 Setting, Study Design and Participants.

This retrospective, cross-sectional study, conducted between 2017 and 2019, focused on the genomic classification and analysis of DR-TB cases from three major National TB Program (NTP) diagnostic centers in Yangon, Myanmar (Aung San, Latha, and North Oakkalapa). We utilized 285 pulmonary TB isolates, previously identified as MDR-TB using the GeneXpert MTB/RIF cartridge from our previous study [1]. These samples were further cultured using the Mycobacterium Growth Indicator Tube (MGIT) and Löwenstein-Jensen (LJ) systems.

Comprehensive patient metadata was collected, including demographics, residential addresses, TB treatment history, HIV status, and random blood glucose levels for diabetes mellitus. Patients were excluded if their residential address was outside Yangon or if they did not provide informed consent. Positive TB identifications via pulmonary chest X-ray were referred to the National TB Reference Laboratory under the Myanmar NTP, where cases were confirmed using the GeneXpert MTB/RIF cartridge from sputum samples.

Upon detection of rifampicin resistance with GeneXpert, routine phenotypic drug susceptibility testing (pDST) for first-line antimicrobials (rifampicin, isoniazid, pyrazinamide, streptomycin, and ethambutol) was conducted. Resistance patterns were defined per updated WHO classifications [18], utilizing in silico identification of resistance mutations. Mono-isoniazid resistant TB (Hr-TB) was confirmed through genotypic DST (gDST) via whole-genome sequencing with next-generation sequencing data.

This study was approved by the Institutional Review Boards of the Department of Medical Research, Ministry of Health and Sports of Myanmar, and the Human Health Ethics Review Committee of the University of Otago, Dunedin, New Zealand.

4.2.2 Laboratory Procedures, DST and Whole Genome Sequencing.

Clinical sputum samples were collected at the time of diagnosis and before treatment commencement. Upon receipt, samples were decontaminated and heat-inactivated at 80°C for 20 minutes to ensure deactivation of TB cells for safe handling and to facilitate downstream processing. Subsequently, deactivated samples were sent to the National Tuberculosis Reference Laboratory in Yangon for comprehensive drug susceptibility testing (DST).

DST for first-line anti-TB drugs, including isoniazid, rifampicin, ethambutol, and streptomycin, as well as second-line drugs such as para-aminosalicylic acid, ethionamide, D-cycloserine, fluoroquinolones (ofloxacin, levofloxacin, capreomycin), and aminoglycosides (amikacin, kanamycin), was performed using the proportion method on LJ and MGIT medium. MGIT medium was prepared for WGS analysis to provide rapid and accurate results on isolate susceptibility to various anti-TB drugs. Additionally, resistance to new and repurposed drugs, including pyrazinamide, bedaquiline, pretomanid, delamanid, linezolid, and clofazimine, was determined based on genomic markers associated with resistance [19].

For the WGS workflow, genomic DNA was extracted from MGIT cultures of single sputum specimens less than one-week old using MoBio Microbial DNA Isolation Kits, following the manufacturer's protocol. DNA sequencing was performed using a modified Nextera-XT DNA Library Preparation Protocol optimized for MTB genomic DNA extracts. Libraries were sequenced on an Illumina MiSeq platform, following the manufacturer's protocol to generate high-quality genomic data for subsequent analysis.

4.2.3 Sequencing Reads – Quality Control.

FastQC (version 0.12.1) was used to assess initial quality of raw sequencing reads. Trimmomatic (version 0.36) was employed to remove Illumina sequencing primers and adapters, applying a base quality cutoff of Q20 (Phred score) to filter out low-quality bases. This step specifically targeted removal of Illumina Nextera-XT PE clip adapters (CTGTCTCTTATACACATCT). After adapter trimming, a second quality control check was conducted with FastQC to confirm improved read quality. MultiQC was used to compile quality control reports into a comprehensive, standardized format.

4.2.4 Drug-resistance mutation and variant calling.

Processed reads were aligned to the MTB H37Rv reference genome using Bowtie2 (version 2.5.4) with maximum sensitivity options (–very-sensitive). This included analyzing samples with low mycobacterial burden, characterized by low (<20x) coverage and high (>50%) levels of contamination. Contamination check was performed to remove non-MTB sequences including human DNA sequences. A custom MTB H37Rv masking Browser Extensible Data (BED) file was incorporated to exclude highly repetitive, GC-rich conserved domains, and proline-rich regions to prevent erroneous alignments. Variant calling was performed using established protocols for MTB genomic analysis, with particular attention to minor variants that might be missed in standard analysis pipelines. Drug resistance predictions were made using the latest WHO drug resistance catalogue [18], incorporating recent advances in genomic diagnostics [23,24].

4.2.5 Phylogenetics and Statistical Analysis.

The final step in our analysis involved constructing phylogenetic trees to infer the evolutionary relationships among the samples. High-quality variants identified through the GATK genomic analysis were used to generate multiple sequence alignments (MSA) from each isolate's lineage consensus file, which were then combined in FASTA format for phylogenetic analysis. The resulting MSA was processed using MAFFT (version 7.490) for alignment, curated with the Noisy model (version 1.5.12) to filter out unreliable positions, and inferred via PhyML (version 3.3) under a maximum likelihood framework utilizing the GTR-G4 model. This approach included 1,000 bootstrap iterations to provide confidence scores for the generated phylogenetic tree. The online platform iTOL (version 6.9.1) was employed for the annotation, management, and visualization of the phylogenetic trees, allowing for detailed representation of evolutionary relationships and associated drug-resistance profiles. Isolates were considered closely related, or genomically linked, if the pairwise distance between them was fewer than 12 SNPs. This approach was adopted to ensure robust inference of phylogenetic relationships, which is essential for investigating the evolutionary dynamics and resistance mechanisms of the MTB strains studied.

Association analyses were performed using RStudio (version 4.1.0) to evaluate the relationship between isolates and drug resistance, as well as their evolutionary relationships. The χ^2 test was employed to determine statistical significance, with a p-value of less than 0.05 considered significant in assessing associations between each patient and their drug resistance profiles. Additionally, logistic regression models were utilized to assess the association between genetic

variants and drug resistance phenotypes, adjusting for potential confounders. Graphical representations and visualizations of the data were generated using the ggplot2 package (version 3.3.3) in RStudio. Complementary statistical calculations were performed using Microsoft Excel to ensure robustness and validation of results. Excel datasets were imported into RStudio using readxl and openxlsx, facilitating seamless integration and comprehensive data analysis. This multifaceted approach provided insights into the genetic and phenotypic relationships influencing drug resistance in MTB isolates.

4.3 Results.

4.3.1 Hr-TB Prevalence and Resistance Patterns

The present study conducted a comprehensive re-analysis of 285 *Mycobacterium tuberculosis* (MTB) clinical isolates collected between 2016 and 2019 (Figure 4-1), revealing critical insights into the prevalence and characteristics of drug-resistant tuberculosis TB in Yangon, Myanmar. These findings expand upon the foundational work by Aung et al. (2021), offering a more detailed understanding of the evolving drug resistance patterns in this region.

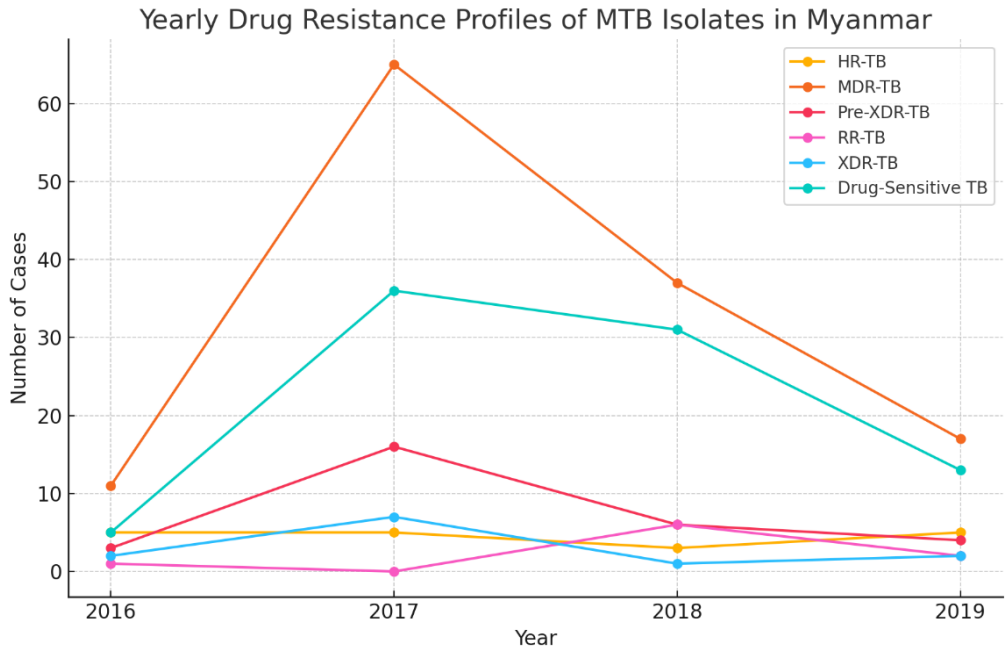


Figure. 4-1 – The number of cases of Hr-TB, MDR-TB, Pre-XDR-TB, RR-TB, XDR-TB, and drug-sensitive TB in Yangon, Myanmar from 2016 to 2019 are shown. Highlighted is the fluctuation in yearly drug resistance profiles of MTB isolates.

Comparison of the drug-resistant profiles in Figure 4-1 reveals significant annual variations in the prevalence and characteristics of drug-resistant TB in Yangon, Myanmar. In 2016, among the 285 MTB clinical isolates, drug-resistant TB, comprising MDR-TB, Hr-TB, pre-XDR-TB, RR-TB, and XDR-TB, accounted for 82 cases, representing 28.8% of the total (Figure 4-1).

The remaining 203 cases were drug-sensitive TB, accounting for 71.2% of the total. In 2017, there was a notable increase in drug-resistant TB, with 144 cases (50.5% of the total) (Figure 1). Drug-sensitive TB cases decreased to 141, representing 49.5% of the total. The year 2018 saw a decrease in drug-resistant TB, with 66 cases (23.0% of the total) (Figure 4-1). Drug-sensitive TB cases increased to 219, accounting for 77.0% of the total. In 2019, drug-resistant TB cases increased again to 98 (34.6% of the total) (Figure 4-1). Drug-sensitive TB cases decreased to 187, representing 65.4% of the total. Among the isolates, 18 (6.32%) were resistant to isoniazid only (Hr-TB) (Figure 4-2), with a significant majority (100%) of these cases being newly diagnosed since the study by Aung et al. (2021). Over the study period, MDR-TB emerged in Yangon, Myanmar as the most prevalent form of resistance, accounting for 44.2% of the cases (Figure 4-2). This was followed by pre-extensively drug-resistant TB (pre-XDR-TB) at 14.5%, and rifampicin-resistant TB (RR-TB) at 5.3% (Figure 4-2). Additionally, extensively drug-resistant TB (XDR-TB) was observed in 6.1% of the isolates, indicating a concerning level of resistance that poses significant challenges for treatment and control (Figure 4-2).

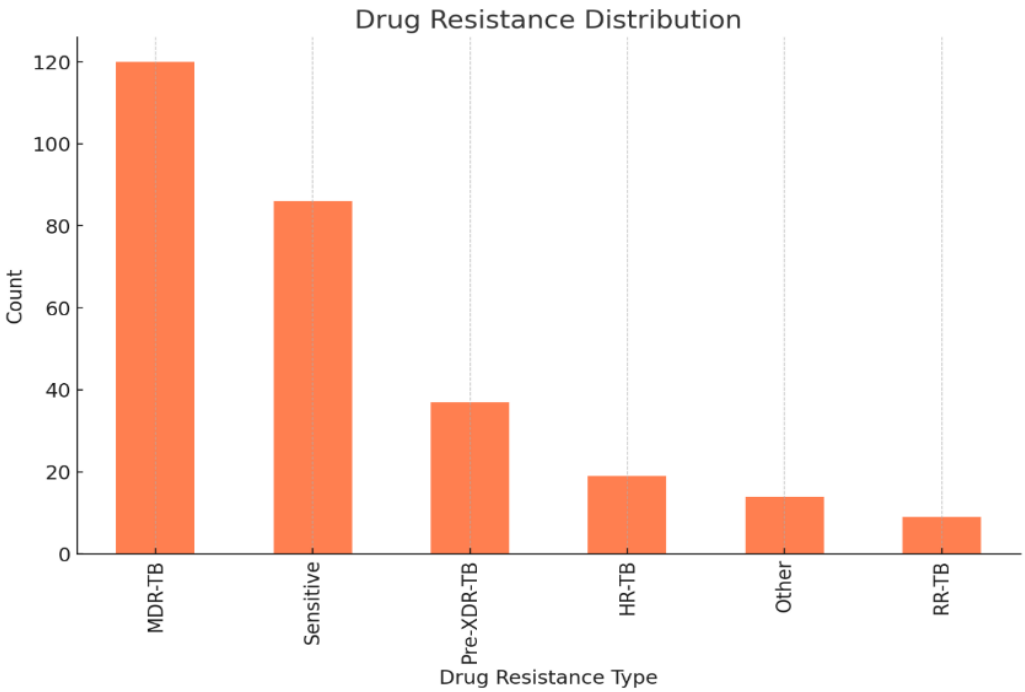


Figure. 4-2 - The figure illustrates that MDR-TB was the most common form of drug resistance between 2016-2019, observed in 120 (42.11%) patients. Drug-sensitive cases accounted for 86 (30.18%) samples, followed by Pre-XDR-TB (pre-Extensively Drug-Resistant TB) in 37 (12.98%) samples. HR-TB (High-Risk TB) was identified in 18 (6.32%) samples, other forms of drug resistance in 14 (4.91%) samples, and RR-TB (Rifampicin-Resistant TB) in 9 (3.16%) samples. These observations indicating a diversity of resistance types underscore the significant challenge posed by MDR-TB in this population.

Further genetic analysis of the isolates in the present study highlighted the predominant presence of the L2-Beijing lineage, which accounted for 70.4% of the cases. This finding is consistent with previous findings of Aung et al. (2021) on the genetic diversity of MTB strains circulating in Yangon. The dominance of the L2-Beijing lineage is particularly concerning due to its association with increased transmissibility and drug resistance. Key mutations associated with drug resistance were identified, including katG Rv1908c and KatG Rv1483 in 6.32% of Hr-TB cases. These mutations are well-documented markers of resistance and provide valuable targets for diagnostic and therapeutic interventions.

In summary, drug-resistant TB cases fluctuated over the years, with notable peaks in 2017 and 2019 (Figure 4-1 and 4-2). Conversely, drug-sensitive TB cases showed a corresponding inverse trend, accounting for 71.2% in 2016, 49.5% in 2017, 77.0% in 2018, and 65.4% in 2019 (Figure 4-1). These fluctuations highlight the changing and dynamic nature of TB resistance and the need for continuous surveillance to effectively track and respond to strains circulating. For more information on statistical analyses, please refer to the supplementary information.

4.3.2. Demographic characteristics of patients.

The median age of the patients was 37 years, with an interquartile range (IQR) of 21 years, indicating a relatively young cohort predominantly affected by TB (Table 4-1). Age distribution between drug-resistant and drug-susceptible TB patients showed no significant difference (P-value = 0.5314) (Table 4-1). Patients aged 21-40 years constituted the largest age group in both categories, with 50.0% (n=100) of drug-resistant and 55.29% (n=47) of drug-susceptible cases (Table 4-1). The gender distribution was similar between the two groups (P-value = 0.7506), with males being more frequently affected in both categories, comprising 64.0% (n=128) of drug-resistant and 61.18% (n=52) of drug-susceptible cases (Table 4-1).

The geographical distribution of TB cases showed no significant difference between the groups (P-value = 0.4913), with the North district having the highest number of cases (Table 4-1). The year of sample collection also did not significantly differ between the groups (P-value = 0.2923), with the majority of samples collected in 2017 (Table 4-1). Treatment history showed a significant difference (P-value < 0.0001), with all retreatment cases being drug-resistant. HIV co-infection rates were higher among drug-resistant patients, though not statistically significant (P-value = 0.16), and blood glucose levels did not show significant differences between groups (P-value = 0.3498) (Table 4-1). There was a significant difference in lineage distribution (P-

value < 0.0001), with Lineage 2 being predominant among drug-resistant cases (Table 4-1). These demographic characteristics provide a detailed overview of the patient population and highlight important factors that may influence TB transmission and treatment outcomes.

Characteristic	Drug-Resistant (n=200)	Drug-Susceptible (n=85)	Overall (n=285)	P-value (χ^2 test)
Age of Patients				**0.5314**
≤ 20 years	11 (5.50%)	5 (5.88%)	16 (5.61%)	
21-40 years	100 (50.0%)	47 (55.29%)	147 (51.58%)	
41-60 years	78 (39.0%)	26 (30.59%)	104 (36.49%)	
≥ 61 years	11 (5.5%)	7 (8.24%)	18 (6.32%)	
Sex				**0.7506**
Male	128 (64.00%)	52 (61.18%)	180 (63.16%)	
Female	72 (36.00%)	33 (38.82%)	105 (36.84%)	
Region District				**0.4913**
North	95 (47.50%)	44 (51.76%)	139 (48.77%)	
South	6 (3.00%)	2 (2.35%)	8 (2.81%)	
East	61 (30.50%)	29 (34.12%)	90 (31.58%)	
West	38 (19.00%)	10 (11.76%)	48 (16.84%)	
Year Collected				**0.2923**
2016	22 (11.00%)	5 (5.88%)	27 (9.47%)	
2017	94 (47.00%)	36 (42.35%)	130 (45.61%)	
2018	54 (27.00%)	31 (36.87%)	85 (29.82%)	
2019	30 (15.00%)	13 (15.29%)	43 (15.09%)	
Treatment History				**<0.0001**
Retreatment	72 (36.00%)	0 (0.00%)	72 (25.56%)	
New	128 (64.00%)	85 (100%)	213 (74.74%)	
HIV				**0.16**
Positive	15 (7.50%)	2 (2.35%)	17 (5.96%)	
Negative	185 (92.50%)	83 (97.65%)	268 (94.04%)	
Blood Glucose, mg/dL				**0.3498**
≥ 200	17 (8.50%)	11 (12.94%)	28 (9.82%)	
≤ 201	183 (91.50%)	74 (87.06%)	257 (90.18%)	
Lineage				**<0.0001**
Lineage 1	17 (8.50%)	38 (44.71%)	55 (19.30%)	
Lineage 2	176 (88.00%)	23 (27.06%)	199 (69.82%)	
Lineage 3	3 (1.50%)	9 (10.59%)	12 (4.21%)	
Lineage 4	4 (2.00%)	15 (17.65%)	19 (6.67%)	

Table. 4-1 - Details the socio-demographic and clinical characteristics of patients with genomic drug-resistant TB along with Pearson's chi-squared test score.

In comparison to the study by Aung et al. (2021), which also focused on drug resistance profiles of TB patients in Yangon, several similarities and differences were noted. Both studies identified a relatively young cohort predominantly affected by TB, with the median age being in the mid-to-late 30s. Aung et al. reported a high prevalence of drug-resistant TB, particularly MDR-TB, similar to our findings where MDR-TB comprised 45.6% of the cases. The gender distribution in both studies showed a higher prevalence of TB among males. Aung et al. (2021) also observed a significant burden of drug-resistant TB in the Yangon region, particularly in the northern districts, which aligns with our findings showing the North district as having the highest number of cases (Figure 4-3).

