# Prevalence of Injectable Anti-Tuberculosis Drugs and Drug Resistance Amongst TB Patients in The Vhembe District (Limpopo, South Africa)

by

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A dissertation submitted in fulfilment for the requirements of the Master of Science (M.Sc.) Degree in Microbiology

to the

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February 2024

# TABLE OF CONTENTS

# DECLARATION

I, Patel SM with student number 18021768, hereby declare that this dissertation for the award of Master's degree in Microbiology at the University of Venda belongs to me. This document has not been submitted before at University of Venda or in any other institution. I declare that it is my own work and all the reference materials contained herein have been properly acknowledged.

Signature……  …… Date: 22/02/2024

# DEDICATION

In the name of God The most Kind and Immensely merciful.

This research is dedicated to my family. My family's remarkable resilience and their prayers and tremendous support encouraged me to keep on moving forward. This research would have been incomplete without them. I am deeply grateful to the Almighty for blessing me with the best support system.

My wonderful husband Sakib was my greatest support system, my parents Mr Mustakahmed Patel and Mrs Zarina Mustakahmed Patel. My in-laws Mr Idris Bapu and Mrs Kousar Bapu, their unconditional love and encouragement kept me going throughout this challenging research.

My siblings Imran, Salman, Saima, Aasiya, Hussen and Mohammed were my backbone of strength. My little niece and nephews Aisha, Issa and Hamdan also kept me going.

# ACKNOWLEDGEMENT

In the name of God The most Kind and Immensely merciful.

I aspire my supervisors, my mentors and my seniors. Prof Afsatou Traore, Dr Mpho Magwalivha, Prof Natasha Potgieter and Dr Mpumelelo Casper Rikhotso for their valuable insights and guidance throughout this research. I owe them my deepest gratitude as they never gave up on me during my lowest times. This research was truly incomplete without them.

My colleagues, friends and lab mates also contributed in the completion and success of this research, Hafsa, Musoliwa, Marry, Sara and Thelma.

I am also grateful for my friends, Hafsa, Faith, Rivha, Takie, Ms Legodi, Chantelle, Shifa, Raisah, Asma, Firdaus, Phathu, Damien, Lutendo and Fhumulani for their moral support and motivational words.

I am immensely grateful to my family for believing in me and keeping me motivated.

I highly appreciate the National Research Foundation (NRF) for their financial support. This research would not be possible if it was not for their funding, thus I thank them.

# ABSTRACT

**Background:** Tuberculosis is one of the most infectious diseases and a major contributor to high mortality and morbidity rates worldwide. The rise of human immunodeficiency virus cases also compounds to the tuberculosis burden, as human immunodeficiency virus patients are thought to be more susceptible to tuberculosis, since the disease is an opportunistic infection and makes an individual's immune system less capable of fighting off infections. Drug resistance tuberculosis makes transmission and combatting of disease challenging, particularly multi-drug resistant strains that are resistant to at least isoniazid and rifampicin, pre-XDR, resistant to isoniazid, rifampicin and a fluoroquinolone and extensively-drug resistant, a rare form of multidrug resistance tuberculosis that is resistant to isoniazid, rifampicin, fluoroquinolone, and a second-line injectable (amikacin, capreomycin, and kanamycin). Resistance to fluoroquinolone and injectable drugs is not frequently tested due to budget constraints and the ongoing high tuberculosis burden in developing nations. Furthermore, there is insufficient knowledge about extensively-drug resistant strains and mutations connected to fluoroquinolone and injectable drugs in South Africa. Thus, the purpose of this study was to determine the frequency of genetic mutations in *M. tuberculosis* obtained from tuberculosis positive patients attending medical facilities in the Vhembe region of the province of Limpopo, South Africa, with respect to genotype conferring resistance for INH, RIF, EMB, PZA, FQs, all of which are linked to resistance to second-line drugs.

**Methods:** Ethical clearance was obtained, a total of 50 morning sputum samples were collected from health-care facilities. Structured questionaries were also administered to collect additional information. Collected data was analysed to investigate the risk factors associated with drug resistance tuberculosis. Allplex™ Multiplex polymerase chain reaction permitted simultaneous amplification and detection of target sequence of *Mycobacterium tuberculosis*. In order to determine genetic mutations, 8 single-nucleotide polymorphisms were targeted using MassARRAY (Agena) system.

**Results:** Among the 50 tuberculosis recruited patients (from the pool of main study August 2022-June 2023), 60% were males and 40% were females. HIV was highly (66%) prevalent amongst study population; susceptible tuberculosis 4%, MTB & NTM 28% (co-infection), NTM 58% and 4% drug resistance were observed. Overall, of the SNPs designed only 8 related gene mutations could be successfully detected. Overall, from the study population, 2 samples were detected to be pre-XDR (FQ-R and RIF-R + INH-R + FQ-R).

**Conclusion:** The findings of the study provide valuable insights into the characteristics and prevalence of tuberculosis, along with associated risk factors and co-morbidities among the study population in Vhembe district. The emergence of drug resistance highlights the necessity for continued pattern monitoring and the application of suitable treatment approaches in order to stop the spread of drug-resistant strains. To draw firm conclusions about drug-resistant tuberculosis risk factors in patients in the Vhembe region, further information is still required.

**Keywords**: Allplex™; Drug resistant; Extensively drug resistance; Risk factors; Single-nucleotide polymorphisms; Tuberculosis.

# LIST OF ABBREVIATIONS

| | |
|---|---|
| A | Adenine |
| AFB | Acid fast bacilli |
| AIDS | Acquired immunodeficiency syndrome |
| AMK | Amikacin |
| BCG | Bacille Calmette-Guerin |
| CAP | Capreomycin |
| CDC | Centre of Disease Control and Prevention |
| CT | Computed tomography |
| ddNTPs | Dideoxyribonucleosides |
| DNA | Deoxyribonucleic acid |
| DR | Drug resistance |
| DST | Drug susceptibility testing |
| EMB | Ethniomide |
| F | Forward |
| FQs | Fluoroquinolones |
| G | Guanine |
| HIV | Human immunodeficiency virus |
| INH | Isoniazid |
| KAN | Kanamicin |
| KAS | Knowledge, attitude and stigma |
| LTBI | Latent TB infection |
| MDR | Multi-drug resistance |
| MTB | Mycobacterium tuberculosis |
| NAAT | Nucleic acid amplification testing |
| NaOH | Sodium hydroxide |
| NTM | Nontuberculous mycobacteria |
| OFX | Ofloxacin |
| PCR | Polymerase Chain Reaction |
| PPD | Pure protein derivative |
| PZA | Pyrazinamide |

| | |
|---|---|
| QRDR | Quinolone Resistance Determining region |
| R | Reverse |
| RIF | Rifampicin |
| SLDs | Second line drugs |
| SNPs | Single nucleotide polymorphisms |
| T | Thymine |
| TB | Tuberculosis |
| TNF | Tumour necrosis factor |
| TST | TB skin test |
| WHO | World Health Organization |
| XDR | Extensively drug resistance |
| °C | Degree Celsius |
| Ml | Mililitre |
| A | Alpha |
| B | Beta |
| µl | Microlitre |

# LIST OF TABLES

# LIST OF FIGURES

# CHAPTER 1
# GENERAL INTRODUCTION

## 1.1 BACKGROUND

Globally, 10.6 million cases of tuberculosis (TB) were reported in 2021 with 6,400,000 for men, 3,400,000 in women, and 1.2,000,000 among kids. TB is prevalent in all nations and in all age groups. In 2021, 1.6 million individuals worldwide (including 187 000 persons living with HIV) passed away from TB. The World Health Organization (WHO) reported TB as the second most lethal infectious disease in the world, behind COVID-19, and it is the 13th greatest cause of death globally (behind HIV and AIDS) (WHO, 2022).

The Centers for Disease Control and Prevention (CDC) stated that, TB is an airborne pathogen, transmitted when an individual with active TB disease coughs or sneezes and another individual inhales the expelled droplets, which contain TB bacteria (CDC, 2014). This disease can be latent TB, also known as TB infection (Latent TB is a state wherein a person might have tuberculosis bacterium in their body but not develop symptoms; most individuals' immune systems are capable of curtailing the bacterium by preventing it from replicating and causing disease) or active TB, also known as active tuberculosis (TB Disease), wherein an individual may contract TB, usually contagious to immunocompromised individuals (HIV/AIDS, Diabetes and Cancer) (Dietrich and Doherty, 2009).

Although TB often affects the lungs, it may also damage the brain, intestines, kidneys, or spine. The location of TB bacteria in the body determines the TB symptoms. Pulmonary TB may manifest as symptoms including a persistent cough, chest discomfort, coughing of blood (haemoptysis), weakness or fatigue, loss of weight, a high temperature (fever), and sweats at night (Zaman, 2010), TB can be a severe disease and become lethal causing death (Wallis *et al.,* 2016).

TB is one of the most serious diseases among immunocompromised people due to its high frequency, morbidity, and mortality (Yates *et al.,* 2016). Weak immune systems increase the risk of LTBI and TB (Singh *et al.,* 2020). Immunosuppressed people (including those with AIDS), extreme age groups (old age and vulnerable children), some

ethnic groups, migrants, and those who have been exposed to animals that may have TB (*Mycobacterium bovis*) infection are most at risk (Thoen *et al.,* 2009). In 2015, according to estimates, those living with HIV had a 26–31 times higher risk of having TB disease than people without the infection. HIV positive people were responsible for almost 28% of tuberculosis deaths in 2015, suggesting a strong association between the two diseases that is HIV/AIDS and tuberculosis (WHO, 2016).

Several diagnostics techniques are used to identify whether the bacterium replicated successfully (TB disease). The most frequently used is Mantoux tuberculin skin test, also known as TB skin test (TST), Direct observed therapy (DOT), other techniques includes: chest radiography (X-ray), microscopy (acid-fast bacilli (AFB) staining), interferon gamma release assay, computed tomography scans (CT), other laboratory diagnostics techniques includes: bronchial washing, liquid culture, solid culture and sputum (Gupta *et al.,* 2020). After a patient is diagnosed with TB, the doctor prescribes a regimen that can last 6-8 months (first-line drugs: Isoniazid, Rifampicin, Pyrazinamide, and Ethambutol). After a few weeks, the patient may feel better and stop taking the drugs, which can cause the bacteria to develop resistance to the drugs, making tuberculosis much more deadly and difficult to treat. For 20–30 months, drug-resistant TB patients get fluoroquinolone antibiotics and injectable medicines such amikacin or capreomycin (Dooley *et al.,* 2021). Bedaquiline and linezolid may be added to these second-line drugs (SLDs) to address drug resistance (Lui *et al.,* 2020).