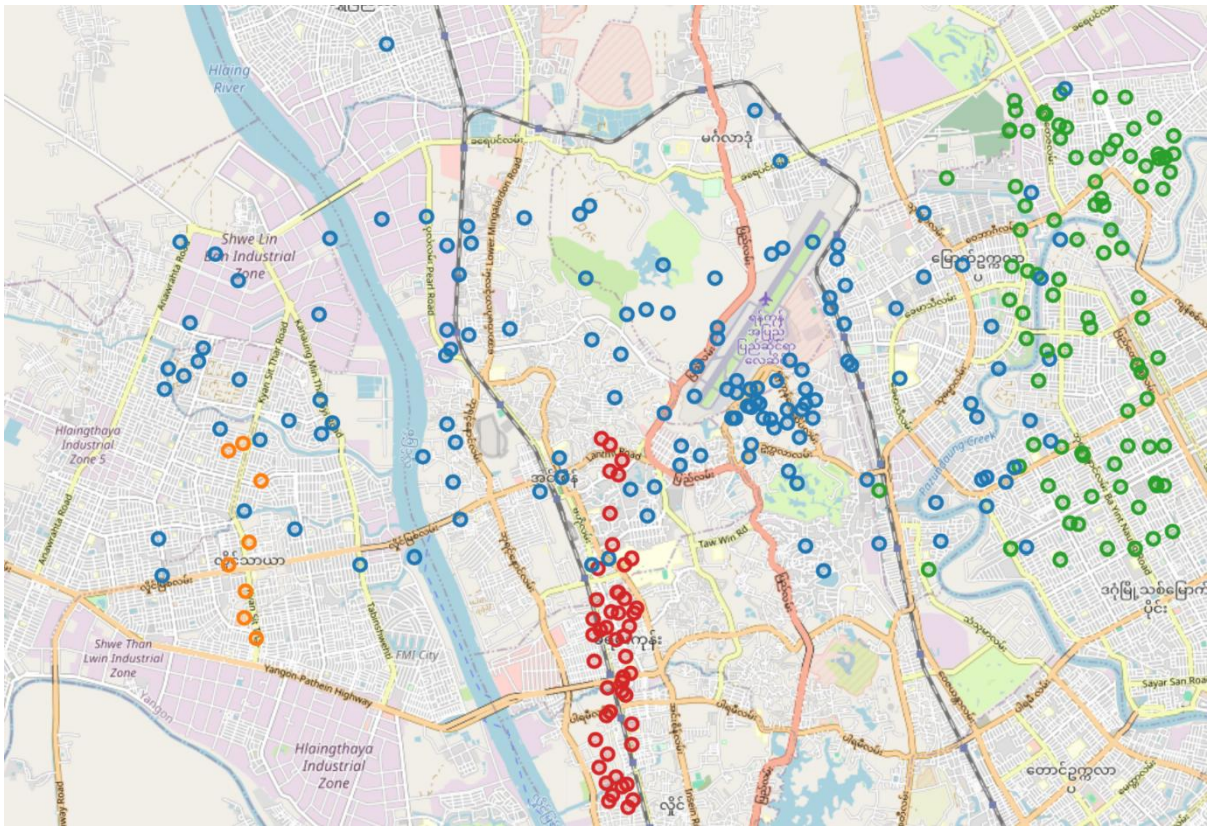


Figure. 4-3 - The map shows TB cases in Yangon, with blue circles marking high prevalence in North and East districts, particularly in industrial zones. Red circles highlight a dense cluster in Central Yangon, while green circles indicate lower prevalence areas. This color-coded map emphasizes the link between industrial activity, high population density, and TB incidence in the city.

In our present investigation, we identified a high prevalence of drug-resistant TB in specific areas of Yangon, which is closely linked to industrial regions in Yangon districts (Figure 4-3). The analysis of our dataset shows that these areas, particularly the Hlaingthaya and Shwepyithar Industrial Zones in Yangon-North, are significant contributors to the TB cases due to their dense industrial activity and large worker populations (Figure 4-3). Similarly, in

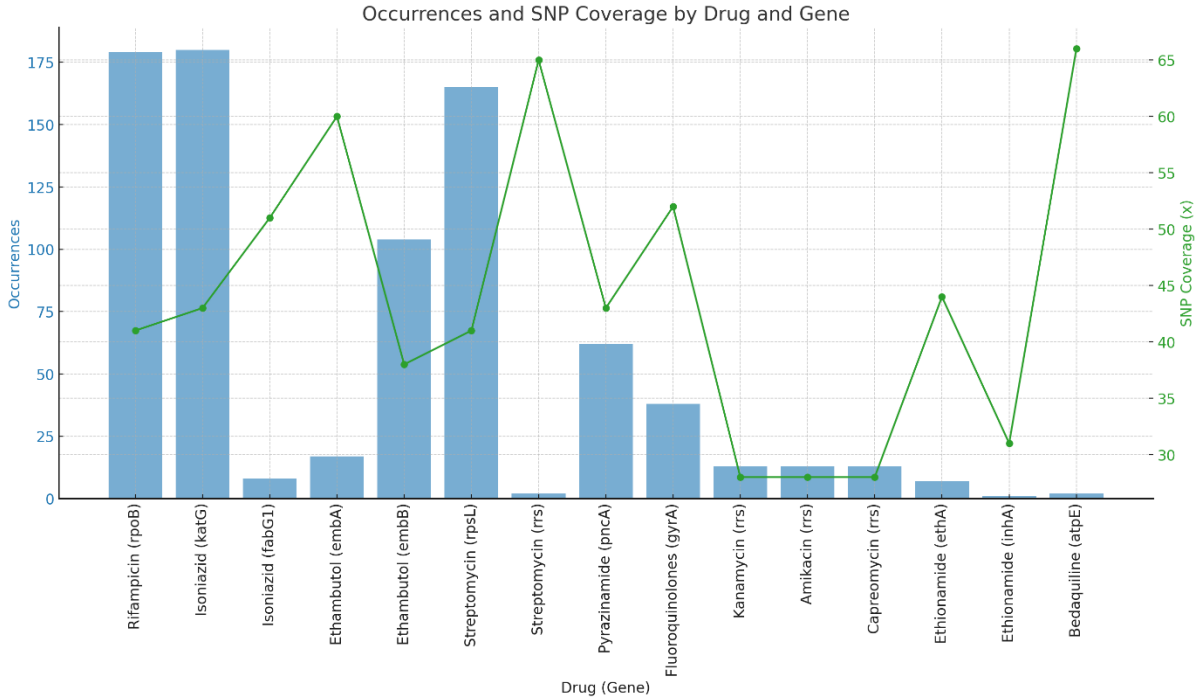
Yangon-East, areas like South Okkalapa and North Okkalapa exhibit notable TB prevalence, likely due to a combination of residential density and light industrial activities (Figure 4-3). The correlation between industrial zones and TB cases is further supported by the presence of the Lineage 2 - Beijing strain, which has been identified as prevalent in these regions, as reported in our study and by Aung et al. (2021).

In the densely populated areas of Yangon-South, such as Dagon Seikkan and Thilawa Special Economic Zone, the prevalence of TB is marked by the red circles on our map, reflecting the link between high population density and increased TB incidents (Figure 4-3). The industrial and economic activities in these zones contribute to higher rates of TB due to the movement and interaction of workers. Conversely, Yangon-West, with areas like Hlaing and Insein Townships, shows varying TB prevalence with yellow circles on the map (Figure 4-3). These regions have a mix of industrial and residential areas that may influence TB cases, but to a lesser extent than the northern industrial hubs. Our results highlight the socioeconomic and environmental factors associated with these industrial zones, emphasizing the need for targeted TB control strategies in high-risk areas.

4.3.3 Drug Resistance Against First Line Drugs.

In our present investigation, we identified significant resistance mechanisms against first-line TB drugs in MTB. Rifampicin resistance was primarily associated with mutations in the rpoB gene, encoding the beta subunit of RNA polymerase. We identified 179 occurrences of these mutations, with an average SNP coverage of 41x, accounting for 22.25% of the total SNPs (Figure 4-4). The Ser531Leu mutation in the rpoB gene was the most frequently observed variant in the present study, demonstrating a significant impact on drug efficacy [4,7]. Additional mutations of note included His526Asp and Asp516Val within the rpoB gene [4]. Resistance to isoniazid was associated with mutations in the katG, fabG1, and inhA genes [4]. The katG gene, located at the Rv1908c locus, plays a critical role in the activation of isoniazid, a key mechanism in the drug's response [4,7]. The study identified 180 instances of katG mutations, with an average single nucleotide polymorphism (SNP) coverage of 43x, accounting for 22.25% of the total SNPs (Figure 4-4). The Ser315Thr mutation in katG was the most prevalent, leading to impaired activation of the drug response enzyme [4,7]. Additionally, mutations in fabG1, such as the -15C>T promoter mutation, and inhA mutations like S94A, which are associated with ethionamide resistance, were observed [4,7]. These findings further corroborate that the S94A mutation is sufficient to confer resistance to both isoniazid and ethionamide in *Mycobacterium tuberculosis* clinical isolates [4-7].

Ethambutol resistance was associated with mutations in the embB gene, located at the Rv3795 locus [4]. Seventeen occurrences of embB mutations were identified, with an average single nucleotide polymorphism (SNP) coverage of 60x, accounting for 2.18% of the total SNPs (Figure 4-4). The Met306Ile substitution, resulting from an A→G transition at nucleotide position 916, was notably prevalent, indicating its significant role in conferring resistance to ethambutol [4]. Other significant mutations included Gly406Ala and Met306Val [4]. Streptomycin resistance in *Mycobacterium tuberculosis* (MTB) was frequently associated with mutations in the rpsL and rrs genes [4-7]. The rpsL gene encodes the ribosomal protein S12, while the rrs gene encodes 16S rRNA [4]. Significant mutations identified at the Rv0682 locus included Lys43Arg and Lys88Arg, both of which are implicated in resistance to streptomycin [4-7]. A total of 165 occurrences of rpsL mutations were observed, with an average SNP coverage of 41x, alongside 2 occurrences of rrs mutations with an average SNP coverage of 65x (Figure 4-4). These mutations interfere with streptomycin binding to the ribosome, reducing the drug's efficacy and leading to resistance [4-7]. Additionally, the A1401G mutation in the rrs gene was noted for its contribution to high-level resistance to multiple drugs, including streptomycin [2,4-7]. Pyrazinamide resistance was associated with mutations in the



pncA gene, which encodes the pyrazinamidase enzyme [4]. Sixty-two occurrences of pncA mutations were identified, with an average SNP coverage of 43x, resulting in a loss of enzyme activity and consequent reduction in drug effectiveness (Figure 4-4).

Figure. 4-4 – A combined figure with bar and line chart illustrating SNP occurrences (blue bars) and coverage (green line) for MTB resistance genes associated with various anti-tuberculosis drugs. The figure highlights significant SNP mutation occurences frequencies and its coverage for anti-TB drugs, offering insights into the genetic basis of drug resistance coverage.

4.3.4 Drug Resistance Against Second Line Drugs.

In the present study, significant resistance mechanisms against second-line tuberculosis (TB) drugs were identified, involving mutations associated with fluoroquinolone, aminoglycoside, ethionamide, and bedaquiline resistance. Fluoroquinolone resistance was linked to mutations in the gyrA and gyrB genes [4], with 38 occurrences observed and an average SNP coverage of 52x (Figure 5). Key mutations included Ala90Val, Ser91Pro, and Asp94Gly in gyrA, as well as Asp461Asn in gyrB which play a significant role of resistance towards second-line TB drugs [4]. Aminoglycoside resistance was primarily associated with mutations in the eis promoter for kanamycin (C-14T and G-37T), rrs gene mutations for amikacin (A1401G and C1484T), and similar rrs gene mutations for capreomycin, in addition to tlyA gene mutations such as C491A [4]. A total of 167 occurrences of rpsL mutations were identified, with an average SNP coverage of 49x, alongside 2 occurrences of rrs mutations with an average SNP coverage of 65x (Figure 4-5).

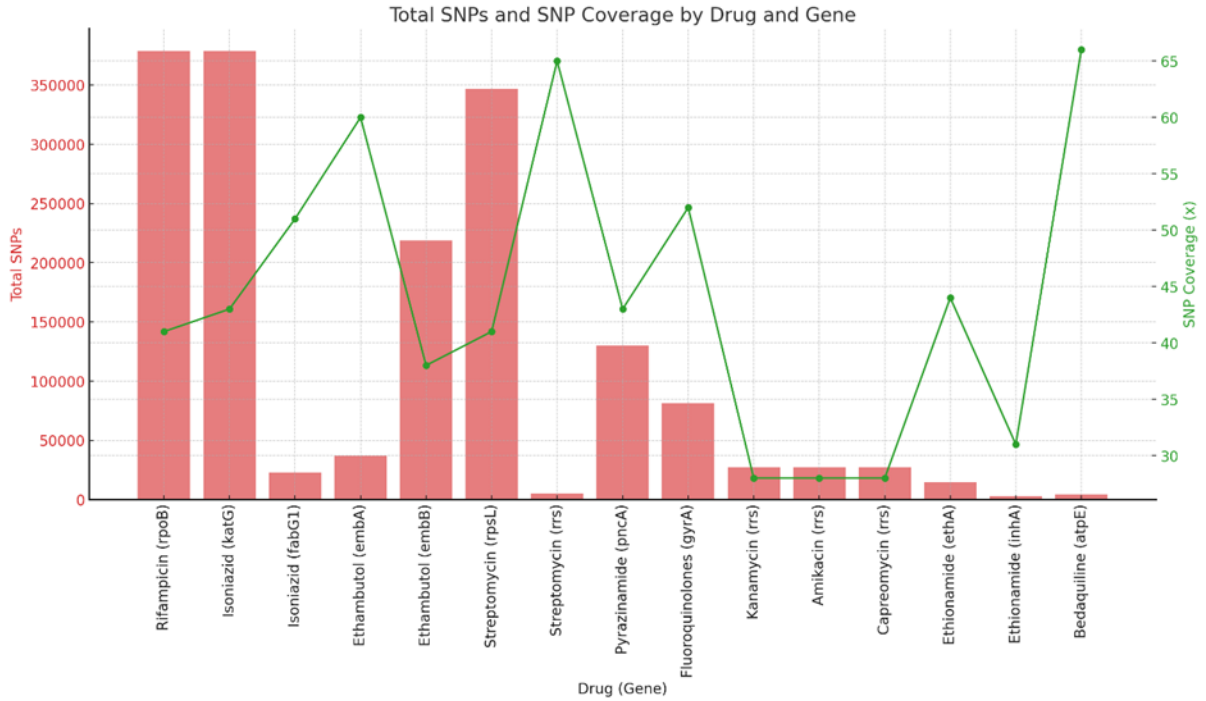


Figure. 4-5 – A combined figure bar and line chart displaying total SNPs (red bars) and SNP coverage (green line) for various anti-tuberculosis drugs and their resistance genes. The figure highlights high SNP counts and SNP coverage for key drugs like Rifampicin (rpoB) and Isoniazid (katG), providing insights into genetic diversity and sequencing coverage for each TB drug resistance genes.

Ethionamide resistance was associated with mutations in the *ethA* gene, specifically -15C>T and -8T>C, as well as mutations in *inhA*, such as S94A [4,7]. Sixty-two occurrences of these mutations were identified, with an average single nucleotide polymorphism (SNP) coverage of 43x (Figure 4-5). Bedaquiline resistance was linked to mutations in the *atpE* gene, such as

Ala63Pro, and mutations in the Rv0678 gene, such as Gly66Asp [4,6]. These mutations were less frequently observed, with a 20x SNP coverage (Figure 4-5). Aung et al. (2021) conducted a similar examination of second-line drug resistance, reporting a high prevalence of mutations in the *gyrA* gene for fluoroquinolone resistance, particularly D94G and A90V, which aligns with our findings [1,4]. Additionally, resistance to aminoglycosides in their study was primarily linked to *rrs* mutations, including A1401G and G1484T, which is consistent with our results. Their study also reported mutations in the *embB* and *rpsL* genes as primary mechanisms of resistance to ethambutol and streptomycin, respectively, corroborating our observations [4].

4.3.5 Phylogenetic Analysis and Lineage Distribution of MTB in Yangon, Myanmar.

The phylogenetic analysis of 285 *Mycobacterium tuberculosis* (MTB) isolates from Yangon, Myanmar, identified four primary lineages: Lineage 1 (L1) - East African-Indian (EAI), Lineage 2 (L2) - Beijing, Lineage 3 (L3) - Delhi-CAS, and Lineage 4 (L4) - Euro-American (Figure 4-6). The analysis revealed that L2 was the predominant lineage, representing 181 isolates (63.5%), followed by L1 with 71 isolates (24.9%), L4 with 21 isolates (7.4%), and L3 with 12 isolates (4.2%) (Figure 4-6). The evolutionary tree indicated an early divergence of L1 from the other three lineages, which shared a common ancestor before subsequently diverging. The clustering of L2 isolates into distinct groups suggests recent and rapid clonal expansion, indicating strong evolutionary linkage and potential adaptation advantages in the local population.

The analysis of antimicrobial resistance (AMR) mutational profiles revealed lineage-specific differences. Mutations in the rpoB gene, such as Leu452Pro and Leu430Pro, and in the katG gene, such as Thr380Ile, Thr394Ala, and Asn238Lys, were exclusively present in L2. Other mutations, including rpoB Ser450Leu, katG Ser315Thr, and rpsL Lys43Arg, were more common in L2 compared to other lineages. Conversely, mutations such as fabG1 -15C>T and gid deletions were more frequent in L1. Notably, no fluoroquinolone resistance-associated mutations were detected in L1 or L4, indicating potential differences in resistance mechanisms across lineages. Drug resistance profiling of the MTB isolates demonstrated a significant prevalence of MDR-TB and Pre-XDR-TB. MDR-TB was detected in 97 isolates (34.0%), while Pre-XDR-TB was observed in 47 isolates (16.5%). Additionally, cases of XDR-TB were identified, albeit less frequently. The distribution of drug resistance patterns was consistent across different regions of Yangon, consistently with previously conducted study by Aung et al., 2021 [1].

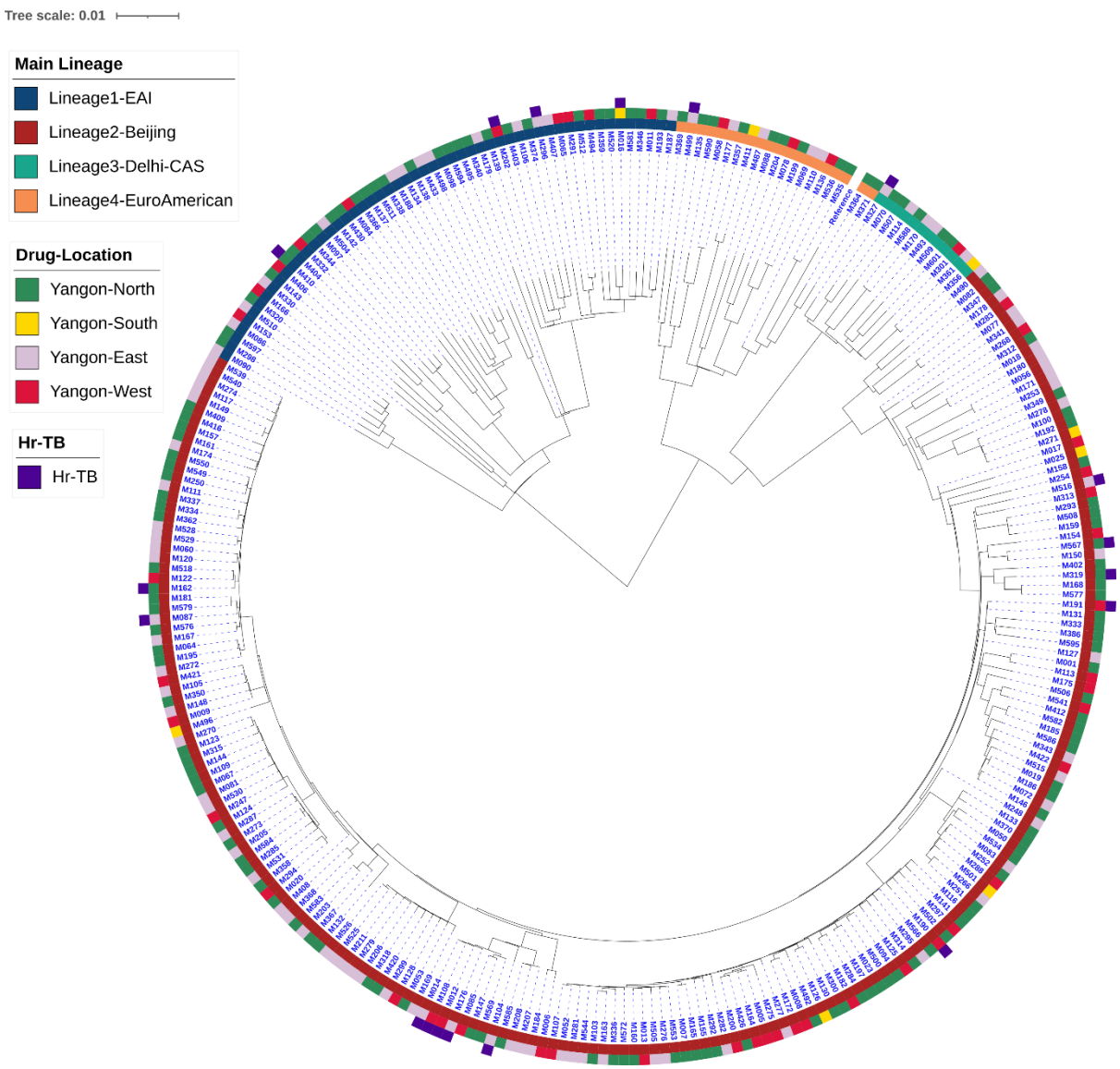


Figure. 4-6 – A circular rooted maximum likelihood (GTR) phylogenetic tree illustrating the distribution of 285 MTB isolates from Yangon, Myanmar, across four primary lineages: L1 to L4, with L2 being the predominant lineage. The tree also shows the geographic distribution of isolates from different districts: North, South, West, and East Yangon. Lineage-specific antimicrobial isoniazid resistance mutations (Hr-TB) are highlighted, showing a higher association with L2. This emphasizes the genetic diversity, evolutionary relationships, and regional prevalence of the isolates.