MDR-TB is resistant to first-line anti-TB drugs isoniazid, rifampicin, ethambutol, and pyrazinamide (commonly known as Rifafour), according to Shah et al. (2007). MDR-TB requires 18–20 months of less toxic second-line treatment (Sharma and Basu 2019). XDR-TB is a rare, lethal form of tuberculosis that resists fluoroquinolone, one of three second-line injectable (SLDs) therapies (capreomycin, amikacin, and kanamycin), and both first- and second-line anti-drugs (Isaboke, 2016). MDR-TB and XDR-TB are treated with diagnostics and medications, but the process is more expensive and time-consuming and has a greater incidence of treatment failure and mortality (Kirirabwa *et al.,* 2016).

The emergence of extensively drug-resistant tuberculosis (XDR-TB) has raised public health concerns for global TB control (Rodriguez *et al.,* 2023). Although Multi-drug-resistant tuberculosis (MDR-TB) prevalence and associated genetic mutations are well

documented, little is known about XDR TB (Chowdhury *et al.,* 2023). Therefore, the prevalence of pre-XDR and XDR, as well as the mutation status of genotypes conferring resistance for INH, RIF, EMB, PZA, FQs, all of which are linked to resistance to second-line drugs (SLDs); are of great significance in order to combat and treat extensively drug-resistant tuberculosis (XDR-TB) (Momen *et al.,* 2021).

## 1.2 STUDY RATIONALE

TB is the fourth leading cause of mortality in Limpopo, South Africa (Statistics South Africa, 2018). The 2009 Limpopo Provincial TB Annual Report (O'Donnell *et al.,* 2013) reported on 2194 TB cases in Vhembe district, moreover, Vhembe was recorded to have the most TB patients in Limpopo in 2012 (Sukumani *et al.,* 2012). Tshitangano *et al.,* (2014), also noted that Vhembe district hospitals lacked TB infection control programs, which contributed to the high TB rate. It has been suggested that infection prevention should involve human and material assistance (Maputle *et al.,* 2013). While Gursimrat (2011), noted that insufficient basic healthcare infrastructure makes TB control difficult. Lack of space and beds in wards (especially in rural based health care facilities) regions) makes it difficult to separate Multi-Drug Resistant TB (MDR-TB) and TB patients, increasing the risk of infection and healthcare professionals face a growing risk of TB (Zelnick *et al.,* 2013; Zhang *et al.,* 2020).

Despite the significant prevalence of MDR and XDR-TB in South Africa, limited information is available nationally on the drug-resistant strains that are currently circulating (Brudey *et al.,* 2006; Mathema *et al.,* 2015), numerous genotypes have been implicated in the worldwide TB epidemiology, and studies have revealed that these genotypes exist at rates that vary among districts, cities, nations, and continents (Warren *et al.,* 2014; Supply *et al.,* 2017; Van Hout and Hope, 2019). The distribution of drug-resistant strains varies by province in South Africa. However, the majority of South Africa's provinces, particularly those in the northern part of the country, do not have enough available statistics. Therefore, studies on the characterization of drug-resistant strains are necessary in order to combat the transmission dynamics, determining prevalent strains of circulating drug-resistant *M. tuberculosis* strains, help to reduce the paucity of data needed in making accurate assessments about the population structure of drug-resistant strains (Barlow *et al.,* 2013; Kamerbeek *et al.,* 2017).

Despite significant advancements in XDR-TB diagnosis and therapy (WHO, 2019), little is still known about the dynamics of XDR-TB transmission and sequence in which drug resistance was acquired. The social and behavioural patterns that govern where and with whom people spend their time can have an impact on transmission dynamics. It is crucial to understand the incidences of resistance in *Mycobacterium tuberculosis* isolates in developing nations where TB is widespread, and drugs are often administered. Thus, molecular genotyping of resistance isolates will aid in epidemiological study by identifying the predominant genotypes within a certain geographic area, enhancing understanding of TB transmission dynamics, and assisting in the evaluation of TB outbreaks. Therefore, this study aims to determine the prevalence and genetic mutation status of genotypes conferring resistance to INH, RIF, EMB, PZA, FQs, all of which are linked to resistance to second-line drugs (SLDs); from patients in the Vhembe region Limpopo province, South Africa.

## 1.3 RESEARCH QUESTIONS

The following questions need to be addressed,

- What are the risk factors commonly associated with XDR-TB?
- What is the extent of XDR-TB amongst patients?

## 1.4 OBJECTIVES OF THE STUDY

### 1.4.1 Primary objective

To determine drug resistant *M.tuberculosis* amongst TB patients in the Vhembe district, Limpopo, South Africa.

### 1.4.2 Secondary objectives

- To correlate the risk factors associated with drug resistance TB using a survey.
- To assess the presence of drug resistance using Allplex™ Multiplex PCR protocol.
- To evaluate resistance to second-line injectable anti-TB drugs targeting the mutation status of genotypes conferring resistance to INH, RIF, EMB, PZA, FQs, all of that are linked to resistance to second-line drugs (SLDs); using MassARRAY (Agena) technique.

## 1.5 HYPOTHESIS

- There is no drug resistance to second line drugs (SLDs) in rural settings.

# CHAPTER 2
# LITERATURE REVIEW

## 2.1 BACKGROUND ON TUBERCULOSIS

Before Robert Koch made the discovery of *Mycobacterium* in 1882, TB was thought to be a vampire illness. *Mycobacterium tuberculosis* (MTB) is a bacterium that causes the infectious illness tuberculosis (TB). Although it mostly affects the lungs, it may also influence other body regions. The organ that is damaged by TB may be related to the signs and symptoms. However, fever, chills, night sweats, loss of appetite, weight loss, and exhaustion are the common signs and symptoms of pulmonary TB (affects the lungs) and extra pulmonary TB (TB that affects other regions of the body). The fact that TB is an airborne pathogen makes the pathogen highly contagious. However, it is treatable, curable and can be prevented (Gehre *et al.,* 2016).

Every nation in the globe has tuberculosis infected people (Singh *et al.,* 2020). According to estimates from the World Health Organization (WHO), two billion individuals, or almost one-third of the global population, are infected with MTB (WHO, 2022). Combinations of several antibiotics are used for effective treatment of active TB. Furthermore, drug-resistance also compounds the burden of active TB disease, drug resistance arises when the *Mycobacterium* survives in an individual who is on drug therapy (Hameed *et al.,* 2018).

The resistance to first line TB drugs has existed since 1950 and as a result the ongoing emergence of Multidrug-resistant (MDR), Pre-extensively drug-resistant (Pre-XDR) and Extensively drug-resistant (XDR) TB. When an individual (the bacterial pathogen is not sensitive does not react to at least isoniazid and rifampicin, which are the two most potent anti-TB medications, such individual is referred to be MDR-TB). Pre-XDR TB is when an individual is resistant to Rifampicin (RIF) and Isoniazid (INH) and either a fluoroquinolone (FQs) [which includes: ofloxacin, moxifloxacin; and levofloxacin] or one of the three second-line injectable that includes: amikacin (AMK), capreomycin (CAP) and kanamycin (KAN)], but not both. Extensively drug-resistant (XDR) TB can be defined as MDR-TB with resistance to fluoroquinolone (FQs) and at least one of the three injectables (referred to as injectable aminoglycosides, which is a powerful group of drugs that inhibit protein

synthesis) (CAP, AMK, KAN) (Lienhardt *et al.,* 2012) OR mycobacterium that is resistant to INH, RIF, a FQ and bedaquiline or linezolid (CDC, 2023).

Additionally, linezolid, delamanid, bedaquiline and pretomanid were recently included in the second line drugs (SLDs) for the treatment of drug resistance TB. Even though the use of SLIs is gradually being discouraged due to their unfavorable side effects, they are still necessary in cases when the treatment regimen is shorter and there are less new SLDs available in resource-constrained or in developing nations (Blair *et al.,* 2015).

The world was astonished by the first case of extensively drug-resistant tuberculosis (XDR-TB) in 2006 (Cohen, 2006; Ghandhi *et al.,* 2006; WHO 2007). At the Tugela Ferry hospital in KwaZulu-Natal, South Africa, more than 50 patients were diagnosed with XDR-TB over a period of 7 months, with resistance to at least rifampicin (RIF), isoniazid (INH), a fluoroquinolone (FQ), and an injectable second-line treatment (SLID) (WHO 2007). After a median of 16 days following sputum collection, wherein one patient passed away (Singh *et al.,* 2007). Since then, instances of XDR-TB have been reported in 128 nations (WHO, 2019), making transmission prevention essential. A total of 553 confirmed cases of XDR-TB were reported in South Africa in 2018; this represents 0.18% of all TB infections and 5% of all MDR/RR-TB cases (WHO, 2019).

The inability to successfully treat drug-resistant TB might cause prolonged infectiousness, which can result in MDR-TB and XDR-TB outbreaks in heavily populated regions (Ghandhi *et al.,* 2013; Valway *et al.,* 2014; Sullivan *et al.,* 2016). Drug-resistant TB continues to threaten worldwide public health and TB treatment, despite the first sequencing of *M.TB's* genome in 1998. Numerous issues concerning the pathogen and host-pathogen interactions still need to be resolved, although it is obvious that MTB takes advantage and manipulates human host responses to facilitate its' survival and ongoing transmission (Oostvogels *et al.,* 2022). Therefore, it is crucial to understand the biochemical, genetic, and molecular causes of resistance in order to develop novel therapeutic approaches and thus combat drug resistance (Raviglione and Sulis 2016; WHO, 2018).

Due to the fact that, tubercle *bacilli* experience spontaneous, predictable rates of chromosomal alterations that confer drug resistance, drug-resistant TB develops. Since

these mutations are unconnected, resistance to one medicine is not correlated with resistance to a different drug. The fundamental idea behind TB treatment stems from the finding that these mutations are not connected. A susceptible strain of *M. tuberculosis* may quickly develop drug resistance if it receives monotherapy or has inconsistent drug compliance, skips one or more medications, inadequate dosage, poor drug absorption, or has insufficient amounts of active medications in its regimen (Toms *et al.,* 2017).

## 2.2 DEFINITIONS OF SURVEILLANCE FOR TUBERCULOSIS THAT IS EXTENSIVELY DRUG RESISTANT (XDR) AND PRE-XDR.

The World Health Organization's Global TB Programme updated its definition of XDR TB in January 2021 and formally defined pre-XDR TB for the first time. Definitions were changed for three reasons: to define more precisely groups of TB patients who require complex treatment regimens, to stimulate the development of better treatment regimens for these dangerous forms of TB disease, and to lead to better reporting, surveillance and monitoring of drug-resistant TB.

- Pre-XDR tuberculosis (TB): caused by mycobacterium resistant to isoniazid (INH), rifampicin (RIF) and a fluoroquinolone (FQ). Alternatively, by an organism resistant to amikacin, capreomycin, and kanamycin, that are second-line injectable drugs (SLID).
- XDR TB: caused by an organism resistant to isoniazid, rifampicin, a fluoroquinolone, and a second-line injectable (amikacin, capreomycin, and kanamycin). Alternatively, mycobacterium resistant to isoniazid, rifampicin, a fluoroquinolone, and bedaquiline or linezolid.

Table 2.1 presents the drug prescribed for treatment, including their classification against the acquired resistance by the TB strains. Every row on the table below displays a single drug resistance combination that satisfies the pre-XDR or XDR TB definition.