Geographical analysis was conducted to assess the distribution of MTB lineages across various regions of Yangon, Myanmar. The analysis revealed that lineage 2 (L2) was prevalent across all regions, with a higher concentration observed in Yangon-North and Yangon-East (Figure 4-6). Lineage 1 (L1) exhibited a higher prevalence in Yangon-West, while lineages 3 (L3) and 4 (L4) were more uniformly distributed throughout the city (Figure 4-6). This distribution pattern reflects the genetic diversity of MTB in Yangon and highlights the widespread presence of L2, particularly in industrial zones such as Yangon-North and Yangon-East.

Further analysis identified a high prevalence of isoniazid-resistant tuberculosis (Hr-TB) within the study population, with 18 isolates (6.3%) demonstrating resistance. Notably, Hr-TB was predominantly associated with L2, suggesting a potential link between this lineage and isoniazid mono-resistance (Figure 4-6). This finding warrants further investigation to understand the implications for public health management and the development of targeted interventions for mono-isoniazid resistance.

Clustering analysis was performed using pairwise SNP distances to investigate the potential for recent transmission events within the local population. Clusters were defined by SNP distances of less than 12, indicating close genetic relationships among isolates. Several clusters meeting this criterion were identified, including isolates M005, M006, M007, and M009, which were primarily associated with L2 (Figure 4-6). The identification of these clusters, characterized by SNP distances of <12, supports the hypothesis of recent and rapid clonal expansion within L2, indicating strong evolutionary clusters within this lineage.

4.4 Discussion.

The identification of Hr-TB through comprehensive genomic analysis reveals a cascading crisis that encompasses diagnostic failures, economic devastation, and the collapse of international funding mechanisms, creating what can only be described as a silent epidemic threatening global health security. This crisis represents the intersection of clinical misdiagnosis, prohibitive treatment costs, and the systematic underfunding of tuberculosis control programs in an era of unprecedented global economic uncertainty.

4.4.1 The Catastrophic Economics of Misdiagnosis.

Traditional rifampicin-focused screening approaches, while effective for detecting MDR-TB, systematically miss Hr-TB cases, creating a diagnostic blind spot with devastating clinical and economic consequences. Patients with undetected Hr-TB are often treated with first-line regimens containing isoniazid, effectively creating ideal conditions for resistance amplification and progression to increasingly complex and costly forms of drug-resistant tuberculosis.

The economic trajectory of misdiagnosed Hr-TB cases follows a predictable but devastating pattern. The median cost of treating drug-susceptible TB remains relatively manageable at USD 783 per patient, but this figure represents only the beginning of a potential economic catastrophe. Hr-TB cases that progress to MDR-TB due to inappropriate initial treatment require USD 4,877 per patient—a sixfold increase that represents just the direct treatment costs [5]. When these cases further progress to pre-extensively drug-resistant TB (Pre-XDR-TB) or

extensively drug-resistant TB (XDR-TB), treatment costs can exceed USD 15,000 per patient, creating an economic burden that is simply unsustainable for low- and middle-income countries.

In resource-constrained settings like Myanmar, where the gross domestic product per capita is approximately USD 1,400, a single XDR-TB case requiring prolonged treatment represents more than ten times the annual income of an average citizen. The polyresistance patterns observed in 55.6% of our Hr-TB isolates likely represent, in part, the consequences of this systematic misdiagnosis and suboptimal treatment cycle. Our comprehensive phylogenetic analysis provides critical insight into the genetic architecture underlying these economically devastating resistance patterns (Figure 4-7).

The phylogenetic tree Figure 4-7 reveals the complex genetic landscape of drug resistance evolution, with significant clustering within Lineage 2 strains suggesting recent clonal expansion and active transmission of costly-to-treat resistant variants. Different MTB lineages exhibit varying propensities for resistance development and treatment complexity: Lineage 1 (EAI) cases often require extended treatment regimens, Lineage 2 (Beijing) strains show higher rates of MDR-TB progression, Lineage 3 (Delhi-CAS) variants present unique diagnostic challenges, and Lineage 4 (Euro-American) cases demonstrate variable treatment responses. The tree illustrates how each resistance category from sensitive cases through Hr-TB, STM-TB, MDR-TB, Pre-XDR-TB, to XDR-TB, represents not only increasing clinical complexity but exponentially escalating treatment costs. The clustering patterns observed in our phylogenetic reconstruction demonstrate how misdiagnosed Hr-TB cases serve as epidemiological amplifiers, generating increasingly expensive treatment scenarios while fueling community transmission of drug-resistant strains.

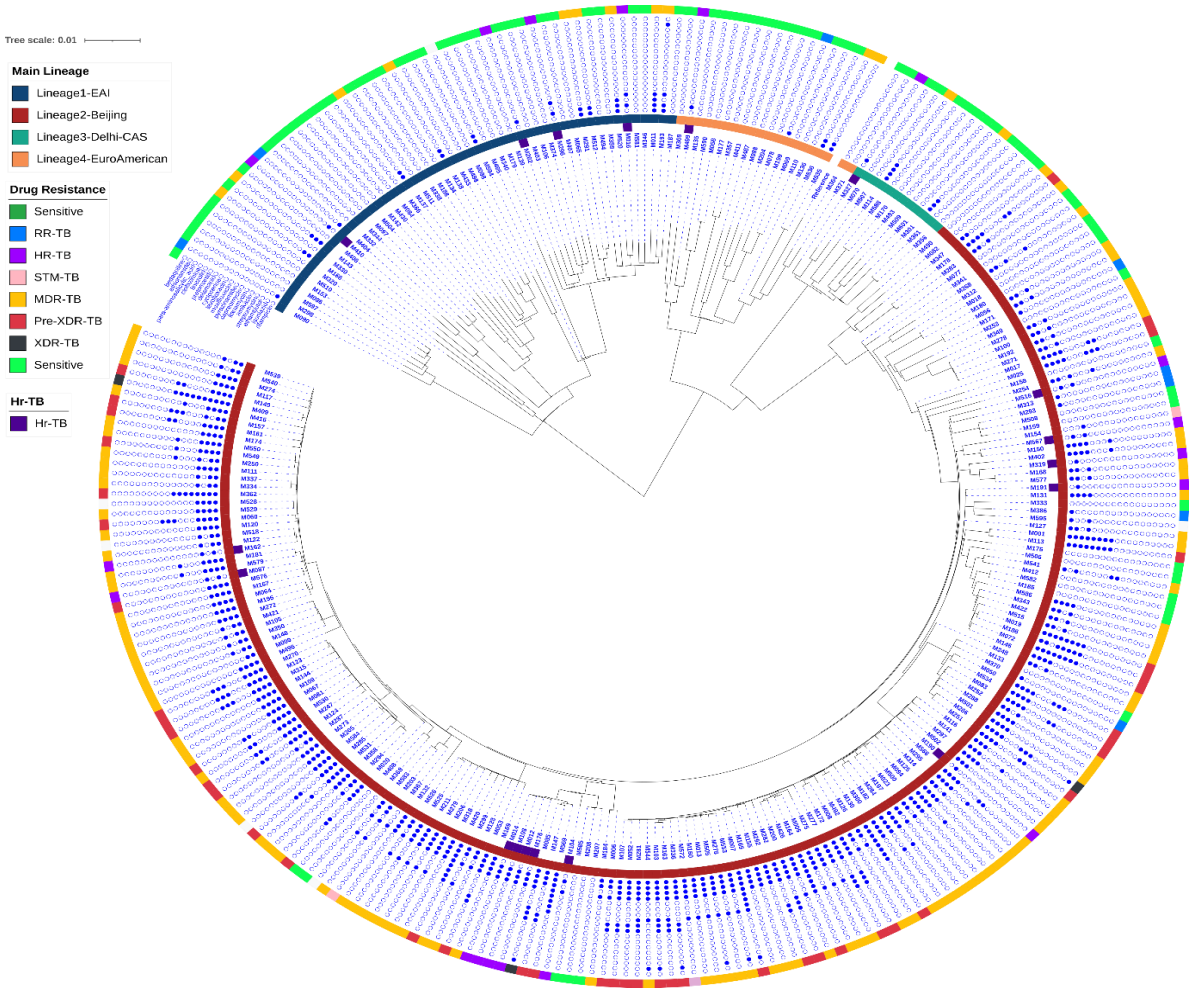


Figure 4-7 – Circular rooted maximum-likelihood phylogenetic tree (GTR) based on WGS of 285 MTB strains from Yangon, Myanmar. The tree displays the main lineages: Lineage 1 (EAI), Lineage 2 (Beijing), Lineage 3 (Delhi-CAS), and Lineage 4 (Euro-American). The drug resistance status of isolates is indicated: sensitive, rifampicin-resistant TB (RR-TB), high-level isoniazid-resistant TB (Hr-TB), streptomycin-resistant TB (STM-TB), multidrug-resistant TB (MDR-TB), pre-extensively drug-resistant TB (Pre-XDR-TB), and extensively drug-resistant TB (XDR-TB). Scale bar indicates nucleotide substitutions per site.

4.4.2 Global Health Financing in Crisis.

The implementation of comprehensive diagnostic approaches capable of preventing this costly progression requires substantial financial investment that is increasingly unavailable in the current global economic climate. The 2024 WHO Global TB Report reveals a catastrophic funding crisis that directly threatens Hr-TB detection capabilities worldwide. Despite global targets calling for USD 22 billion annually by 2027, only 26% of required funding is currently available in LMICs [5]. Sixty countries reported combined funding gaps totaling USD 1.7 billion for 2024 alone, with the largest gaps concentrated in African and South-East Asian regions where Hr-TB burden is highest [28].

The median cost of comprehensive drug susceptibility testing, including whole-genome sequencing approaches demonstrated in our study, can exceed USD 200 per sample in low-resource settings. For countries like Myanmar, where the entire national TB program budget may be less than USD 10 million annually, implementing Hr-TB detection for all presumptive cases would require doubling or tripling current diagnostic expenditures. This economic reality forces programs into a devastating paradox: they cannot afford the diagnostic tools needed to prevent the expensive treatment scenarios that drain their already inadequate budgets.

4.4.3 The Post-COVID-19 Collapse of International Support.

The global TB response in LMICs has historically relied heavily on international donor funding, with organizations such as the Global Fund to Fight AIDS, Tuberculosis and Malaria providing 76% of international donor funding [28]. However, the post-COVID-19 global economic landscape has fundamentally altered international aid priorities and availability, creating an unprecedented crisis for TB control programs precisely when enhanced diagnostic capabilities are most needed. The Global Fund, the primary international financier of TB programs globally recently announced devastating cuts of USD 1.4 billion from already allocated grants in 2024, affecting over 100 countries [7]. These cuts, largely attributed to donor shortfalls particularly from the United States, threaten to dismantle years of progress in TB control and force countries to abandon the very diagnostic approaches that could prevent costly treatment scenarios. The United States, which has contributed only USD 1.8 billion of its USD 6 billion pledge to the Global Fund, faces increasing domestic pressure to prioritize national economic recovery over international development assistance [7].

Concurrent geopolitical tensions, including trade disputes and shifting international relationships, have further constrained global health funding. European Union members

similarly report reduced capacity for global health investments amid economic challenges and regional security concerns. The Stop TB Partnership has already implemented staff layoffs and curtailed advocacy activities due to funding constraints [7], signaling broader system-wide reductions in TB control capacity.

4.4.4 The Vicious Cycle of Diagnostic Poverty.

This funding crisis creates a vicious cycle that perpetuates the most expensive treatment scenarios while undermining the diagnostic capabilities needed to prevent them. When external funding is reduced or discontinued, TB programs revert to less expensive but less comprehensive diagnostic approaches, systematically missing Hr-TB cases and creating the conditions for resistance amplification. Each missed Hr-TB case represents not only immediate clinical failure but also a future economic catastrophe, as these patients progress through increasingly expensive treatment categories while serving as sources of ongoing community transmission.

The phylogenetic evidence presented in Figure 4-7 demonstrates the critical need for comprehensive genomic approaches, as traditional screening methods fail to capture the complex resistance evolution and transmission dynamics that drive treatment costs skyward. The clustering patterns visible in our phylogenetic reconstruction represent not just scientific curiosities but economic time bombs, each cluster potentially representing a future outbreak of costly-to-treat resistant tuberculosis that could overwhelm already strained health systems.

4.5 Study Limitations and Recommendations.

This study is subject to several limitations that should be considered when interpreting the findings. The most notable limitation relates to the geographical scope of sampling, which was confined to three National TB Program diagnostic centres in Yangon, Myanmar (Aung San, Latha, and North Oakkalapa). Although these sites represent important referral centres within the capital, they do not adequately capture the diversity of tuberculosis epidemiology across the country. Rural and remote regions often differ substantially in terms of disease burden, diagnostic infrastructure, and access to treatment. Consequently, the concentration of sampling in an urban setting introduces potential selection bias and restricts the generalizability of the results to other parts of Myanmar.

A further limitation arises from the temporal heterogeneity of data collection, which spanned 2015 to 2019, a period marked by major changes in diagnostic technology and national treatment guidelines. The gradual shift from the GeneXpert MTB/RIF assay to the more

advanced GeneXpert Ultra platform introduced inconsistencies in the sensitivity and specificity of drug resistance detection. This evolution may have influenced the reported prevalence of multidrug-resistant TB and rifampicin-resistant TB, thereby introducing temporal bias in the classification and interpretation of resistance patterns.

In addition, challenges related to diagnostic standardization across the study period affected the consistency of drug resistance profiling. WGS was progressively incorporated into routine workflows during the study, but variations in its application for drug susceptibility testing created methodological heterogeneity. While these developments reflect clinical progress, they also limited the comparability of results across time and may have influenced the reliability of genomic resistance characterizations.

To address these limitations, future studies should expand sampling beyond Yangon to achieve nationwide representation. Incorporating diagnostic centres from diverse geographical regions will enable the identification of regional variations in TB epidemiology, resistance dynamics, and healthcare capacity, thereby informing region-specific control strategies. Efforts should also be directed toward establishing standardized diagnostic protocols across all participating sites. Ensuring universal access to advanced tools such as GeneXpert Ultra and WGS-based drug susceptibility testing will enhance diagnostic accuracy, minimize technological disparities, and improve the comparability of data across different centres. Finally, integration of WGS-based molecular surveillance into routine diagnostic practice should be prioritized. Embedding real-time resistance monitoring into interconnected local, national, and regional databases will strengthen early outbreak detection, facilitate tracking of transmission pathways, and provide robust evidence to support timely, evidence-based public health interventions.

4.6 Study Impact and Clinical Significance.

This study provides critical insights into the management of isoniazid-mono-resistant tuberculosis (Hr-TB) in Yangon, Myanmar, with important implications for both clinical practice and public health policy. The genomic epidemiological analysis demonstrated a striking predominance of Lineage 2-Beijing (L2) strains among Hr-TB cases, highlighting a concerning epidemiological pattern. The clonal expansion of this lineage points toward possible transmission hotspots within the urban population, underscoring the urgent need for targeted public health interventions.

The resistance profiling conducted in this study further identified key genetic mutations, particularly within the katG and fabG1 genes, as well as alterations in Rv1908 and Rv1483.

These biomarkers represent valuable tools for the development of rapid diagnostic assays and hold promise for advancing point-of-care testing. Incorporating such genetic markers into diagnostic workflows could accelerate treatment initiation, improve patient outcomes, and ultimately reduce the risk of onward transmission.

The clinical and public health implications of these findings are significant. Resistance patterns observed in Hr-TB strains extended beyond simple isoniazid mono-resistance, with frequent co-resistance to other first-line drugs. This complexity challenges existing treatment protocols and highlights the need for revising national treatment guidelines to account for multi-drug resistance beyond the traditional Hr-TB definition. Additionally, our results strongly support a diagnostic strategy that integrates both phenotypic and genotypic drug susceptibility testing. Such a dual approach reduces diagnostic uncertainty, ensures more accurate resistance detection, and facilitates more effective treatment selection—key steps toward minimizing treatment failure and preventing the emergence of further resistance.

Looking forward, several future directions and policy priorities emerge from this work. First, strengthening Myanmar's TB surveillance systems with whole-genome sequencing–based monitoring is essential for tracking resistance emergence and understanding transmission dynamics in real time. Proactive surveillance will enable earlier outbreak detection and faster implementation of targeted interventions. Second, the predominance of the L2-Beijing lineage calls for tailored strategies that address the unique resistance mechanisms and transmission pathways associated with this strain. Enhanced case detection, improved contact tracing, and lineage-focused interventions should be prioritized in urban areas where L2 predominates. Third, research should advance the development of rapid diagnostic tools based on identified genetic biomarkers, refine treatment regimens that address complex resistance profiles, and investigate lineage-specific transmission drivers within Myanmar's epidemiological context.

Finally, integration into clinical practice requires equipping healthcare providers with training in genomic resistance interpretation and the application of combined diagnostic approaches. Embedding these findings into updated clinical guidelines will improve the precision of treatment strategies, support more effective patient management, and reduce the likelihood of extensively drug-resistant TB emergence.

Overall, this study contributes foundational evidence to transform Hr-TB management in Myanmar by providing an evidence-based framework for diagnostic and treatment strategies. The integration of genomic insights into clinical and public health practice not only advances

the control of Hr-TB but also offers a scalable model for TB management in other resource-limited settings.

4.7 Ethical Statement.

This study was approved by the Institutional Review Boards of the Department of Medical Research, Ministry of Health and Sports of Myanmar, and the Human Health Ethics Review Committee of the University of Otago, Dunedin, New Zealand (Ethics approval number: H18/123).

4.8 Acknowledgments.

We thank the staff and supervisor from TB clinics and collection laboratory from the Aung San, Latha, and North Oakkalapa diagnostic centres and the National Tuberculosis Reference Laboratory in Yangon, for their invaluable assistance. We also thank the Massey Genome Service for sample preparation and next-generation sequencing support. This study was supported by the New Zealand Health Research Council through the e-ASIA funding scheme, New Zealand Marsden Fund, Massey University PhD Scholarship, and Massey Ventures for additional research, development support, and commercialization.

4.9 About the Author.

Yang Fong is a genomics specialist at the Massey Genome Service and is also enrolled as a PhD student at Massey University, Palmerston North, New Zealand. His major research interests include antimicrobial resistance, genomics, and translational research addressing health inequalities. Yang Fong was responsible for the project design, sample preparation and methodology, data analysis and curation, data visualization, and writing.

4.9 Supplementary Material.

4.9.1 Reporting Guideline for Genotypic MTB WGS Analysis.

Adopted and modified from Tornheim et al., (2019) for Standardized TB Clinical Laboratory Reporting WGS Data [26].

NGS offers a rapid, comprehensive method for drug susceptibility testing (DST), reducing the time and cost of phenotypic tests and improving global TB surveillance. As NGS becomes more accessible, clear and consistent result communication, comparability across laboratories, and precise clinical interpretation are crucial. This guideline presents a standardized template for reporting NGS results for MTB, based on key variables, terminology, and essential elements identified in clinical setting.

Data.

Clinical Isolates.

- Operation in accordance with Lab Quality Management System (LQMS)
- In accordance to international ISO15189-mandated elements: Include patient- and specimen-level identifiers and assay details.
- Source of sequenced material: Indicate whether the material was sourced directly from the specimen or from culture.
- Organism identification/speciation: Provide detailed identification of the organism.
- *Mycobacterium tuberculosis* lineage: Specify the lineage of the *Mycobacterium tuberculosis.*
- Predicted antibiotic susceptibility profile: List drug names, resistance genes, and amino acid mutations.
- Susceptibility interpretative comments: Include the confidence level of mutation-resistance phenotype associations.
- Appropriate disclaimers: For instance, "For research use only" if the WGS assay is not approved for clinical use.

Surveillance & Monitoring.

- Geographic location: Provide the location where the sample was collected.
- Date of sampling: Use the format YYYY-MM-DD for consistency.
- SNV threshold to define clusters: Specify the single nucleotide variant (SNV) threshold used to define clusters.
- Cluster characteristics summary: Provide a summary of the characteristics of identified clusters.

Research.

- Source of sequenced material: Indicate whether the material was sourced directly from the specimen or from culture.
- Instrument, technology/chemistry: Specify the sequencing technology used, such as paired-end and read length.
- Assembly: Indicate whether the assembly was de novo or reference-based (include accession number) and specify the assembly software and version.
- Metrics: Provide coverage breadth and depth metrics.
- Coordinates of filtered sequences: Include the coordinates.
- Variant-calling software: Specify the software used, its version, and parameters.

- Treatment of indels: Explain how insertions and deletions were treated.
- Treatment of ambiguous/low-quality calls: Describe the handling of these calls.
- Cut-off threshold for calling minor variants: Indicate the threshold used.
- Accession numbers: Provide the accession numbers of FASTQ files and assemblies.
- Any in-house scripts used: Include details of any in-house scripts employed.

Visualisation.

Clinical

Emphasis: Use shading and other types of emphasis to highlight key details.

Image handling: Recognize that images may not be integrated into laboratory or electronic health record databases.

Surveillance & Monitoring

Input data: Provide details such as concatenated SNVs.

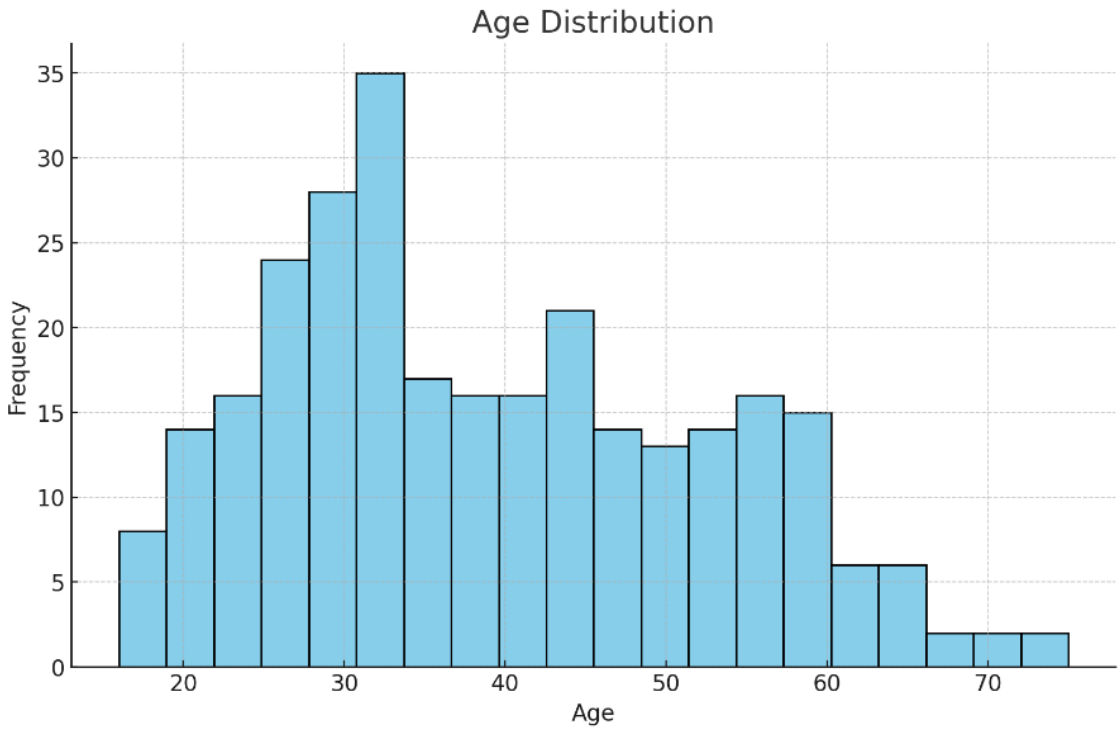
Genetic distance indication: Use phylogenetic trees or other visual representations to indicate genetic distance.

Isolate-level metadata: Include metadata in the phylogeny for comprehensive visualization.

4.9.2 Supplementary Figure Visualization.

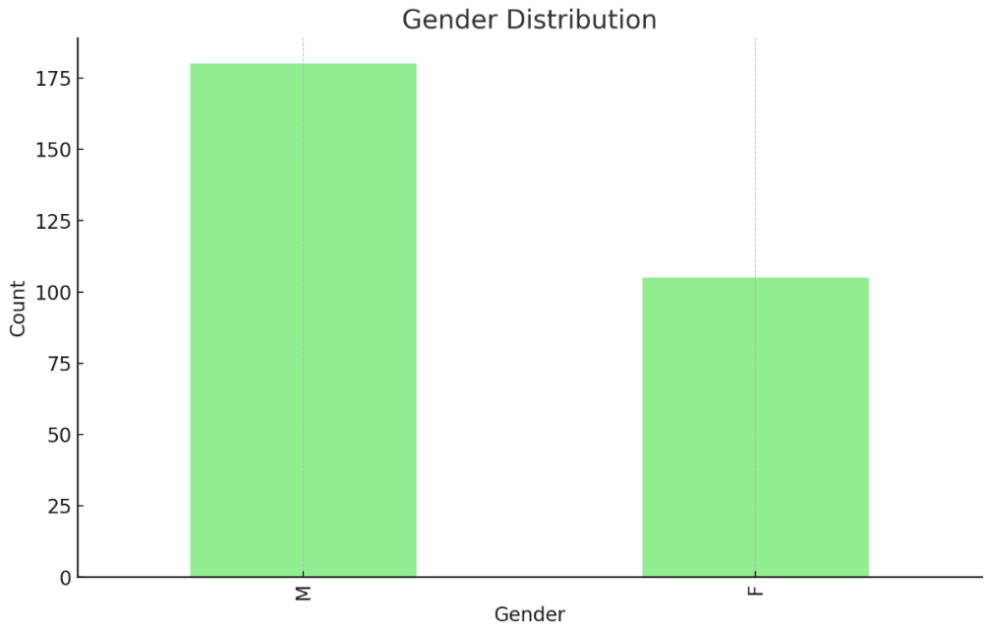
4.9.2.1 Supplementary Figure 1.

Supp. Fig 4-1 - The age of individuals in the dataset ranged from 16 to 75 years, with a mean age of 39.14 years (SD = 13.48), indicating a broad age distribution. The interquartile range (IQR) was 29 to 50 years, with a median age of 37 years, showing that half the individuals were younger, and half were older than 37 years. This wide age range suggests a varied demographic representation in the study.



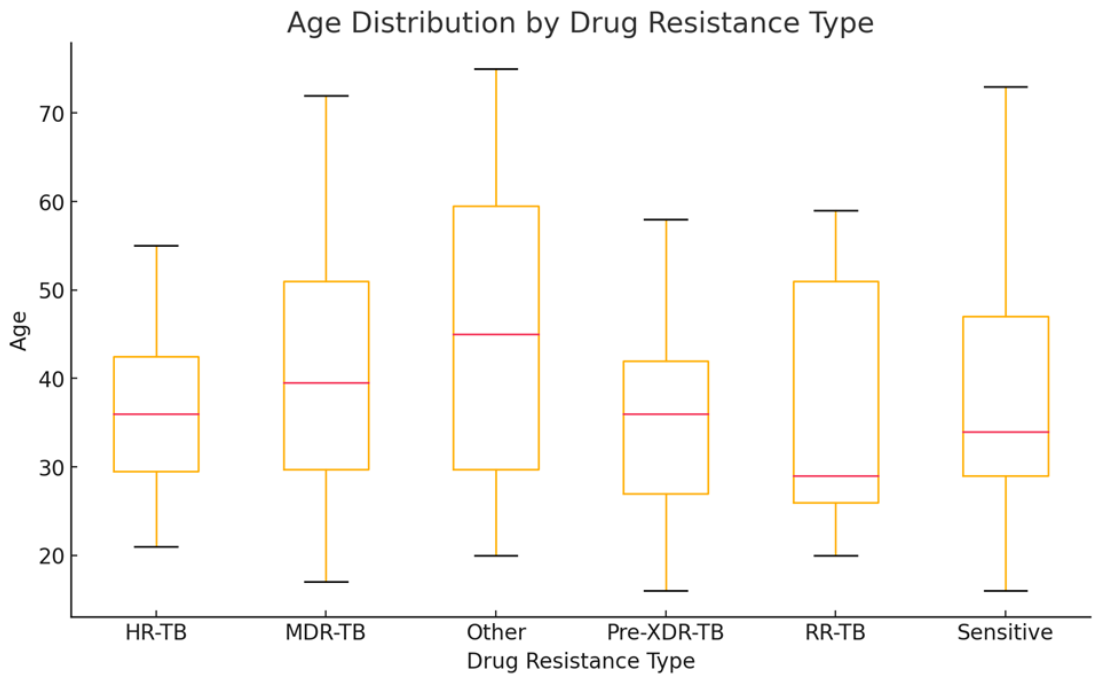
4.9.2.2 Supplementary Figure 2.

Supp. Fig 4-2 – The gender distribution showed a higher prevalence of male samples compared to female samples. Out of the 285 samples, 180 (63.16%) were male and 105 (36.84%) were female. This significant gender imbalance, with nearly two-thirds of the samples being male, may reflect gender biases in sample collection or differing rates of drug-resistant TB infection among genders.



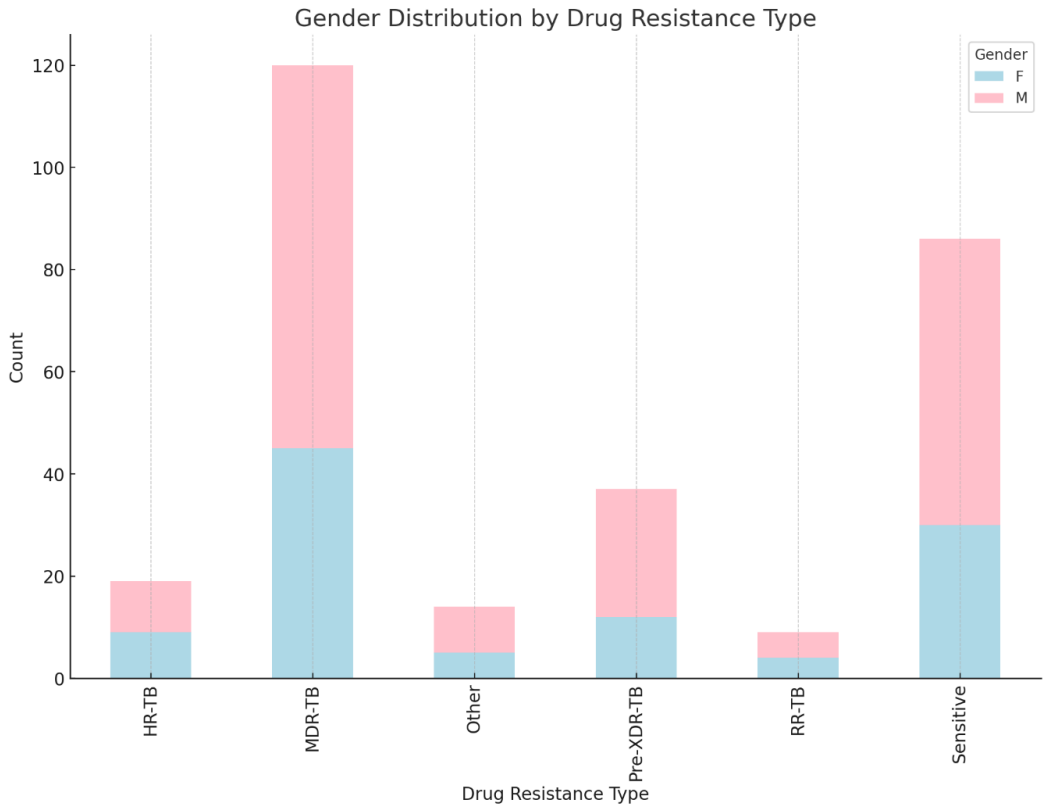
4.9.2.3 Supplementary Figure 3.

Supp. Fig 4-3 - A box plot analysis of age distribution by drug resistance type revealed some variation across different types. MDR-TB cases exhibited a slightly wider age range compared to other types, indicating that this form of drug resistance affects a diverse age group. In contrast, Pre-XDR-TB and HR-TB cases were more concentrated within specific age ranges, suggesting potential age-related susceptibility or risk factors.



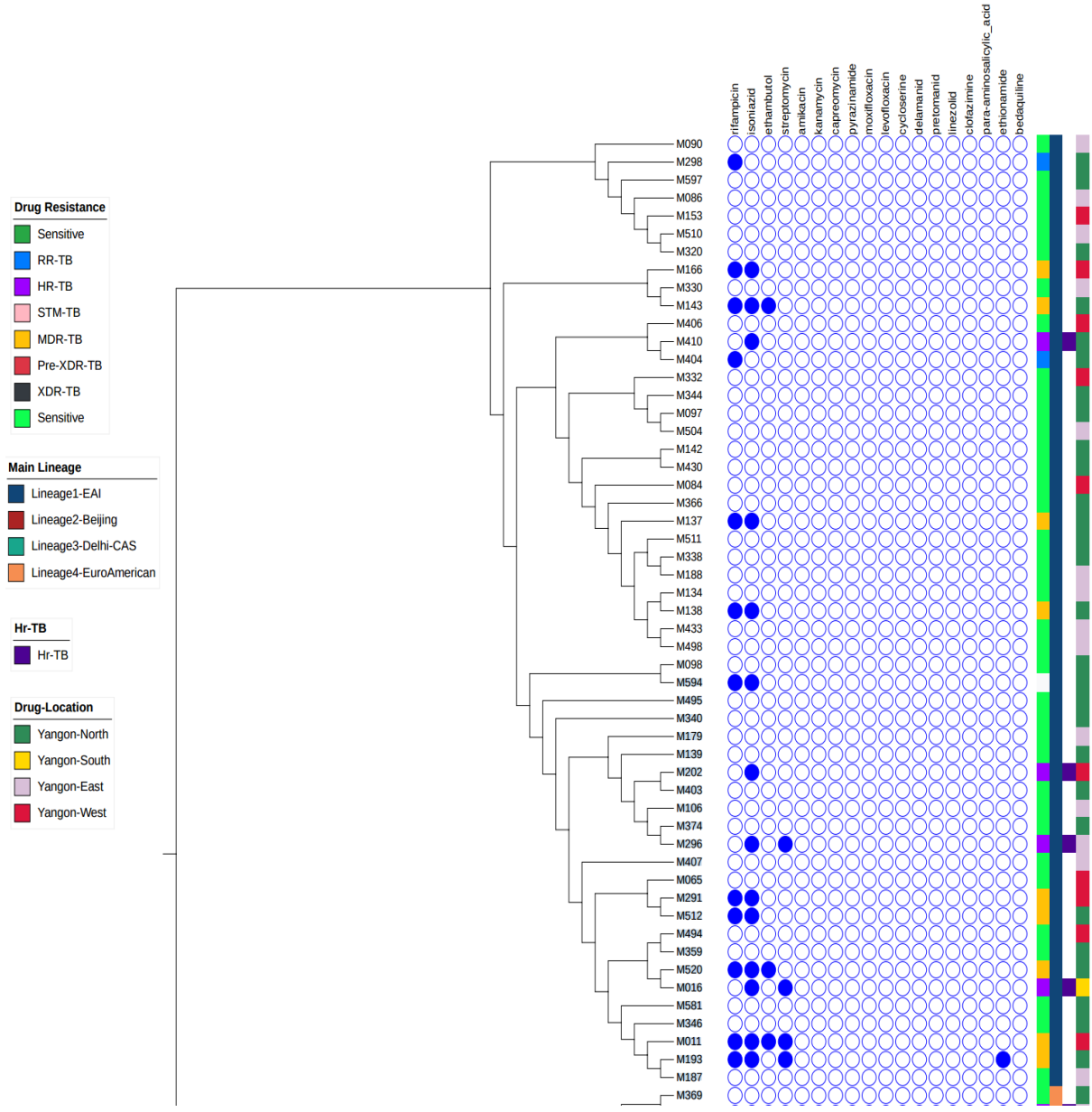
4.9.2.4 Supplementary Figure 4.

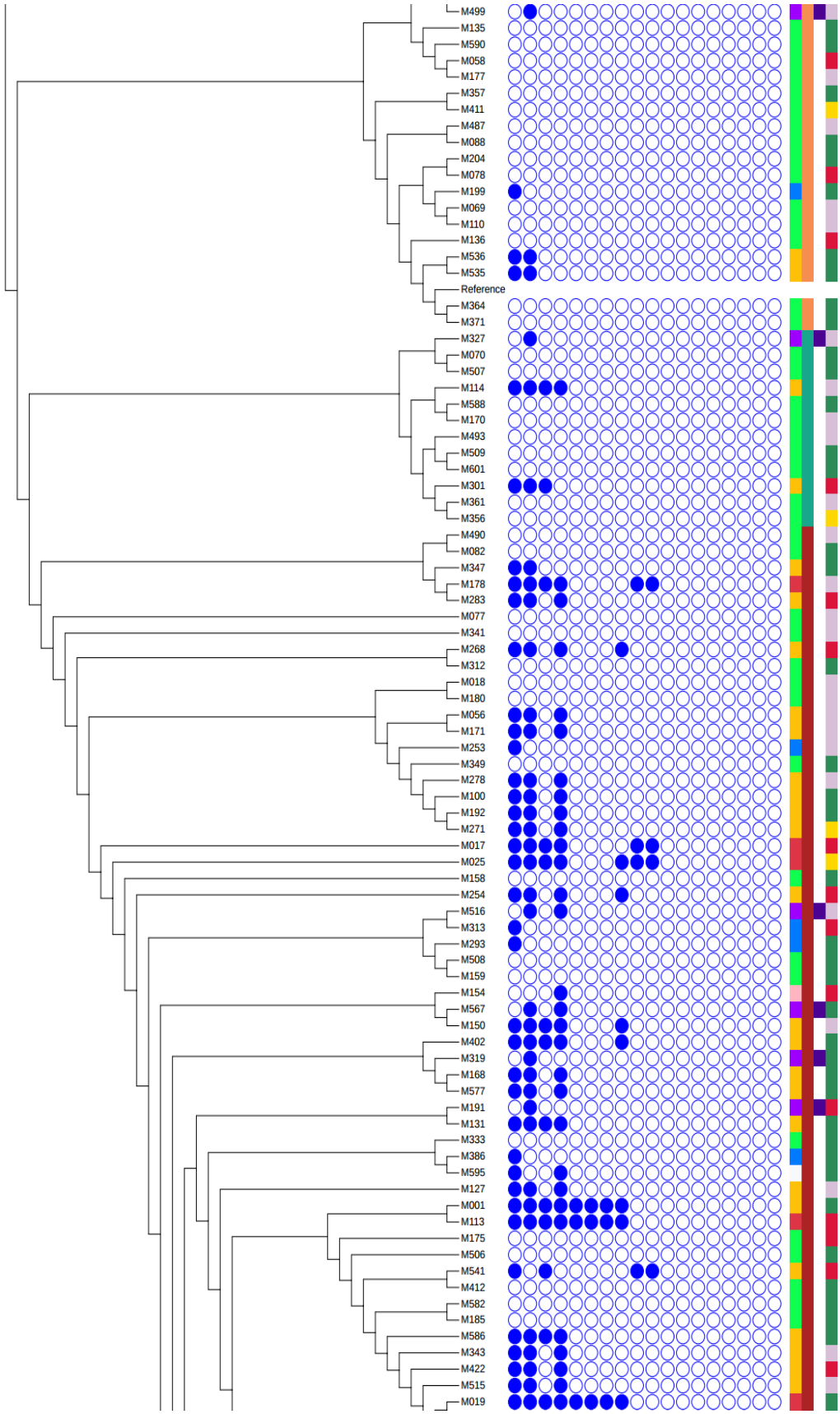
Supp. Fig 4-4 - A stacked bar chart of gender distribution across different drug resistance types indicated that MDR-TB was the most prevalent among both males and females. However, the proportion of males with MDR-TB was higher than that of females. Similarly, drug-sensitive cases were more common among males. Interestingly, Pre-XDR-TB and HR-TB displayed a relatively balanced gender distribution, whereas RR-TB and other types were predominantly observed in males. These patterns may reflect underlying differences in exposure, health-seeking behavior, or biological susceptibility between genders.