**Table 2.1:** Classification of drug resistance (WHO 2021; CDC 2022).

| | DRUG CLASSES | | | | |
|---|---|---|---|---|---|
| **CLASSIFICATION OF RESISTANCE** | INH and RIF | FQ (at least one) | SLID (at least one) | Bedaquiline | Linezolid |
| **MDR-TB** | X | | | | |
| **PRE-XDR TB** | X | | X | | |
| | X | X | | | |
| **XDR-TB** | X | X | X | | |
| | X | X | | X | |
| | X | X | | | X |

## 2.3 BIOLOGICAL CHARACTERISTICS

Active TB is brought on by infection with *Mycobacterium tuberculosis*, an alcohol- and acid-fast bacillus. It belongs to the *M. TB* complex group, which also contains the *M. canettii, M. microti, M. bovis, and M. africanum*, all of which are capable of causing active TB (Miassangoumouka *et al.,* 2020). The intracellular bacterium *M. tuberculosis* is facultatively catalase-negative, nonspore-forming, obligately anaerobic, and nonmotile. The high lipid content of *M. tuberculosis* contributes to its several distinctive clinical traits. These include the capacity to endure a variety of harsh circumstances and resistance to a number of antibiotics. Additionally, it divides slowly (16 to 20 hours approximately), taking far longer than other bacteria (which often divide in less than an hour) (Hayward *et al.,* 2018).

This organism does not fit into either the Gram-positive or Gram-negative categories since it reacts poorly to the Gram stain. However, sometimes faintly positive cells, often known as "ghost cells," may be seen on Gram stain. *M. tuberculosis* has been defined as an acid-fast bacillus because it maintains certain stains even after being treated with acidic solutions. The two most often used methods to identify *M. tuberculosis* are the Ziehl-Neelsen stain and the Kinyoun stain. The acid-fast bacilli are dyed brilliant red by the test, making them stand out against a backdrop of blue (Forrellad *et al.,* 2013).

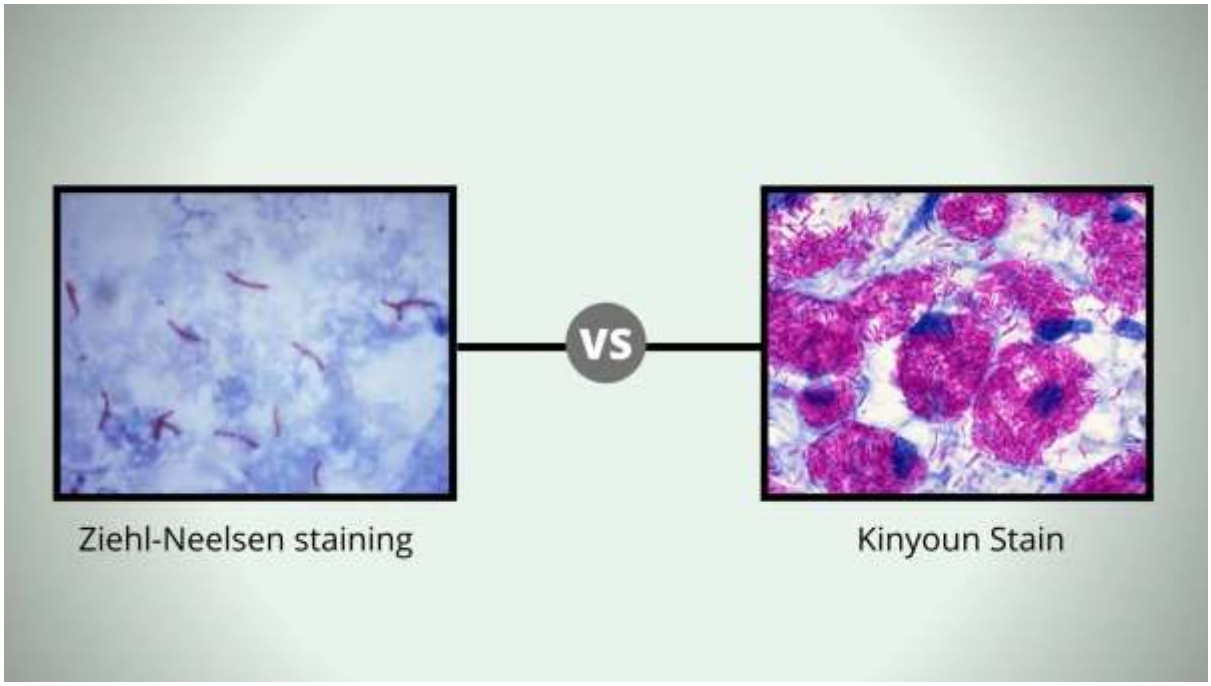


**Figure 2.1:** *M. tuberculosis* under the microscope during Ziehl-Neelsen stain and the Kinyoun stain technique (https://microbiologynote.com/comparison-between-kinyoun-stain-vs-ziehl-neelsen-stain/).

Only humans have been shown to be the native host of *M. tuberculosis*. Although transdermal and gastrointestinal (GI) transmission are also conceivable, airborne aerosols from a patient in the infectious stage of the illness are the main way the organism is transmitted (Jackson *et al.,* 2020).

## 2.4 NONTUBERCULOUS MYCOBACTERIA

Nontuberculous mycobacteria (NTM) are ubiquitous in the environment, especially in soil and water. In contrast to *M*. *tuberculosis*, these bacteria are typically acquired from environmental exposure rather than contact with infected individuals. The NTMs that infect humans most frequently are *Mycobacteria avium, M. kansasii,* and *M. abscessus* (Desai and Hurtado, 2021).