4.9.2.5 Supplementary Figure 5.

Supp. Fig 4-5 - The maximum likelihood phylogenetic tree (n=285) shows the evolutionary relationships among MTB isolates from WGS dataset constructed from SNP core via GTR-GAMMA model with bootstrap value of 1000. Branches are color-coded by lineage, and resistance profiles for isoniazid (INH), rifampicin (RIF), ethambutol (EMB), streptomycin (STR), pyrazinamide (PZA), fluoroquinolones (FLQ), second-line injectables (SLID), and new/repurposed drugs are indicated with colored bars. Geographic origin, sampling date, and significant resistance mutations (e.g., katG S315T, rpoB L430P/L452P, gyrA D94G) are annotated. Clusters of closely related isolates highlight potential transmission events. This figure aids in understanding MTB genetic relationships and drug resistance patterns.

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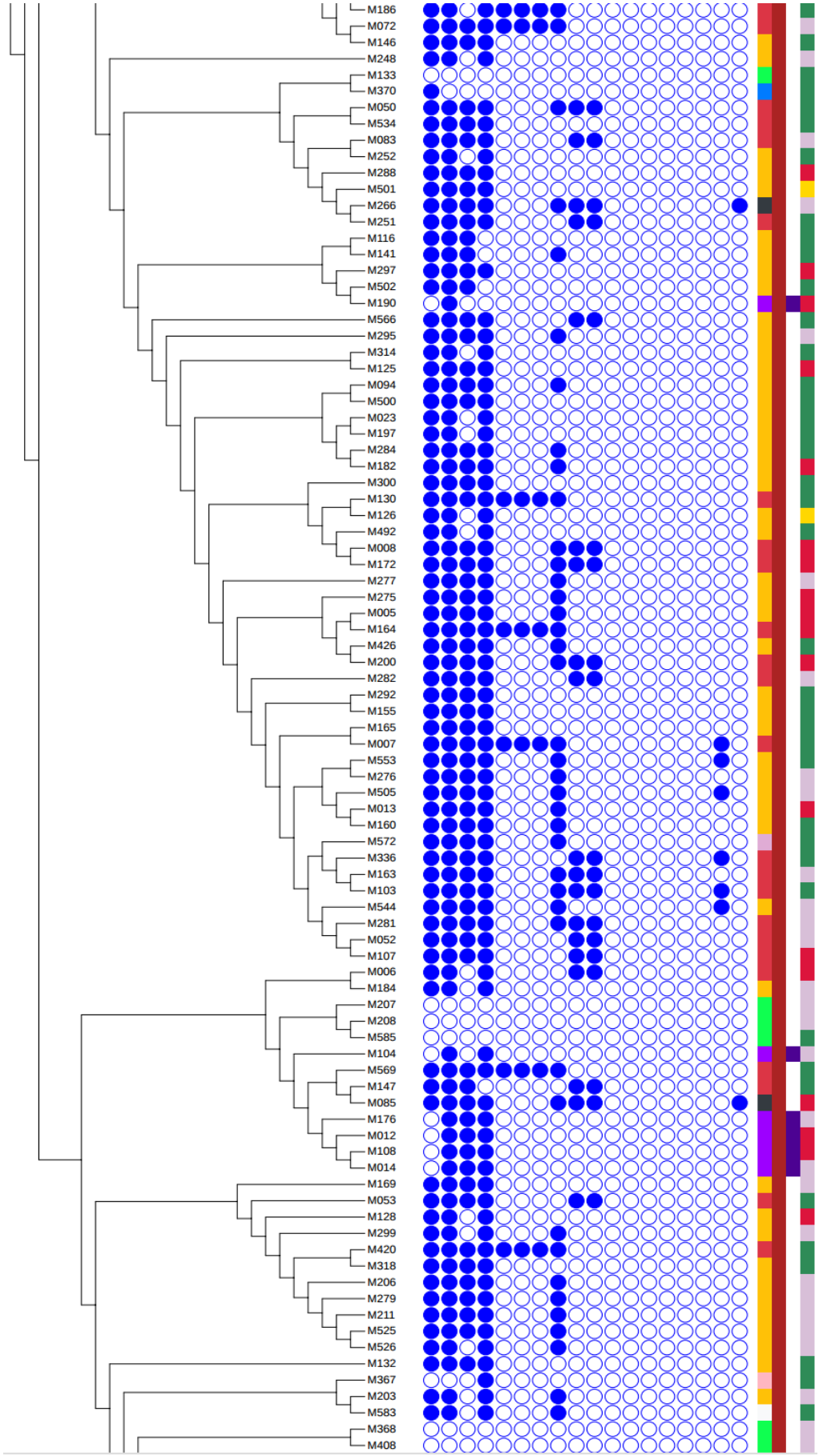
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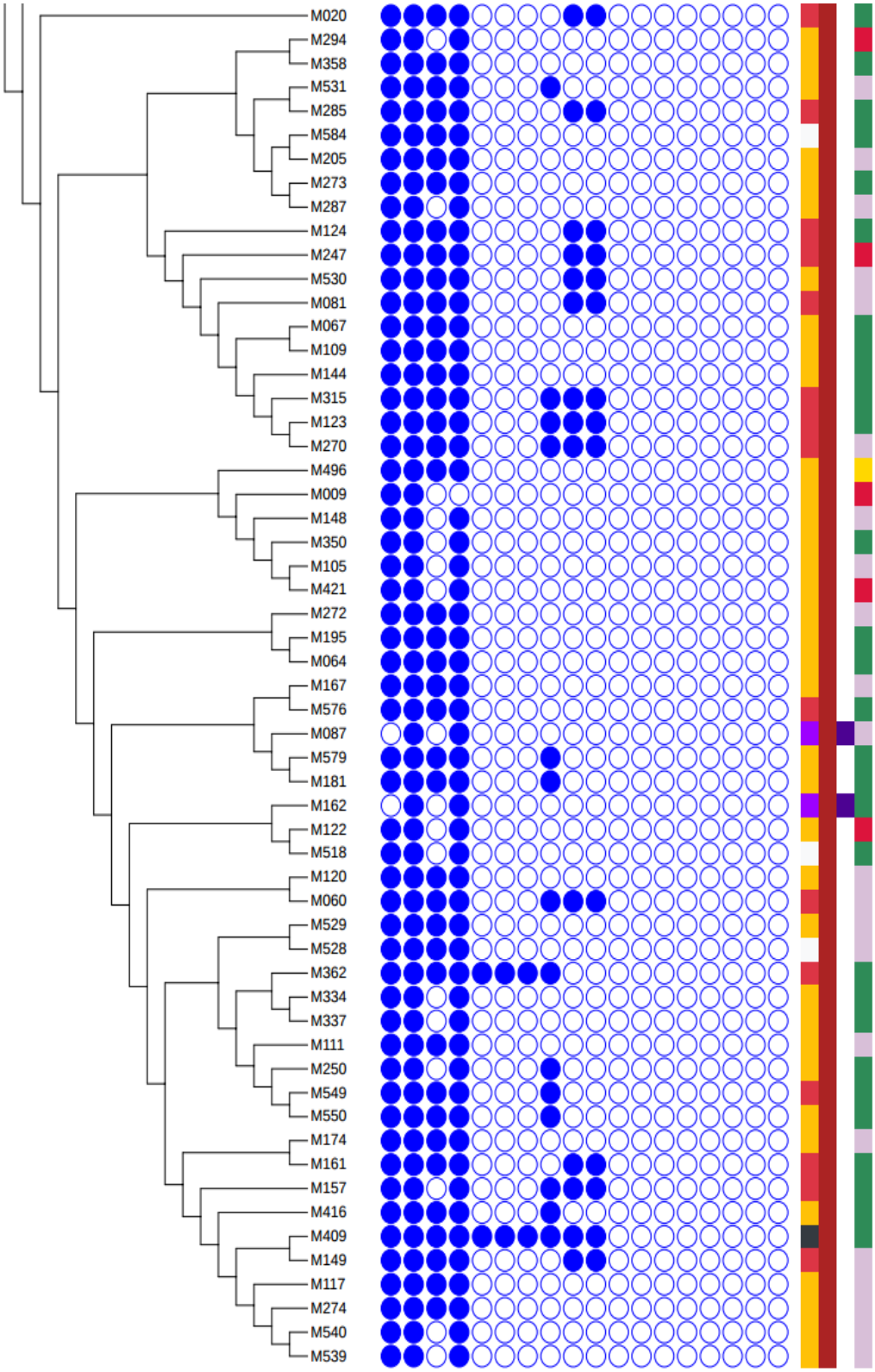
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Chapter 5

General Discussion, Synthesis, and Future Directions.

5.1 Introduction and Thesis Integration.

Tuberculosis (TB) remains a formidable global health challenge, with drug-resistant forms constituting a persistent threat to control efforts [1]. This doctoral research has employed advanced genomic technologies to address critical gaps in TB diagnosis and understanding within Myanmar's high-burden context. The research progression from microbiome characterization (Chapter 2) to comprehensive genomic surveillance (Chapter 3) and detailed analysis of lineage-specific pathogenicity and Hr-TB (Chapter 4) represents a logical trajectory from ecological context to molecular mechanism to applied diagnostic innovation. This final chapter synthesizes these findings, contextualizes them within global TB control strategies, acknowledges limitations, and proposes evidence-based recommendations for clinical practice and future research. Crucially, this work demonstrates how fundamental research can directly inform the development of practical diagnostic solutions, such as the patented Multiple Displacement Amplification (MDA) primers that emerged from microbiome investigations.

5.2 Synthesis of Key Findings: Connecting Microbiome, Genomics, and Diagnostics.

The research program revealed several interconnected findings that advance our understanding of TB in Myanmar. The initial microbiome characterization in Chapter 2 of this thesis provided not merely descriptive data but a foundational resource that enabled the rational design of targeted molecular tools [3]. This translation from basic science to applied technology exemplifies the translational approach characterizing this thesis. The development of MTB-specific MDA primers (Patent: Yang Fong, Massey University) as molecular tool, directly addressed the diagnostic challenge of detecting TB infections, demonstrating how genomic insights can drive innovation [4,5].

Whole-genome sequencing of 309 clinical isolates established the predominance of Lineage 2 (Beijing) strains and revealed a critical pattern of predominantly independent acquisition of resistance mutations rather than clonal transmission [7,8]. This finding fundamentally redirects public health strategy from focusing solely on transmission interruption to emphasizing prevention of resistance acquisition through improved treatment adherence and support systems [10,12]. The phylogenetic dispersion of resistant isolates underscores how programmatic weaknesses in treatment delivery contribute significantly to the drug resistance crisis in resource-limited settings [11,12].

Building on this genomic foundation, the research further elucidated the distinct epidemiological profiles of MTB lineages in Myanmar, associating Lineage 2 with enhanced

virulence and drug resistance [14,15]. Most significantly, the work identified a substantial burden of isoniazid-resistant, rifampicin-susceptible TB (Hr-TB) that is systematically missed by current diagnostic algorithms relying on rifampicin resistance as a proxy for MDR-TB [7,17]. This diagnostic blind spot has severe consequences, as patients with Hr-TB receive ineffective first-line regimens containing isoniazid, leading to poor outcomes and potentially amplifying resistance [19,20]. The finding underscores the urgent need for diagnostic approaches that specifically detect isoniazid resistance to guide appropriate therapy [21,28].

5.3 Public Health Implications and Global Relevance.

With the establishment of the nasopharyngeal and oropharyngeal microbiota profiles, we proceeded to the genomic profiling of MTB strains in Myanmar using WGS as our primary diagnostic method with recent published paper by Aung et al., (2021) [9]. We conducted and compared WGS dataset with traditional methods like the GeneXpert MTB/RIF cartridge to understand the genetic composition of antimicrobial MDR-TB with total of 309 clinical isolates collected from 2016 to 2019. The results highlighted a high prevalence of rifampin resistance with Lineage 2 -Beijing clade as dominance strain, and further genomic analysis revealed that most rifampin-resistant isolates were not genomically related [9]. This finding suggests that resistance primarily arises through spontaneous acquired mutations rather than the transmission of resistant strains [9]. This is likely due to inproper treatment or patient who stop the treatment regime halfway which suggested such issue may aid and drive the mutation rate in Yangon, Myanmar.

This insight has profound implications for Myanmar TB control strategies, emphasizing the need for rapid and accurate diagnostic tools that can detect drug resistance early and guide appropriate treatment regimens. WGS provides a more comprehensive understanding of drug resistance mechanisms by identifying specific genetic mutations associated with resistance. This level of detail allows for the development of targeted therapies that can address specific resistance mechanisms, potentially improving treatment outcomes in Myanmar. Additionally, WGS can further identify emerging resistance patterns, enabling public health officials to respond more quickly to new threats. Our findings underscore the significance of advanced genomic tools like WGS in the fight against TB. The ability to pinpoint specific genetic mutations associated with drug resistance offers significant advantages over traditional diagnostic methods, which often fail to capture the full scope of resistance mechanisms [9]. This comprehensive approach enhances understanding of TB's genetic landscape and can facilitates the development of more effective, targeted treatment strategies. Future studies

should aim to expand the application of WGS in TB diagnostics across different regions of Myanmar and as well as internationally. By increasing the geographic scope, there is a potential to gain further understanding of the genetic diversity and resistance patterns of MTB strains nationally and compare to global MDR-TB output. Additionally, there is a need to develop cost-effective and scalable WGS platforms that can be readily implemented in resource-limited settings. This will make advanced diagnostic tools accessible to a broader range of healthcare providers and patients.

Expanding on the genomic profiling, our research should also investigate the impact of environmental, co-infections and social factors on the emergence and spread of drug-resistant TB. This integrated approach could revolutionize the way we diagnose and treat TB, particularly in cases involving co-infections with other pathogens like HIV. Our results revealed that most MDR-TB cases are concentrated in the densely populated slum areas of Yangon, Myanmar, particularly in the South and North Okkalapa regions. These regions are characterized by crowded living conditions, poor healthcare infrastructure, and low socioeconomic status, all of which facilitate TB transmission and resistance. Limited access to healthcare services leads to late diagnoses and incomplete treatments, while poverty and lack of education contribute to the spread of TB and development of drug-resistant strains. Addressing these issues requires improved healthcare accessibility, public health education, and better living conditions to reduce TB transmission and resistance effectively. Additionally, exploring the role of host genetics in TB susceptibility and treatment response could provide deeper insights into the interaction between human hosts and MTB. Studies focusing on the genetic variations in human populations that affect immune responses to TB can inform personalized medicine approaches, potentially leading to more effective treatments based on individual genetic profiles.

Furthermore, implementing robust surveillance systems that utilize WGS could significantly enhance our ability to monitor TB outbreaks and resistance trends. Establishing centralized databases for genomic data can facilitate real-time tracking of TB cases and enable rapid response to emerging threats. International cooperation and data sharing will be crucial in managing TB at a global level. In terms of computational advancements, there is a need to improve the sensitivity and specificity of genomic analyses. Developing more diverse and sensitive computational analysis software tools can help accurately identify resistance mutations and other genetic markers. Enhanced algorithms for data analysis and interpretation will aid in understanding the genetic landscape of TB, leading to more effective monitoring and control strategies.

Lastly, public health education campaigns should be strengthened to raise awareness about TB prevention, diagnosis, and treatment. Educating communities about the importance of early diagnosis and adherence to treatment regimens can reduce the spread of TB and improve treatment outcomes. Engaging with local communities and leveraging the expertise of healthcare workers can ensure that these educational efforts are culturally appropriate and effective.

5.4 Methodological Strengths, Limitations, and Diagnostic Implications.

This PhD research exhibits significant methodological strengths that directly support its potential for advancing rapid tuberculosis diagnosis and improving clinical outcomes. The integrative approach connecting microbiome science, bacterial genomics, and diagnostic development represents a particular innovation, as it demonstrates a clear translational pathway from fundamental microbial ecology to applied clinical tool development. This connected methodology is especially valuable for addressing complex pathogens like MTB that operate within host-microbe environments [47,48].

The progression from initial microbiome characterisation to the development of targeted Multiple Displacement Amplification primers exemplifies a rational design process that leverages ecological data for diagnostic innovation [2,49]. By first establishing baseline microbiota profiles in the Myanmar population, the research team identified specific genetic targets that could be exploited for MTB-specific enrichment, thereby addressing the critical diagnostic challenge of detecting paucibacillary infections and degraded DNA samples commonly encountered in clinical practice [50,51]. This approach demonstrates how understanding microbial community dynamics can directly inform the development of more sensitive diagnostic tools.

The whole-genome sequencing component employed in this research provides a level of resolution that surpasses conventional diagnostic methods, enabling comprehensive resistance profiling that is essential for guiding appropriate treatment decisions [25,26]. The ability to identify specific resistance-conferring mutations across the entire genome, rather than targeting limited genetic regions as in most molecular assays, offers significant advantages for detecting emerging resistance patterns and complex resistance profiles that might otherwise be missed [52,53]. This comprehensive approach is particularly crucial for managing isoniazid-resistant TB, where conventional methods provide incomplete information that can lead to inappropriate treatment regimens [11,15].

The methodological approach also demonstrates scalability and adaptability across different healthcare settings. The successful application of these genomic techniques to both the high-burden context of Myanmar and the distinct epidemiological situation in New Zealand indicates that the underlying methodologies are robust and transferable [29,30]. This cross-context validation strengthens the potential for these approaches to be implemented in diverse clinical settings, from reference laboratories in low-resource areas to advanced facilities in high-income countries [54,55].

However, several methodological limitations must be acknowledged. The facility-based, cross-sectional design of the primary studies limits our ability to assess longitudinal strain evolution or long-term patient outcomes associated with different resistance profiles [56,57]. While the genomic data provide detailed snapshots of resistance patterns at specific time points, they cannot capture the dynamic processes of resistance acquisition and transmission that occur over extended periods. Future research should incorporate longitudinal sampling designs to better understand these evolutionary dynamics and their clinical implications.

Furthermore, while the development of MDA primers represents a significant technical advance, their full clinical validation and integration with portable sequencing platforms constitute essential future work [58,59]. The transition from laboratory-based validation to clinical implementation requires addressing practical challenges related to sample processing, workflow integration, and result interpretation in resource-limited settings [60,61]. Additional studies are needed to establish the analytical and clinical performance characteristics of these primers across diverse patient populations and specimen types, as well as to develop standardised protocols for their use in point-of-care settings [62,63].

The methodological approach also faces challenges related to cost and infrastructure requirements for implementation in low-resource settings. While WGS provides unparalleled resolution for resistance detection, its current cost and technical requirements may limit widespread adoption in precisely the settings where it is most needed [64,65]. Future work should focus on streamlining sequencing protocols, reducing reagent costs, and developing simplified bioinformatics pipelines that can be operated by personnel with limited technical training [66,67].

Despite these limitations, the methodological framework established in this research provides a strong foundation for developing rapid, comprehensive TB diagnostic solutions that can significantly impact clinical outcomes. By enabling earlier detection of drug resistance and more targeted treatment selection, these approaches have the potential to reduce treatment

failure rates, prevent further resistance development, and ultimately improve patient outcomes in high-burden settings like Myanmar [68,69].

5.5 Policy Implications and Recommendations for Practice.

Based on the outcome from this research, several evidence-based recommendations emerge for national and regional TB control programs. First, National TB programs should urgently incorporate WHO-recommended molecular assays that detect isoniazid resistance alongside rifampicin resistance to prevent the systematic misdiagnosis and consequent mismanagement of Hr-TB cases [15,70]. The current reliance on rifampicin resistance as a proxy for MDR-TB represents a dangerous oversimplification that leads to inappropriate treatment and potentially amplifies drug resistance [11,13]. While implementing WGS widely remains a longer-term goal, establishing centralised sequencing services for complex cases and surveillance would provide valuable resistance data for guiding treatment decisions and public health policy [25,71]. These reference services could serve as regional hubs for comprehensive resistance testing, particularly for pre-XDR and XDR-TB cases that require complex drug regimens [72,73]. The data generated would also support the development of local resistance databases that reflect the specific epidemiological context of Myanmar and the Southeast Asia region [74,75].

Futher investigation should prioritise validating and scaling rapid, low-cost technologies such as the MDA primers developed in this research, particularly for resource-limited settings where traditional laboratory infrastructure may be limited [49,76]. These technologies could be integrated with emerging portable sequencing platforms to create comprehensive diagnostic solutions that provide rapid resistance profiling at or near the point of care [58,77]. Operational research is needed to assess the feasibility, cost-effectiveness, and health systems requirements for implementing such tools within the routine workflow of the Myanmar National TB Reference Laboratory and regional laboratories [60,78]. The finding that resistance often emerges during treatment underscores the critical need for robust patient support systems to prevent acquisition of resistance [7,79]. Policy must therefore advocate for and fund interventions such as dedicated counselling, nutritional support, transportation vouchers, and digital health reminders to mitigate default rates and protect the efficacy of first-line regimens [80,81]. These support systems are particularly important in the context of Myanmar's challenging socioeconomic environment, where multiple barriers to treatment adherence exist [17,21].

Finally, Myanmar should establish a formal framework for a national genomic surveillance system for MTB [35,82]. This system would enable real-time tracking of resistant and virulent strains, facilitate the identification of outbreaks as they occur, and inform targeted public health responses to emerging threats [83,84]. The integration of genomic data with conventional epidemiological information would create a powerful surveillance tool that could significantly enhance TB control efforts and provide early warning of new resistance threats [85,86].

5.6 Future Perspectives: Next-Generation Diagnostics and Implementation Science.

The future of TB diagnostics is advancing toward ultra-sensitive, rapid, and field-deployable solutions that could dramatically improve case detection and treatment monitoring. The MDA primers developed in this thesis represent a crucial step toward integration with third-generation sequencing platforms, but several other technological directions show particular promise for transforming TB diagnosis in the coming years [58,87]. CRISPR-Cas based diagnostic platforms such as SHERLOCK and DETECTR offer exceptional sensitivity and specificity for detecting resistance mutations directly from clinical samples in under an hour [88,89]. These systems can be designed for multiplex detection, allowing comprehensive resistance profiling at point-of-care settings that currently lack advanced laboratory capabilities [90,91]. The programmability of CRISPR systems makes them particularly adaptable for detecting emerging resistance mutations, providing a flexible platform that can be rapidly updated as new resistance mechanisms are identified [92,93].

Parallel developments in nanopore sequencing continue to evolve toward true same-day, near-patient whole genome sequencing, with ongoing improvements in accuracy, throughput, and cost-effectiveness [94,95]. The MiniON platform and similar devices are becoming increasingly suitable for deployment in reference laboratories in high-burden countries, providing near-complete genomic information that surpasses the capabilities of targeted molecular assays [96,97]. Future integration of isothermal amplification methods with streamlined sequencing in cartridge-based formats could potentially enable complete resistance profiling during a single clinical visit, dramatically reducing the time to appropriate treatment initiation [98,99].

Complementary technologies including digital PCR for detecting heteroresistance [100,101], microfluidic devices for automated sample processing [102,103], and AI-assisted diagnosis for interpreting complex data [104,105] offer additional avenues for innovation. These technologies could be integrated into comprehensive diagnostic systems that provide end-to-end solutions from sample processing to result interpretation, making advanced diagnostics

accessible in settings with limited technical expertise [106,107]. Implementation of these technologies requires parallel research efforts beyond technical development. Health economic analyses comparing the long-term costs of novel diagnostics versus current care are essential for convincing policymakers and securing funding [108,109]. These analyses must account for the often-hidden costs of misdiagnosed Hr-TB and MDR-TB, including extended infectious periods, need for more expensive second-line drugs, and associated loss of productivity [110,111].

Geographical expansion of genomic surveillance is needed to understand regional strain diversity and transmission dynamics across Myanmar and Southeast Asia [74,112]. This expanded surveillance would provide crucial data for ensuring that diagnostic tests are calibrated for local epidemiology and for detecting emerging threats before they become widespread [113,114]. Host-directed biomarker discovery represents another important direction, with potential for developing rapid tests based on serum or urine biomarkers that could detect active TB and treatment response, particularly in challenging cases like paediatric TB and extrapulmonary TB [115,116]. Finally, implementation science research is critically needed to study how to optimally integrate these advanced technologies into diverse healthcare settings [117,118]. This includes understanding barriers to adoption, developing appropriate training programs for healthcare workers, creating sustainable maintenance and supply chains, and ensuring that new technologies actually improve patient outcomes in real-world conditions [119,120]. Without attention to these implementation challenges, even the most promising technologies may fail to achieve their potential impact on TB control.

5.7 Conclusion.

This thesis has demonstrated the transformative potential of genomic approaches for understanding and combating tuberculosis in Myanmar. The research has moved beyond simply documenting drug resistance to uncovering its fundamental drivers including programmatic weaknesses in treatment delivery and critical diagnostic blind spots, while simultaneously developing innovative solutions to address these challenges. The integrative approach connecting microbiome science, bacterial genomics, and diagnostic development provides a model for translational research that addresses real-world problems in resource-limited settings. The findings presented here make a compelling case for the transition toward precision medicine in TB care, enabled by comprehensive genomic information that exceeds the capabilities of conventional diagnostic methods. The identification of a substantial burden of isoniazid-resistant TB that is systematically missed by current algorithms highlights a

critical gap in TB management that requires urgent attention. The development of targeted molecular tools such as MDA primers represents a practical response to this challenge, demonstrating how fundamental research can directly inform diagnostic innovation.

The integration of these genomic insights into public health policy represents an urgent priority for changing the trajectory of TB epidemics in high-burden settings. By embracing a comprehensive strategy that combines precision diagnostics, personalised treatment, and strengthened patient support, Myanmar and similar countries can address both the biomedical and social dimensions of the TB crisis. The findings and technologies presented here offer a roadmap for this transition, contributing to the global effort to end TB through innovation, equity, and evidence-based practice. The continued development and implementation of advanced diagnostic technologies, coupled with strengthened health systems and attention to social determinants of health, will be essential for achieving lasting progress against this ancient disease. As these technologies evolve and become more accessible, they have the potential to dramatically improve case detection, enable more targeted treatment, and ultimately reduce the burden of drug-resistant tuberculosis in Myanmar and beyond.

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Addendum

Additional Publications and Information

This addendum brings together the supporting publications and additional materials that have been part of my research journey throughout this PhD. While the core chapters of this thesis present my central findings, the works included here represent important complementary studies that have informed my methodological development, deepened my contextual understanding, and expanded the applications of the genomic approaches I've explored.

These publications trace my growing expertise in microbial genomics and its application to diagnostic challenges, particularly in detecting antimicrobial resistance. Although they don't form the fundamental chapters of this thesis, they reflect the intellectual pathway and technical growth that supported my primary research. The inclusion of work extending beyond *Mycobacterium tuberculosis* demonstrates how I've tested the transferability of genomic approaches across different pathogens, ultimately strengthening the methodological foundation of my core thesis research.

By including these supplementary works, I aim to provide a complete picture of the research ecosystem in which I've developed this thesis, offering additional context for my methodologies and showing the broader potential of the genomic strategies I've advanced during my doctoral studies.

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STATEMENT OF CONTRIBUTION
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We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Yang Fong
Name/title of Primary Supervisor:	Peter Lockhart, Professor
In which chapter is the manuscript /published work:	Appendix

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Title: Association between anti-tuberculosis drug resistance-conferring mutations and treatment outcomes in Myanmar (2017).
DOI: 10.1080/23744235.2017.1404632.

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:
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Candidate's Signature:	
Date:	24-Jan-2024
Primary Supervisor's Signature:	Peter Lockhart Digitally signed by Peter Lockhart Date: 2024.01.30 17:18:23 +13'00'
Date:	24-Jan-2024

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GRS Version 5 – 13 December 2019
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Supplementary Publication 1:

Title: Association between anti-tuberculosis drug resistance-conferring mutations and treatment outcomes in Myanmar.

Publisher: Taylor and Francis, Infectious Disease.

Authors: Htin Lin Aung, Thanda Tun, Wint Wint Nyunt, Yang Fong, John A Crump, Kyi Kyi Thinn, Si Thu Aung, Gregory M Cook.

DOI: 10.1080/23744235.2017.1404632

Status: Published, 2018.

Hyperlink: https://www.tandfonline.com/doi/abs/10.1080/23744235.2017.1404632

Contribution: Yang Fong; Methodology, sample preparation, DNA sequencing, data analysis and curation, writing original draft and review.

Summary:

Drug-resistant tuberculosis, including multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains, poses a significant global health challenge due to its association with poor treatment outcomes. This study investigates the relationship between mutations conferring drug resistance and treatment outcomes among tuberculosis patients in Myanmar. Whole-genome sequencing (WGS) was performed on MDR-TB isolates to identify resistance-conferring mutations. Our findings reveal a strong correlation between specific genetic mutations and unfavorable treatment outcomes, particularly in cases involving resistance to second-line drugs. The study underscores the importance of incorporating clinical outcomes and patient demographics in future WGS studies to enhance the effectiveness of treatment regimens. This research highlights the potential of WGS as a diagnostic tool to guide treatment and improve patient outcomes, ultimately contributing to the reduction of tuberculosis-related mortality.

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Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Title: First 2 Extensively Drug-Resistant Tuberculosis Cases From Myanmar Treated with Bedaquiline.
DOI: 10.1093/cid/cix365

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:
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Supplementary Publication 2:

Title: First 2 Extensively Drug-Resistant Tuberculosis Cases From Myanmar Treated With Bedaquiline.

Publisher: Oxford Academic, Clinical Infectious Diseases

Authors: Htin Lin Aung, Wint Wint Nyunt, Yang Fong, Gregory M. Cook, Si Thu Aung

DOI: 10.1093/cid/cix365

Status: Published, 2017.

Hyperlink: https://academic.oup.com/cid/article/65/3/531/3749833

Contribution: Yang Fong; Conceptualization, methodology, sample preparation, DNA sequencing, data analysis and curation, writing original draft and review.

Summary:

This study reports the first two cases of extensively drug-resistant tuberculosis (XDR-TB) in Myanmar treated with bedaquiline. Diagnosed through routine GeneXpert testing and confirmed via whole-genome sequencing (WGS) and phenotypic drug susceptibility testing, both patients exhibited resistance to fluoroquinolones and aminoglycosides, but remained susceptible to bedaquiline and linezolid. The failure of conventional MDR-TB treatments prompted the use of bedaquiline under the EndTB program, a collaborative effort between the Myanmar National TB Programme and Médecins Sans Frontières. This study highlights the importance of early and accurate diagnosis of XDR-TB using WGS to guide appropriate treatment, underscoring the potential benefits of incorporating WGS into TB management strategies, especially in low-resource settings.

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Name/title of Primary Supervisor:	Peter Lockhart, Professor
In which chapter is the manuscript /published work:	Appendix

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Title: Acquired Resistance to Antituberculosis Drugs (2018).
DOI: 10.3201/eid2411.180465

( ) The manuscript is currently under review for publication – please indicate:

- The name of the journal:
- The percentage of the manuscript/published work that was contributed by the candidate:
- Describe the contribution that the candidate has made to the manuscript/published work:

( ) It is intended that the manuscript will be published, but it has not yet been submitted to a journal

Candidate's Signature:	
Date:	24-Jan-2024
Primary Supervisor's Signature:	Peter Lockhart Digitally signed by Peter Lockhart Date: 2024.01.30 17:21:12 +13'00'
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GRS Version 5 – 13 December 2019
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Supplementary Publication 3:

Title: Acquired Resistance to Antituberculosis Drugs.

Publisher: Centers for Disease Control and Prevention, Emerging Infectious Diseases.

Authors: Htin Lin Aung, Wint Wint Nyunt, Yang Fong, Bruce Russell, Gregory M. Cook, and Si Thu Aung.

DOI: 10.3201/eid2411.180465

Status: Published, 2018.

Hyperlink: https://wwwnc.cdc.gov/eid/article/24/11/18-0465_article

Contribution: Yang Fong; Conceptualization, methodology, sample preparation, DNA sequencing, data analysis and curation, writing original draft and review.

Summary:

This study investigates the association between mutations that confer drug resistance and treatment outcomes in tuberculosis (TB) patients in Myanmar. By employing whole-genome sequencing (WGS) on multidrug-resistant TB (MDR-TB) isolates, the research identifies specific genetic mutations linked to poor treatment outcomes. Our findings reveal that strains resistant to second-line drugs often lead to unfavorable results, highlighting the necessity for early and precise diagnosis using WGS. Additionally, the study emphasizes the importance of integrating clinical outcomes and patient demographics into future WGS research to improve treatment regimens. The results underscore WGS's potential as a diagnostic tool to enhance TB management, reduce mortality rates, and combat drug-resistant TB effectively.

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Name of candidate:	Yang Fong
Name/title of Primary Supervisor:	Peter Lockhart, Professor
In which chapter is the manuscript /published work:	Appendix

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Title: Evaluation of the Rapid Molecular Diagnostics Test for the New Zealand Mycobacterium Tuberculosis Rangipo Strain in a Clinical Setting (2018).
Issued: The New Zealand Medical Journal 131(1478):70-72

( ) The manuscript is currently under review for publication – please indicate:

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Candidate's Signature:	
Date:	24-Jan-2024
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GRS Version 5 – 13 December 2019
DRC 19/09/10

Supplementary Publication 4:

Title: Evaluation of the rapid molecular diagnostic test for the New Zealand *Mycobacterium tuberculosis* Rangipo strain in a clinical setting.

Publisher: New Zealand Medical Journal.

Authors: Claire V. Mulholland, Duncan Thorpe, Ray T. Cursons, Noel Karalus, Yang Fong, Vickery L. Arcus, Gregory M. Cook, Htin Lin Aung.

DOI: The New Zealand Medical Journal 131(1478):70-72

Status: Published, 2018.

Hyperlink: https://pubmed.ncbi.nlm.nih.gov/30001311/

Contribution: Yang Fong; DNA sequencing, data analysis and curation, writing draft and review.

Summary:

This study evaluates the performance of a rapid molecular diagnostic test for identifying the New Zealand *Mycobacterium tuberculosis* Rangipo strain in clinical settings. The Rangipo strain is responsible for a significant proportion of TB cases among the indigenous Māori population in New Zealand. Utilizing a whole genome sequencing-directed, single nucleotide polymorphism (SNP)-based PCR-RFLP diagnostic, we demonstrated the test's ability to accurately differentiate the Rangipo strain from other M. tuberculosis strains directly from sputum samples within 24 hours. This rapid identification significantly reduces the turnaround time compared to traditional methods, which require 3-4 weeks for strain typing. Our findings suggest that this affordable and reliable diagnostic tool can be effectively used in clinical settings to facilitate timely intervention and control of the Rangipo strain, thereby preventing further transmission and outbreaks. This study underscores the importance of rapid diagnostics in managing TB, especially in socioeconomically disadvantaged populations with high disease burden.

Massey University Statement of Contribution (DRC 16) Declaration Form Supp 5.

DRC 16

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Yang Fong
Name/title of Primary Supervisor:	Peter Lockhart, Professor
In which chapter is the manuscript /published work:	Appendix

Please select one of the following three options:

(●) The manuscript/published work is published or in press

- Please provide the full reference of the Research Output:

Title: High Rate of Reinfection and Possible Transmission of Mycobacterium avium complex in Northeast Thailand (2022).
DOI: 10.1016/j.onehlt.2022.100374

( ) The manuscript is currently under review for publication – please indicate:

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Primary Supervisor's Signature:	Peter Lockhart Digitally signed by Peter Lockhart Date: 2024.01.30 17:19:55 +13'00'
Date:	24-Jan-2024

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GRS Version 5 – 13 December 2019
DRC 19/09/10

Supplementary Publication 5:

Title: High rate of reinfection and possible transmission of *Mycobacterium avium* complex in Northeast Thailand.

Publisher: Science Direct, Elsevier, One Health.

Authors:
Wicharajit Boonjetsadaruhk, Orawee Kaewprasert, Arnone Nithichanon, Pimjai Ananta, Prajuab Chaimanee, Kanin Salao, Wisitsak Phoksawat, Marut Laohaviroj, Auttawit Sirichoat, Yang Fong, Suwin Wongwajana, Wises Namwat, Viraphong Lulitanond, Ploenchan Chetcho tisakd, Kiatichai Faksri.

DOI: 10.1016/j.onehlt.2022.100374.

Status: Published, 2022.

Hyperlink: https://www.sciencedirect.com/science/article/pii/S2352771422000064

Contribution: Yang Fong; DNA sequencing, data analysis and curation and review draft.

Abstract:

The *Mycobacterium avium* complex (MAC) includes two main species of non-tuberculous mycobacteria (NTM), M. avium and Mycobacterium intracellulare. These can cause serious disease, especially in immunocompromised patients. Little information is available concerning genetic diversity of NTM. We used multilocus sequence typing (MLST) based on a highly discriminative gene set to analyze MAC serially isolated from patients to determine the rate of MAC reinfection. Genomic DNA was sequenced from 49 MAC isolates (15 cases comprised of 11 true infections and 4 instances of colonization). More than half of the MAC isolates tested were found to be multidrug resistant. The discriminatory power was assessed of 24 house-keeping genes (fusA, atpD, pheT, glnA, topA, secA, argH, glpK, murC, cya, pta, rrl, rrs, hsp65, rpoB, 16S-23S rRNA ITS, recF, lipT, pepB, gnd, aspB, groEL, sodA and est) previously used for genotyping of MAC and other NTM. Seven genes (fusA, secA, rpoB, hsp65, 16S rRNA, 23S rRNA, 16S-23S rRNA ITS) had a discriminatory power index higher than 0.9 and were included in the optimized set that we used. This set was significantly better for genotyping and diagnosis of MAC than previously used 4-gene, 5-gene and 9-gene sets. MLST using our 7-gene set indicated that the rate of reinfection was 54.55% (6/11 cases). Persistent infections (n = 5 cases, 45.45%) were found. A changing of clone in the same patient was found in 1/4 (25%) of the colonization cases. Two small clusters of possible MAC transmission between humans

were found. Our study demonstrated that the high frequency of apparent treatment failure of MAC might be artefactual, as a consequence of a high rate of MAC reinfection in Thai population. Our useful highly discriminative gene set for MAC species and clonal strain analysis could be further applied for the diagnosis and patient management.