Nontuberculous mycobacteria can infect many parts of the body. These bacteria belong to a different class of mycobacteria, while having a relationship to the type that causes tuberculosis, *M. tuberculosis*. The lungs are where infections occur most frequently, although blood, lymph nodes, and skin and soft tissue infections can all happen. It is uncommon for a nontuberculous mycobacterial infection to simultaneously produce

illness outside and inside the lungs. Certain nontuberculous mycobacterial infections are brought on by contaminated medical equipment, like pacemakers or catheters that are put into circulations (Tortoli, 2017; Fedrizzi *et al.,* 2017; Parte, 2018).

## 2.5 INTERACTION OF HOST AND *M.tuberculosis* BACTERIUM

When the bacteria are phagocytosed by macrophages in the lungs, they survive the body's natural anti-microbial activities and cause infection. Infected macrophages often perish while trying to kill the bacterium, delivering live germs into respiratory passages (Ferguson *et al.,* 2004). The illness takes a little over four to twelve weeks to incubate, and it progresses gradually. Fever, exhaustion, nighttime sweats, and loss of weight are all signs of TB. The cough that is a sign of pulmonary involvement may cause bloody sputum to be expectorated (Korf *et al.,* 2009).

MTB possess distinctive traits that add to its pathogenicity. MTB's cell envelope contains special lipids and glycolipids that are directly poisonous to eukaryotic cells and that also help the bacterium to be impermeable to antimicrobial treatments and resistant to them by forming a hydrophobic barrier around it. This further guards the bacteria from being killed by lysozyme, acidic and alkaline substances, and osmotic lysis (Clay *et al.,* 2008). By making use of the macrophage mannose receptors, cell wall glycolipids interact with mannose and provide MTB control over entrance into these cells. Once inside, MTB modifies the phagosome membrane to prevent fusion of phagosome-lysosome. This mechanism makes the bacterium superior and thus contributes to the survival of MTB within macrophages also include resistance to oxidative death, prevention of phagosome-lysosome fusion, and inhibition of diffusion of lysosomal enzymes (Glickman and Jacobs, 2001).

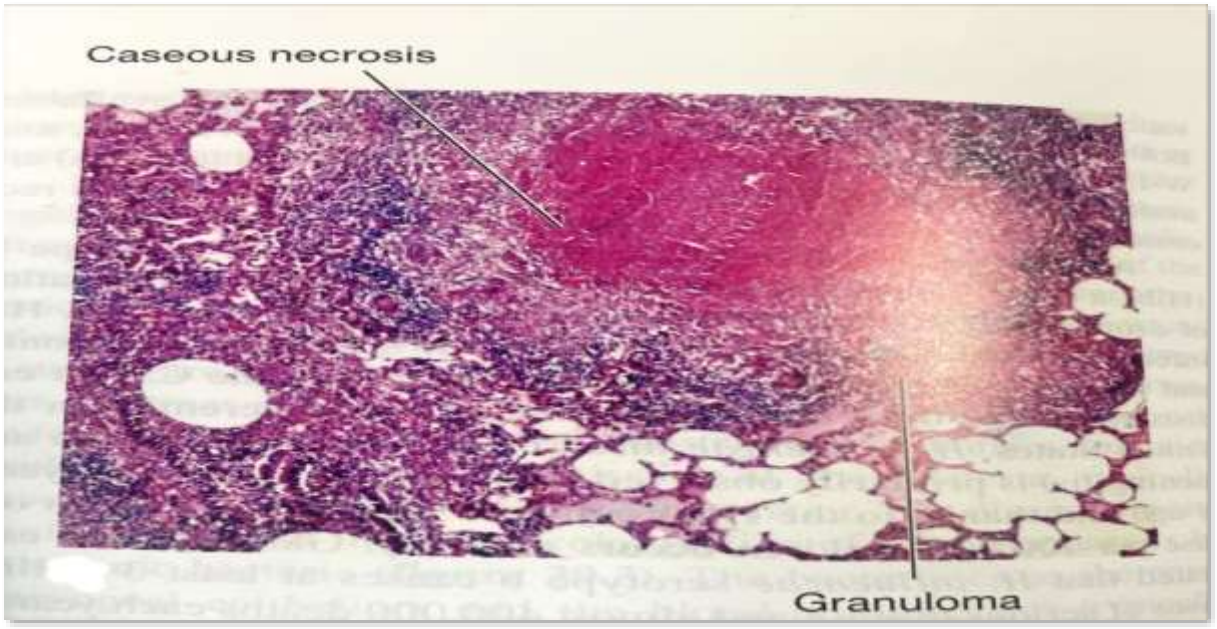


**Figure 2.2:** In the lungs, TB is identified by the tubercle, a granuloma of white blood cells, bacteria, fibroblasts and epithelioid cells (https://images.app.goo.gl/28dsF88NqupXVmhE6).

When an infection occurs, cytokines generated by the reacting macrophages attract other immune cells to the infection site. A hypersensitive reaction results in the creation of tiny nodules known as tubercles made up of bacteria, macrophages, T cells, and different human proteins when it occurs jointly and in response to numerous mycobacterial products (Gorna *et al.,* 2010). The illness gets its name from the tubercles that are a defining feature of TB. At this point, the disease normally comes to an end, although the bacteria are often still alive within the phagosomes of macrophages (McGrath *et al.,* 2014). Even after several years of latency, the illness may sometimes reactivate. A tubercle may eventually have a cheese-like consistency, at which point it is known as a caseous lesion or granuloma (Figure 2.2) (Franzblau *et al.,* 2012; Adane *et al.,* 2013).

These lesions are known as Ghon complexes if they calcify and are very visible on a chest X-ray. The tubercle lesions may sometimes liquefy and produce air-filled tuberculous chambers. The germs may spread to new foci throughout the body from these cavities. Due to the many millet-seed-sized tubercles that develop in the diseased tissue, this spreading is sometimes referred to as miliary TB. Due to the bacteria's reactivation in the original site of infection, it is also known as reactivation tuberculosis. The majority of infections are contracted from other people by respiratory droplet nuclei, as displayed on Figure 2.3 (Smith *et al.,* 2016).

If an individual contracted MTB or been exposed to MTB, they acquire cell-mediated immunity as a result of the bacteria's potent TH1-cell response. To detect these, the tuberculin skin test is performed, that is based on the sensitized T-cells. The Mantoux test involves injecting a pure protein derivative (PPD of MTB) intracutaneously. Sensitized T- cells react with these proteins in the presence of MTB, and a delayed hypersensitivity reaction takes place within 48 hours. The area around the injection site becomes indurated (hardened) and reddened as a result of this favorable skin reaction. A positive skin test could signal active TB in a young individual. It may be brought on by a past illness, a vaccination, or a false-positive test in elderly people. To confirm the diagnosis in these situations, X-rays and bacterial isolation are recommended (Forrellad *et al.,* 2013).

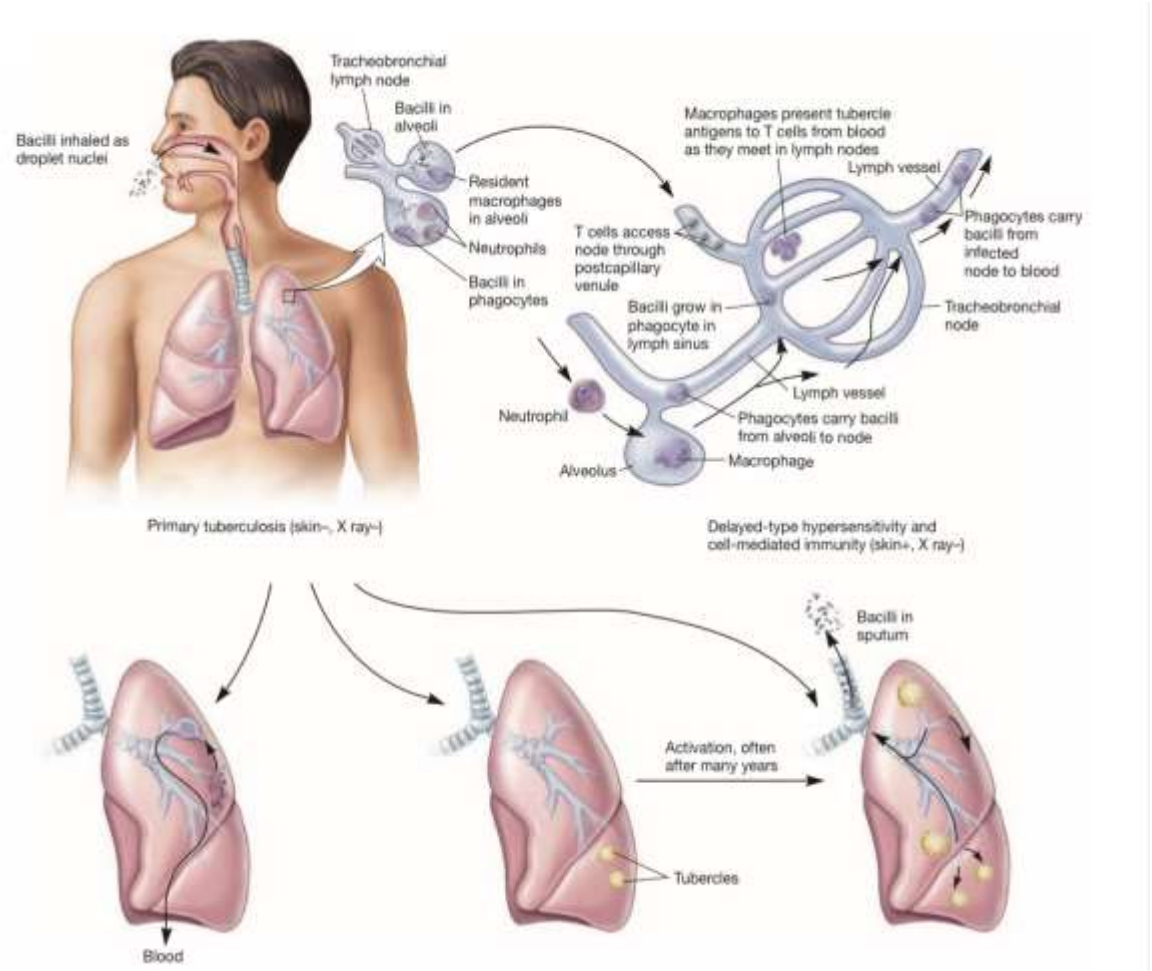


**Figure 2.3:** Mycobacterium tuberculosis infection and its possible outcomes (Woolverton *et al.,* 2008).

## 2.6 PREVALENCE, EPIDEMIOLOGY AND DRUG-RESISTANCE TB

Southeast Asia accounted for 45% of all cases of tuberculosis worldwide, with Africa coming in second with 23% and the Western Pacific with 18% (Fig. 2.4). Other countries like China, Indonesia, India, Nigeria, the Philippines, Pakistan, Bangladesh, the Democratic Republic of the Congo, and Bangladesh account for two thirds of these instances. Males are reported to be more likely afflicted than females, with children making up 11% of almost all TB cases. The projected MDR/RR case rate in 2021 was 18% among subjects who had previously received treatment and 3.6% among new patients (WHO, 2022).