Metadata of Mycobacterium Tuberculosis Samples in Myanmar: Distribution and Drug Resistance (2016-2019).

No	Sample Name	GPS Latitude	GPS Longitude	Year	District_Yangon	District	Gender	Age	HIV	DM	Treatment History	DST	Drug_Resistance	Xpert MTB/RIF	Lineage
1	M001	16.916964	96.1221419	2016	North	N	F	55			New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
2	M005	16.8757	96.1127831	2017	West	W	F	39			New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
3	M006	16.869572	96.1179543	2016	West	W	M	31			Retreatment	R: RIF, INH, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
4	M007	16.922222	96.09425	2017	North	N	M	43			New	R: RIF, INH, EMB, STM, PZA, KAN, AMK, CAP, ETH	Pre-XDR-TB	RR	lineage2-Beijing
5	M008	16.872321	96.115831	2016	West	W	M	33			Retreatment	R: RIF, INH, EMB, STM, FQ, PZA, KAN, AMK, CAP	Pre-XDR-TB	RR	lineage2-Beijing
6	M009	16.847286	96.1154964	2016	West	W	F	33			New	R: RIF, INH	MDR-TB	RR	lineage2-Beijing
7	M011	16.84632	96.1169581	2016	West	W	M	63		1	New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage1-EAI
8	M012	16.869639	96.1157278	2016	West	W	M	55			New	R: INH, EMB, STM	HR-TB	T	lineage2-Beijing
9	M013	16.87135	96.1127243	2016	West	W	M	19	1		New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
10	M014	16.925949	96.1843662	2016	East	E	F	38			New	R: INH, EMB, STM	HR-TB	T	lineage2-Beijing
11	M016	16.89164	96.0603359	2016	South	S	M	48			New	R: INH, STM	HR-TB	T	lineage1-EAI
12	M017	16.890378	96.1162778	2017	West	W	M	52			Retreatment	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
13	M018	16.881763	96.1800524	2017	East	E	M	75		1	New	Sensitive	Sensitive	T	lineage2-Beijing
14	M019	16.919905	96.0948425	2017	North	N	M	30			Retreatment	R: RIF, INH, EMB, STM, PZA, KAN, AMK, CAP	Pre-XDR-TB	RR	lineage2-Beijing
15	M020	16.89595	96.1374389	2016	North	N	M	42			Retreatment	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
16	M023	16.908601	96.1688957	2016	North	N	F	38			New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
17	M025	16.879095	96.0631968	2017	South	S	F	33			Retreatment	R: RIF, INH, EMB, STM, FQ, PZA	Pre-XDR-TB	RR	lineage2-Beijing
18	M050	16.880938	96.0697089	2017	North	N	M	36			Retreatment	R: RIF, INH, EMB, STM, FQ, PZA	Pre-XDR-TB	RR	lineage2-Beijing
19	M052	16.939179	96.1721075	2017	East	E	M	44			Retreatment	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
20	M053	16.919954	96.0534231	2016	North	N	F	41			Retreatment	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
21	M056	16.87835	96.1892386	2017	East	E	M	38			New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
22	M058	16.855706	96.1141426	2016	West	W	M	33			New	Sensitive	Sensitive	T	lineage4-Haarlem
23	M060	16.931341	96.192729	2017	East	E	M	37			Retreatment	R: RIF, INH, EMB, STM, FQ, PZA	Pre-XDR-TB	RR	lineage2-Beijing
24	M064	16.888027	96.1077875	2016	North	N	M	28			Retreatment	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
25	M065	16.850351	96.1141824	2017	West	W	M	66		1	New	Sensitive	Sensitive	T	lineage1-EAI
26	M067	16.923994	96.1592555	2017	North	N	F	22			Retreatment	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
27	M069	16.892173	96.1913267	2016	East	E	F	29			New	Sensitive	Sensitive	T	lineage4-EuroAmerican
28	M070	16.877077	96.1143433	2017	North	N	M	26			New	Sensitive	Sensitive	T	lineage3-Delhi/CAS
29	M072	16.931957	96.1946585	2019	East	E	M	47			Retreatment	R: RIF, INH, EMB, STM, PZA, KAN, AMK, CAP	Pre-XDR-TB	RR	lineage2-Beijing
30	M077	16.902284	96.1900486	2017	East	E	M	20			New	Sensitive	Sensitive	T	lineage2-Beijing
31	M078	16.863648	96.1167758	2017	West	W	F	26			New	Sensitive	Sensitive	T	lineage4-EuroAmerican
32	M081	16.935429	96.1890419	2017	East	E	M	42			Retreatment	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
33	M082	16.899561	96.1326252	2017	North	N	F	32			New	Sensitive	Sensitive	T	lineage2-Beijing
34	M083	16.906934	96.173832	2017	East	E	M	21	1		New	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
35	M084	16.876994	96.1177347	2016	West	W	F	34			New	Sensitive	Sensitive	T	lineage1-EAI

36	M085	16.867776	96.1173336	2017	West	W	M	37		Retreatment	R: RIF, INH, EMB, STM, FQ, PZA, BDQ	XDR-TB	RR	lineage2-Beijing
37	M086	16.929531	96.1942184	2017	East	E	F	52	1	New	Sensitive	Sensitive	T	lineage1-EAI-Manila
38	M087	16.921673	96.18581	2016	East	E	M	21		New	R: INH, STM	HR-TB	T	lineage2-Beijing
39	M088	16.918359	96.0583989	2017	North	N	M	50	1	New	Sensitive	Sensitive	T	lineage4-EuroAmerican
40	M090	16.916131	96.1740037	2016	East	E	M	31	1	New	Sensitive	Sensitive	T	lineage1-EAI-Manila
41	M094	16.887207	96.1411399	2017	North	N	M	66	1	New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
42	M097	16.882243	96.0932271	2017	North	N	M	28		New	Sensitive	Sensitive	T	lineage1-EAI
43	M098	16.923834	96.1103503	2017	North	N	F	23		New	Sensitive	Sensitive	T	lineage1-EAI
44	M100	16.908388	96.1299367	2017	North	N	M	32		Retreatment	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
45	M103	16.924979	96.1116108	2017	North	N	M	42		Retreatment	R: RIF, INH, EMB, STM, FQ, PZA, ETH	Pre-XDR-TB	RR	lineage2-Beijing
46	M104	16.916752	96.1718225	2016	East	E	F	50		New	R: INH, STM	HR-TB	T	lineage2-Beijing
47	M105	16.886871	96.1769132	2017	East	E	M	61	1	New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
48	M106	16.889487	96.1728473	2017	East	E	M	57		New	Sensitive	Sensitive	T	lineage1-EAI
49	M107	16.860861	96.1164168	2016	West	W	F	42		Retreatment	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
50	M108	16.878797	96.1150047	2017	West	W	F	30		New	R: INH, EMB, STM	HR-TB	T	lineage2-Beijing
51	M109	16.904787	96.1160556	2016	North	N	M	24		Retreatment	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
52	M110	16.940222	96.1786111	2016	East	E	M	41		New	Sensitive	Sensitive	T	lineage4-EuroAmerican
53	M111	16.921649	96.172958	2017	East	E	M	30		Retreatment	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
54	M113	16.86725	96.1131667	2017	West	W	M	27		Retreatment	R: RIF, INH, EMB, STM, PZA, KAN, AMK, CAP	Pre-XDR-TB	RR	lineage2-Beijing
55	M114	16.886773	96.1923842	2017	East	E	F	45	1	New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage3-Delhi/CAS
56	M116	16.886352	96.117579	2016	North	N	M	57		Retreatment	R: RIF, INH, EMB	MDR-TB	T	lineage2-Beijing
57	M117	16.927615	96.1722646	2017	East	E	F	40		Retreatment	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
58	M120	16.881554	96.1811481	2017	East	E	M	30		Retreatment	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
59	M122	16.888363	96.1158646	2017	West	W	F	20		Retreatment	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
60	M123	16.884085	96.1680672	2017	North	N	F	29		Retreatment	R: RIF, INH, EMB, STM, FQ, PZA	Pre-XDR-TB	RR	lineage2-Beijing
61	M124	16.887388	96.1708008	2017	North	N	F	24		Retreatment	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
62	M125	16.859345	96.1142049	2017	West	W	F	35		Retreatment	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
63	M126	16.892527	96.0624554	2017	South	S	M	17	1	Retreatment	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
64	M127	16.928217	96.1835142	2017	East	E	F	50		Retreatment	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
65	M128	16.85898	96.1162804	2017	West	W	F	17		Retreatment	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
66	M130	16.887612	96.1511881	2017	North	N	F	30		Retreatment	R: RIF, INH, EMB, STM, PZA, KAN, AMK, CAP	Pre-XDR-TB	RR	lineage2-Beijing
67	M131	16.919509	96.1470933	2017	North	N	F	45		Retreatment	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
68	M132	16.938	96.1352222	2017	North	N	M	25		Retreatment	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
69	M133	16.904063	96.1770328	2017	North	N	M	22		New	Sensitive	Sensitive	T	lineage2-Beijing
70	M134	16.901079	96.1755682	2017	East	E	M	37		New	Sensitive	Sensitive	T	lineage1-EAI
71	M135	16.930972	96.1388333	2017	North	N	M	25		New	Sensitive	Sensitive	T	lineage4-Haarlem
72	M136	16.860165	96.1162144	2017	West	W	M	29		New	Sensitive	Sensitive	T	lineage4-EuroAmerican
73	M137	16.914735	96.0617578	2017	North	N	M	51		Retreatment	R: RIF, INH	MDR-TB	RR	lineage1-EAI
74	M138	16.898038	96.1659415	2017	North	N	M	57		Retreatment	R: RIF, INH	MDR-TB	RR	lineage1-EAI
75	M139	16.891032	96.1275201	2017	North	N	M	32		New	Sensitive	Sensitive	T	lineage1-EAI

76	M141	16.887411	96.0921996	2017	North	N	M	32		Retreatment	R: RIF, INH, EMB, PZA	MDR-TB	RR	lineage2-Beijing
77	M142	16.915058	96.1295287	2017	North	N	M	32		New	Sensitive	Sensitive	T	lineage1-EAI
78	M143	16.878492	96.1737371	2017	North	N	M	44	1	New	R: RIF, INH, EMB	MDR-TB	RR	lineage1-EAI
79	M144	16.92003	96.143497	2017	North	N	M	37		Retreatment	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
80	M146	16.910973	96.1460622	2017	North	N	M	36		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
81	M147	16.877122	96.086793	2017	North	N	F	24		Retreatment	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
82	M148	16.912514	96.1898076	2017	East	E	M	32		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
83	M149	16.879616	96.1917734	2017	East	E	M	37	1	New	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
84	M150	16.931467	96.1935127	2017	East	E	F	35		New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
85	M153	16.888762	96.1145154	2017	West	W	M	44		New	Sensitive	Sensitive	T	lineage1-EAI-Manila
86	M154	16.86978	96.1147869	2017	West	W	M	29		New	R: STM	STM-TB	T	lineage2-Beijing
87	M155	16.940917	96.1793058	2017	North	N	M	42		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
88	M157	16.903806	96.1484053	2017	North	N	M	35		Retreatment	R: RIF, INH, STM, FQ, PZA	Pre-XDR-TB	RR	lineage2-Beijing
89	M158	16.898448	96.0733589	2017	North	N	M	29		New	Sensitive	Sensitive	T	lineage2-Beijing
90	M159	16.896651	96.1222871	2017	North	N	M	52		New	Sensitive	Sensitive	T	lineage2-Beijing
91	M160	16.901751	96.0540594	2017	North	N	M	33		New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
92	M161	16.876256	96.1119866	2017	North	N	M	17	1	Retreatment	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
93	M162	16.878911	96.1527789	2017	North	N	M	52	1	New	R: INH, STM	HR-TB	T	lineage2-Beijing
94	M163	16.920427	96.1810489	2017	East	E	F	58	1	Retreatment	R: RIF, INH, EMB, STM, FQ, PZA	Pre-XDR-TB	RR	lineage2-Beijing
95	M164	16.84418	96.1177887	2017	West	W	M	31		New	R: RIF, INH, EMB, STM, PZA, KAN, AMK, CAP	Pre-XDR-TB	RR	lineage2-Beijing
96	M165	16.893922	96.0735733	2017	North	N	M	32		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
97	M166	16.866583	96.11225	2017	West	W	M	51		New	R: RIF, INH	MDR-TB	RR	lineage1-EAI
98	M167	16.931469	96.1807559	2017	East	E	M	40		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
99	M168	16.888074	96.1681828	2019	North	N	M	48		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
100	M169	16.878329	96.1853682	2016	East	E	M	50		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
101	M170	16.924926	96.1736417	2019	East	E	M	18		New	Sensitive	Sensitive	T	lineage3-Delhi/CAS
102	M171	16.905244	96.1792806	2019	East	E	M	47		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
103	M172	16.854572	96.1176035	2019	West	W	F	32	1	New	R: RIF, INH, EMB, STM, FQ, PZA	Pre-XDR-TB	RR	lineage2-Beijing
104	M174	16.885328	96.1830611	2019	East	E	M	57	1	New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
105	M175	16.851753	96.117641	2019	West	W	M	32		New	Sensitive	Sensitive	T	lineage2-Beijing
106	M176	16.931479	96.1933403	2019	East	E	M	41		New	R: INH, EMB, STM	HR-TB	T	lineage2-Beijing
107	M177	16.876798	96.1786443	2019	East	E	F	33		New	Sensitive	Sensitive	T	lineage4-Haarlem
108	M178	16.909663	96.1825833	2019	East	E	M	25		Retreatment	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
109	M179	16.878296	96.1714911	2019	East	E	M	34		New	Sensitive	Sensitive	T	lineage1-EAI
110	M180	16.88669	96.1872622	2019	East	E	F	36		New	Sensitive	Sensitive	T	lineage2-Beijing
111	M181	16.894577	96.0591546	2019	North	N	M	58		New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
112	M182	16.872411	96.1155677	2019	West	W	M	18		New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
113	M184	16.927229	96.1932277	2019	East	E	F	69	1	Retreatment	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
114	M185	16.882805	96.1199713	2019	North	N	F	16		New	Sensitive	Sensitive	T	lineage2-Beijing
115	M186	16.923237	96.1024533	2019	North	N	M	27		New	R: RIF, INH, STM, PZA, KAN, AMK, CAP	Pre-XDR-TB	RR	lineage2-Beijing

116	M187	16.927543	96.1900918	2019	East	E	M	49	1	New	Sensitive	Sensitive	T	lineage1-EAI
117	M188	16.891365	96.1817599	2019	East	E	M	21		New	Sensitive	Sensitive	T	lineage1-EAI
118	M190	16.89238	96.1146044	2019	West	W	F	28		New	R: INH Mono	HR-TB	T	lineage2-Beijing
119	M191	16.867647	96.1140808	2019	West	W	F	29		New	R: INH Mono	HR-TB	T	lineage2-Beijing
120	M192	16.88598	96.1046814	2019	North	N	M	63		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
121	M193	16.902844	96.1698764	2019	North	N	F	29		New	R: RIF, INH, STM, ETH	MDR-TB	RR	lineage1-EAI
122	M195	16.920455	96.0746761	2019	North	N	F	27		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
123	M197	16.874639	96.0508611	2019	North	N	M	49		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
124	M199	16.879639	96.0504167	2016	North	N	M	26		New	R: RIF Mono	RR-TB	RR	lineage4-EuroAmerican
125	M200	16.868722	96.1121389	2019	West	W	M	58		Retreatment	R: RIF, INH, EMB, STM, FQ, PZA	Pre-XDR-TB	RR	lineage2-Beijing
126	M202	16.865988	96.1154241	2019	West	W	M	29		New	R: INH Mono	HR-TB	T	lineage1-EAI
127	M203	16.934556	96.1947222	2016	East	E	M	28		New	R: RIF, INH, STM, PZA	MDR-TB	RR	lineage2-Beijing
128	M204	16.910842	96.119813	2019	North	N	M	38		New	Sensitive	Sensitive	T	lineage4-EuroAmerican
129	M205	16.908367	96.1829063	2016	East	E	M	51		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
130	M206	16.887173	96.192187	2019	East	E	M	56		New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
131	M207	16.905194	96.1766987	2019	East	E	F	22		New	Sensitive	Sensitive	T	lineage2-Beijing
132	M208	16.936916	96.1920558	2019	East	E	M	24		New	Sensitive	Sensitive	T	lineage2-Beijing
133	M211	16.884482	96.1788916	2019	East	E	M	37		New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
134	M247	16.8932	96.1133363	2019	West	W	F	23	1	New	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
135	M248	16.932644	96.1855882	2019	East	E	M	37		Retreatment	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
136	M250	16.898034	96.1358065	2019	North	N	M	43		New	R: RIF, INH, STM, PZA	MDR-TB	RR	lineage2-Beijing
137	M251	16.890847	96.0879711	2019	North	N	M	43		Retreatment	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
138	M252	16.906822	96.129838	2019	North	N	F	65		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
139	M253	16.915626	96.1752859	2019	East	E	M	53		New	R: RIF Mono	RR-TB	RR	lineage2-Beijing
140	M254	16.846503	96.1141218	2018	West	W	M	20		New	R: RIF, INH, STM, PZA	MDR-TB	RR	lineage2-Beijing
141	M266	16.903259	96.1896575	2018	East	E	M	24		Retreatment	R: RIF, INH, EMB, STM, FQ, PZA, BDQ	XDR-TB	RR	lineage2-Beijing
142	M268	16.87025	96.1183056	2018	West	W	F	59		New	R: RIF, INH, STM, PZA	MDR-TB	RR	lineage2-Beijing
143	M270	16.937989	96.172222	2018	East	E	F	43		Retreatment	R: RIF, INH, EMB, STM, FQ, PZA	Pre-XDR-TB	RR	lineage2-Beijing
144	M271	16.868902	96.0625775	2018	South	S	M	32		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
145	M272	16.935703	96.1794794	2018	East	E	M	30		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
146	M273	16.876143	96.078981	2018	North	N	F	32		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
147	M274	16.910341	96.1732304	2018	East	E	F	58	1	Retreatment	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
148	M275	16.846004	96.1163647	2019	West	W	F	26		New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
149	M276	16.892473	96.1748347	2017	East	E	M	46		New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
150	M277	16.924899	96.1848345	2018	East	E	F	24		New	R: RIF, INH, EMB, STM, PZA, ETH	MDR-TB	RR	lineage2-Beijing
151	M278	16.930447	96.1907331	2018	East	E	F	34	1	Retreatment	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
152	M279	16.886167	96.1528333	2018	East	E	F	25		New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
153	M281	16.939778	96.1878333	2018	East	E	F	16		Retreatment	R: RIF, INH, EMB, STM, FQ, PZA	Pre-XDR-TB	RR	lineage2-Beijing
154	M282	16.931757	96.1885524	2018	East	E	M	53		Retreatment	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
155	M283	16.876128	96.1166615	2018	West	W	M	27		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing

156	M284	16.919471	96.0914584	2017	North	N	M	53		New	R: RIF, INH, EMB, STM , PZA	MDR-TB	RR	lineage2-Beijing
157	M285	16.889667	96.1245833	2018	North	N	F	21		Retreatment	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
158	M287	16.92875	96.1625278	2018	East	E	M	64		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
159	M288	16.862972	96.1123889	2018	West	W	F	33		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
160	M291	16.843919	96.1143722	2018	West	W	F	25		New	R: RIF, INH	MDR-TB	RR	lineage1-EAI
161	M292	16.893102	96.0647468	2018	North	N	M	52		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
162	M293	16.887663	96.167407	2018	North	N	M	26		New	R: RIF Mono	RR-TB	RR	lineage2-Beijing
163	M294	16.852373	96.1125571	2018	West	W	F	31		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
164	M295	16.89233	96.1882653	2018	East	E	M	31		New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
165	M296	16.935148	96.1714442	2018	East	E	M	48		New	R: INH, STM	HR-TB	T	lineage1-EAI
166	M297	16.856121	96.114496	2018	West	W	F	50		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
167	M298	16.910216	96.0732517	2018	North	N	M	20		New	R: RIF Mono	RR-TB	RR	lineage1-EAI Manila
168	M299	16.892149	96.1787176	2018	East	E	M	57		New	R: RIF, INH, STM, PZA	MDR-TB	RR	lineage2-Beijing
169	M300	16.892857	96.177257	2018	North	N	M	48		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
170	M301	16.883132	96.114642	2018	West	W	F	17	1	Retreatment	R: RIF, INH, EMB	MDR-TB	RR	lineage3-Delhi/CAS
171	M312	16.923543	96.0884357	2018	North	N	F	28		New	Sensitive	Sensitive	T	lineage2-Beijing
172	M313	16.866402	96.1167016	2018	West	W	M	59		New	R: RIF Mono	RR-TB	RR	lineage2-Beijing
173	M314	16.89568	96.0688926	2018	North	N	M	55		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
174	M315	16.915246	96.1592395	2018	North	N	M	46		Retreatment	R: RIF, INH, EMB, STM, FQ, PZA	Pre-XDR-TB	RR	lineage2-Beijing
175	M318	16.910336	96.1228427	2018	North	N	M	52		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
176	M319	16.923169	96.0822084	2018	North	N	F	26	1	New	R: INH Mono	HR-TB	T	lineage2-Beijing
177	M320	16.879267	96.1615385	2018	North	N	M	45		New	Sensitive	Sensitive	T	lineage1-EAI Manila
178	M327	16.932051	96.1924637	2018	East	E	F	40		New	R: INH Mono	HR-TB	T	lineage3-Delhi/CAS
179	M330	16.892343	96.1933949	2018	East	E	M	32		New	Sensitive	Sensitive	T	lineage1-EAI
180	M332	16.844878	96.1148906	2018	West	W	M	30		New	Sensitive	Sensitive	T	lineage1-EAI
181	M333	16.896158	96.1668174	2018	North	N	F	32		New	Sensitive	Sensitive	T	lineage2-Beijing
182	M334	16.918379	96.1375836	2018	North	N	M	45		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
183	M336	16.903169	96.1269458	2018	North	N	M	60		Retreatment	R: RIF, INH, EMB, STM, FQ, ETH	Pre-XDR-TB	T	lineage2-Beijing
184	M337	16.888776	96.1399085	2018	North	N	F	28		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
185	M338	16.890787	96.1343644	2018	North	N	F	32		New	Sensitive	Sensitive	T	lineage1-EAI
186	M340	16.886763	96.1209141	2018	North	N	M	57		New	Sensitive	Sensitive	T	lineage1-EAI
187	M341	16.875444	96.1599167	2018	East	E	M	24		New	Sensitive	Sensitive	T	lineage2-Beijing
188	M343	16.934099	96.1785889	2018	East	E	M	45		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
189	M344	16.946944	96.0827778	2018	North	N	F	47	1	New	Sensitive	Sensitive	T	lineage1-EAI
190	M346	16.903206	96.1489258	2018	North	N	M	46		New	Sensitive	Sensitive	T	lineage1-EAI
191	M347	16.90815	96.100286	2018	North	N	M	24		Retreatment	R: RIF, INH	MDR-TB	RR	lineage2-Beijing
192	M349	16.914072	96.1480356	2018	North	N	M	25		New	Sensitive	Sensitive	T	lineage2-Beijing
193	M350	16.875351	96.1450062	2018	North	N	M	36		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
194	M356	16.887493	96.0649596	2018	South	S	F	54		New	Sensitive	Sensitive	T	lineage3-Delhi/CAS
195	M357	16.902714	96.0517428	2018	North	N	M	33		New	Sensitive	Sensitive	T	lineage4-LAM

196	M358	16.908936	96.1478787	2018	North	N	M	20		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
197	M359	16.898999	96.1266421	2018	North	N	M	71		New	Sensitive	Sensitive	T	lineage1-EAI
198	M361	16.937528	96.1762778	2018	East	E	M	24		New	Sensitive	Sensitive	T	lineage3-Delhi/CAS
199	M362	16.889313	96.1720857	2018	North	N	F	21		New	R: RIF, INH, EMB, STM, PZA, KAN, AMK, CAP	Pre-XDR-TB	RR	lineage2-Beijing
200	M364	16.892831	96.0925261	2018	North	N	F	57	1	New	Sensitive	Sensitive	T	lineage4-LAM
201	M366	16.893463	96.1415867	2018	North	N	M	45	1	New	Sensitive	Sensitive	T	lineage1-EAI
202	M367	16.910222	96.1169078	2018	North	N	M	45	1	New	R: STM	STM-TB	T	lineage2-Beijing
203	M368	16.931471	96.183797	2018	East	E	M	73		New	Sensitive	Sensitive	T	lineage2-Beijing
204	M369	16.911035	96.1553004	2018	North	N	F	44		New	Sensitive	Sensitive	T	lineage4-EuroAmerican
205	M370	16.901434	96.1557028	2018	North	N	M	42	1	New	R: RIF Mono	RR-TB	RR	lineage2-Beijing
206	M371	16.915678	96.0932004	2018	North	N	M	47		New	Sensitive	Sensitive	T	lineage4-EuroAmerican
207	M374	16.906808	96.1119319	2018	North	N	F	25		New	Sensitive	Sensitive	T	lineage1-EAI
208	M386	16.883459	96.0624987	2018	North	N	M	29		New	R: RIF Mono	RR-TB	RR	lineage2-Beijing
209	M402	16.90004	96.0512944	2018	North	N	F	50		Retreatment	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
210	M403	16.9267	96.1744538	2018	North	N	M	35		New	Sensitive	Sensitive	T	lineage1-EAI
211	M404	16.916895	96.1647813	2018	North	N	F	21		New	R: RIF Mono	RR-TB	RR	lineage1-EAI
212	M406	16.871519	96.1165598	2018	West	W	M	35		New	Sensitive	Sensitive	T	lineage1-EAI
213	M407	16.918532	96.1848367	2018	East	E	M	45		New	Sensitive	Sensitive	T	lineage1-EAI
214	M408	16.880611	96.1945611	2018	East	E	M	33		New	Sensitive	Sensitive	T	lineage2-Beijing
215	M409	16.905371	96.0918395	2017	North	N	F	35		Retreatment	R: RIF, INH, EMB, STM, FQ, PZA, KAN, AMK, CAP	XDR-TB	RR	lineage2-Beijing
216	M410	16.901256	96.0619577	2017	North	N	M	56		New	R: INH Mono	HR-TB	T	lineage1-EAI
217	M411	16.872552	96.0627849	2017	South	S	M	37	1	New	Sensitive	Sensitive	T	lineage4-LAM
218	M412	16.92032	96.1785288	2017	North	N	M	31		New	Sensitive	Sensitive	T	lineage2-Beijing
219	M416	16.915065	96.1758649	2017	North	N	M	45		New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
220	M420	16.912544	96.1458663	2017	North	N	M	55		New	R: RIF, INH, EMB, STM, PZA, KAN, AMK, CAP	Pre-XDR-TB	RR	lineage2-Beijing
221	M421	16.848519	96.1129924	2017	West	W	F	27		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
222	M422	16.861344	96.1174825	2017	West	W	F	59		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
223	M426	16.878576	96.1423898	2017	North	N	M	34		New	R: RIF, INH, EMB, STM , PZA	MDR-TB	RR	lineage2-Beijing
224	M430	16.908095	96.0913406	2017	North	N	F	45		New	Sensitive	Sensitive	T	lineage1-EAI
225	M433	16.933903	96.1864804	2017	East	E	M	68		New	Sensitive	Sensitive	T	lineage1-EAI
226	M487	16.898656	96.191022	2017	East	E	M	20		New	Sensitive	Sensitive	T	lineage4-LAM
227	M490	16.935889	96.1783889	2017	East	E	F	52		New	Sensitive	Sensitive	T	lineage2-Beijing
228	M492	16.898887	96.1153169	2017	North	N	F	26		Retreatment	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
229	M493	16.912194	96.1713611	2017	East	E	F	43	1	New	Sensitive	Sensitive	T	lineage3-Delhi/CAS
230	M494	16.843222	96.1172222	2017	West	W	M	59		New	Sensitive	Sensitive	T	lineage1-EAI
231	M495	16.892399	96.1346114	2017	North	N	F	20		New	Sensitive	Sensitive	T	lineage1-EAI
232	M496	16.866102	96.0642954	2017	South	S	M	64		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
233	M498	16.923061	96.1778353	2018	East	E	F	24		New	Sensitive	Sensitive	T	lineage1-EAI
234	M499	16.939804	96.1842212	2019	East	E	F	32		New	R: INH Mono	HR-TB	T	lineage4-Haarlem
235	M500	16.915026	96.1111735	2017	North	N	M	27		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing

236	M501	16.876187	96.0602719	2017	South	S	F	55	1	New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
237	M502	16.907458	96.0945058	2017	North	N	M	39		New	R: RIF, INH, EMB	MDR-TB	RR	lineage2-Beijing
238	M504	16.919201	96.1873726	2017	East	E	M	41		New	R: RIF, INH, STM, PZA	Sensitive	T	lineage1-EAI
239	M505	16.915004	96.1784847	2017	East	E	F	52		New	Sensitive	MDR-TB	RR	lineage2-Beijing
240	M506	16.895744	96.1742978	2017	North	N	M	33		Retreatment	R: RIF, INH, EMB, STM, PZA, ETH	Sensitive	T	lineage2-Beijing
241	M507	16.905536	96.0567543	2017	North	N	F	47		New	Sensitive	Sensitive	T	lineage3-Delhi/CAS
242	M508	16.908992	96.0547887	2017	North	N	M	32		New	Sensitive	Sensitive	T	lineage2-Beijing
243	M509	16.9037	96.0560252	2017	North	N	F	28		New	Sensitive	Sensitive	T	lineage3-Delhi/CAS
244	M510	16.891007	96.1817883	2017	East	E	F	30		New	Sensitive	Sensitive	T	lineage1-EAI-Manila
245	M511	16.890685	96.1073109	2017	North	N	F	30		New	Sensitive	Sensitive	T	lineage1-EAI
246	M512	16.884604	96.1608964	2017	North	N	F	28		New	R: RIF, INH	MDR-TB	RR	lineage1-EAI
247	M515	16.93527	96.1714693	2017	East	E	M	35		Retreatment	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
248	M516	16.912789	96.1775393	2017	East	E	M	31		New	R: INH, STM	HR-TB	T	lineage2-Beijing
249	M518	16.895415	96.0750798	2017	North	N	F	25		New	Sensitive	MDR-TB	RR	lineage2-Beijing
250	M520	16.919235	96.1390479	2017	North	N	M	42		New	R: RIF, INH, EMB	MDR-TB	T	lineage1-EAI
251	M525	16.937371	96.1765563	2017	East	E	M	56		New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
252	M526	16.904312	96.1924518	2017	East	E	M	59		New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
253	M528	16.88681	96.1933287	2017	East	E	M	25		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
254	M529	16.935152	96.173926	2017	East	E	F	72	1	Retreatment	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
255	M530	16.909652	96.1902488	2017	East	E	M	37		Retreatment	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
256	M531	16.92504	96.1835494	2017	East	E	M	40		New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
257	M534	16.90463	96.0912595	2017	North	N	M	63	1	New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
258	M535	16.895192	96.0914376	2017	North	N	M	27		New	R: RIF, INH	MDR-TB	RR	lineage4-EuroAmerican
259	M536	16.892338	96.1247398	2017	North	N	F	60	1	New	R: RIF, INH	MDR-TB	RR	lineage4-EuroAmerican
260	M539	16.883892	96.1896882	2017	East	E	F	60	1	Retreatment	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
261	M540	16.889849	96.1864176	2017	East	E	F	51		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
262	M541	16.858361	96.1168889	2017	West	W	M	27	1	Retreatment	R: RIF, EMB, FQ	Pre-XDR-TB	RR	lineage2-Beijing
263	M544	16.896966	96.1882333	2017	East	E	F	40		New	R: RIF, INH, EMB, STM, PZA, ETH	MDR-TB	RR	lineage2-Beijing
264	M549	16.901094	96.1325461	2017	North	N	F	28		Retreatment	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
265	M550	16.901136	96.1383342	2017	North	N	M	22	1	Retreatment	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
266	M553	16.89542	96.1398615	2017	North	N	F	31		New	R: RIF, INH, EMB, STM, PZA, ETH	MDR-TB	RR	lineage2-Beijing
267	M566	16.898384	96.1353946	2017	North	N	M	29		Retreatment	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
268	M567	16.89725	96.1397764	2017	North	N	F	43		New	R: INH, STM	HR-TB	T	lineage2-Beijing
269	M569	16.90001	96.1423588	2017	North	N	M	55		Retreatment	R: RIF, INH, EMB, STM, FQ	Pre-XDR-TB	RR	lineage2-Beijing
270	M572	16.897945	96.1424559	2017	North	N	M	59		New	R: RIF, INH, EMB, STM, PZA, KAN, AMK, CAP	MDR-TB	RR	lineage2-Beijing
271	M576	16.917705	96.1469357	2018	North	N	F	39	1	Retreatment	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
272	M577	16.899963	96.1345494	2018	North	N	M	45		New	R: RIF, INH, STM	MDR-TB	RR	lineage2-Beijing
273	M579	16.894795	96.1379972	2018	North	N	F	58		New	R: RIF, INH, EMB, STM, PZA	MDR-TB	RR	lineage2-Beijing
274	M581	16.896008	96.1364344	2018	North	N	F	36		New	Sensitive	Sensitive	T	lineage1-EAI
275	M582	16.897448	96.1346926	2018	North	N	M	63	1	New	Sensitive	Sensitive	T	lineage2-Beijing

276	M583	16.897746	96.1341709	2018	North	N	M	54	1	New	R: RIF, INH, STM, PZA	MDR-TB	RR	lineage2-Beijing
277	M584	16.900175	96.1355582	2018	North	N	F	32		New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
278	M585	16.90399	96.1400796	2018	North	N	F	65		New	Sensitive	Sensitive	T	lineage2-Beijing
279	M586	16.896059	96.1318359	2018	North	N	M	61	1	New	R: RIF, INH, EMB, STM	MDR-TB	RR	lineage2-Beijing
280	M588	16.896033	96.1434276	2018	North	N	F	34	1	New	Sensitive	Sensitive	T	lineage3-Delhi/CAS
281	M590	16.896024	96.1324115	2018	North	N	F	58		New	Sensitive	Sensitive	T	lineage4-Haarlem
282	M594	16.899984	96.1312211	2018	North	N	F	51		New	R: RIF, INH	MDR-TB	RR	lineage1-EAI
283	M595	16.902579	96.1418894	2019	North	N	M	48		New	R: RIF, STM	RR-TB	RR	lineage2-Beijing
284	M597	16.897236	96.1419071	2019	North	N	M	47		New	Sensitive	Sensitive	T	lineage1-EAI-Manila
285	M601	16.898541	96.1437592	2019	North	N	M	52		New	Sensitive	Sensitive	T	lineage3-Delhi/CAS

World Health Organization (WHO) TB Drug Resistant Catalogue 2023.

	WHO catalogue (reference)		PhyReSSe v1.0	TB Profiler v5.01
Drug	Variant	Resistance Annotation	Resistance Annotation	Resistance Annotation
Ethambutol (EMB)	embB_p.Met306Val	R	R	R
	embA_c.-590C>T	U	U	U
	embA_c.228C>T	S	U	U
	embC_g-1419a (aftA_171)	U	U	Not Reported
Isoniazid (INH)	inhA_g-154a (fabG1_L203L)	R	R	R
	Rv1258c_c.580_581insC	S	S	S
	katG_p.Arg463Leu	S	S	S
	mshA_p.Ala187Val	S	S	S
Rifampicin (RIF)	rpoB_p.Serine450Leu	R	R	R
	nadD_c.-44A>C	Not Reported	U	U
	rpoC_p.Gly519Ser	Not Reported	U	U
Rifabutin (RFB)	rpoB_p.Serine450Leu	R	Not Reported	Not Reported
	rpoC_c.-339T>C	U	Not Reported	Not Reported
	rpoC_p.Gly519Ser	U	Not Reported	Not Reported
Streptomycin (STM)	rrs_n.514A>C	R	R	R
	gid_c.615A>G	U	U	U
	rrs_n.-187C>T	S	S	S
	Rv1258c_c.580_581insC	S	S	S
	gid_p.Glu92Asp	S	S	S
	rpsL_c.-165T>C	S	S	S
	whiB6_c.-75delG	S	S	S
Ethionamide (ETO)	inhA_g-154a (fabG1_L203L)	R	R	R
	mshA_p.Ala187Val	U	U	U
Prothionamide (PTO)	inhA_g-154a (fabG1_L203L)	R	R	Not Reported
	mshA_p.Ala187Val	U	U	Not Reported
Amikacin (AMI)	fprA_c.-11_-10insA	U	U	U
	rrs_n.-187C>T	S	S	S
	aftB_p.Asp397Gly	S	S	S
	ccsA_p.Ile245Met	S	S	S
	whiB6_c.-75delG	S	S	S
	rrs_n.514A>C	S	S	S
Bedaquiline (BDQ)	mmpS5_c.-710C>G	U	Not Reported	U
	Rv1979c_c.-129A>G	S	Not Reported	S
	mmpL5_p.Asp767Asn	S	Not Reported	S
	mmpL5_p.Ile948Val	S	Not Reported	S
	mmpL5_p.Thr794Ile	S	Not Reported	S
Capreomycin (CAP)	fprA_c.-11_-10insA	U	U	U
	rrs_n.-187C>T	S	S	S
	tlyA_c.33A>G	U	U	U
	aftB_p.Asp397Gly	S	S	S
	ccsA_p.Ile245Met	S	S	S
	whiB6_c.-75delG	S	S	S
	rrs_n.514A>C	S	S	S
Clofazimine (CFZ)	mmpS5_c.-710C>G	U	Not Reported	U

	Rv1979c_c.-129A>G	S	Not Reported	S
	mmpL5_p.Asp767Asn	S	Not Reported	S
	mmpL5_p.Ile948Val	S	Not Reported	S
	mmpL5_p.Thr794Ile	S	Not Reported	S
Cycloserine (CS)	ald_c.-32T>C	U	Not Reported	Not Reported
	cycA_p.Arg93Leu	U	Not Reported	Not Reported
	ddlA_p.Thr365Ala	U	Not Reported	Not Reported
Delamanid (DLM)	fgd1_c.960T>C	U	Not Reported	U
Imipenem (LPM)	nadD_c.-44A>C	U	Not Reported	Not Reported
Kanamycin (KAN)	rrs_n.-187C>T	S	S	S
	rrs_n.514A>C	S	S	S
Linezolid (LZD)	rrs_n.-187C>T	S	Not Reported	Not Reported
	rrs_n.514A>C	U	Not Reported	Not Reported
Meropenem (MPM)	nadD_c.-44A>C	U	Not Reported	Not Reported
Pretomanid (PTA)	fgd1_c.960T>C	U	Not Reported	Not Reported
Terizidone (TRD)	ald_c.-32T>C	U	Not Reported	Not Reported
	cycA_p.Arg93Leu	U	Not Reported	Not Reported
	ddlA_p.Thr365Ala	U	Not Reported	Not Reported
Levofloxacin (LFX)	gyrA_p.Glu21Gln	S	U	S
	gyrA_p.Gly668Asp	S	U	S
	gyrA_p.Ser95Thr	S	U	S
Moxifloxacin (MFX)	gyrA_p.Glu21Gln	S	U	S
	gyrA_p.Gly668Asp	S	U	S
	gyrA_p.Ser95Thr	S	U	S
Pyrazinamide (PZA)	PPE35_p.Leu896Ser	S	S	S
	Rv1258c_c.580_581insC	S	S	S
	Rv3236c_p.Thr102Ala	S	S	S
	rpsA_Arg212_cha/cgc	Not Reported	Not Reported	Not Reported
Para_aminosalicylic_acid (	PPE35_L896S	S	S	S
	Rv1258c_C.581_ins_1_t_tg	S	S	S
	rpsA_Arg212_cha/cgc	U	S	S
	Rv3236c_p.Thr102Ala	S	S	S
Total Drug Detection		23	14	23

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