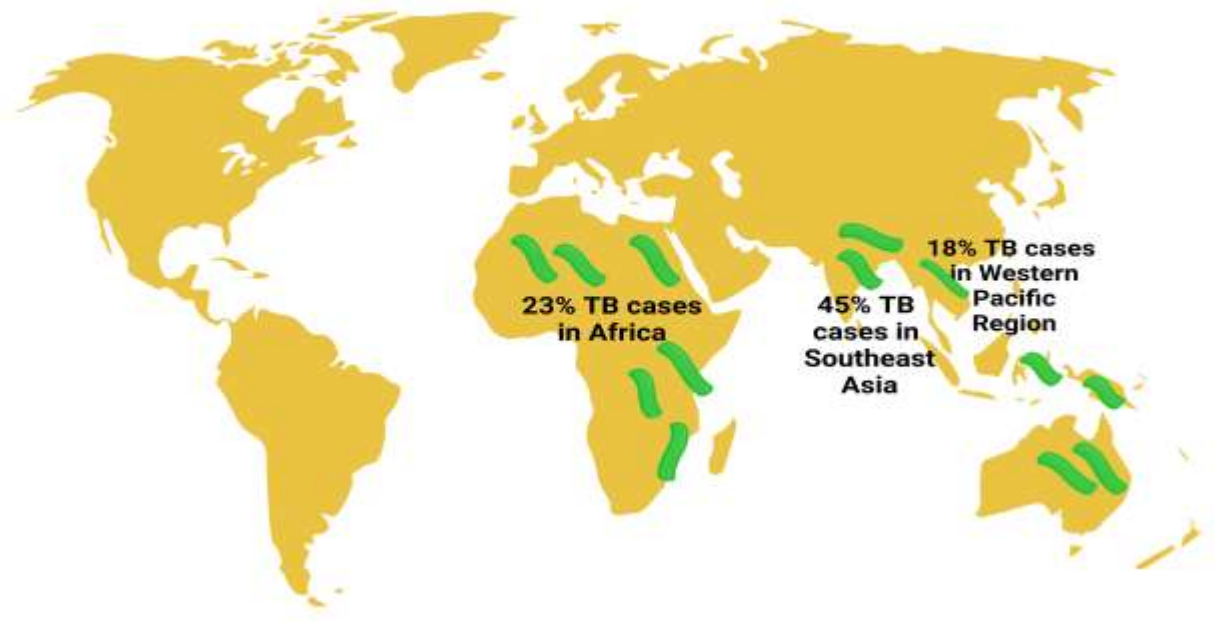


**Figure 2.4:** A map showing the percentage of tuberculosis cases in Southeast Asia, Africa, and the Western Pacific region of the world (Chowdhury *et al.,* 2023).

In terms of MDR/RR TB cases worldwide, the largest contributors are India, Pakistan, and the Russian Federation. Continuous surveillance systems have now been implemented in more nations. This approach tests for drug resistance using quick molecular techniques. Drug susceptibility data for the years 2021–2025 is being kept by thirty-eight countries with greater caseloads (WHO, 2022). Yet there is a sizable disparity in the drug resistance profiles for paediatric TB (Yuen *et al.,* 2015; Roberts *et al.,* 2021;

Malik *et al.,* 2022). According to estimates, 3% of pediatric TB patients roughly 25,000–32,000 cases annually develop resistance. Only 3–4% of them obtain a diagnosis and treatment, and about 21% of them will die (Harichander *et al.,* 2022).

The World Health Organization (WHO, 2018) reports that the overall cure rates for MDR-TB and XDR-TB were only 56% and 39%, respectively. The proportion of patients who are first-time MDR and rifampicin-resistant TB patients has been around 3% to 4% for over ten years, while the proportion of patients who have received prior TB treatment has been between 18% and 21%. Some countries even have higher than 50% proportions of MDR-TB cases that have received prior treatment (WHO, 2021).

In South Africa, TB claimed the lives of over 304,000 individuals (832/day) in 2021, while the disease was treatable and preventable in 56,000 cases. The nation has experienced a more than 50% decrease in new infections and a nearly two-thirds decrease in associated mortality since 2009, despite these concerning statistics stated by National Institute of Communicable Diseases (NICD, 2023). The estimated prevalence of HIV co-infection among TB patients was 28% (Department of Health, 2018). Kwazulu-Natal, Eastern Cape, and Western Cape are the provinces with the highest recorded rates (Pillay *et al.,* 2021). The prevalence of TB increased in Limpopo from 165 per 100,000 in the previous year to 197 per 100,000 in 2019–20. In the province of Limpopo, the number of TB cases fell from 12,709 in 2018 to 11,747 in 2019, and then to 8,874 in 2020 (Mehlape, 2021).

## 2.7 RISK FACTORS

There are several risk factors involved in one contracting active tuberculosis, and the most common is HIV (active TB is 20–30 times more likely to occur in individuals living with HIV) (Habib *et al.,* 2018). As summarized in figure 2.5, TB commonly affects the homeless, old, malnourished, intoxicated men (alcoholic), minorities, immigrants, populations within prisons, and those who live in crowded settings. It may also be nosocomial. It's estimated that between one-fourth and one-third of current TB infections may be the result of recent transmission. Thus, the bulk of current cases are the consequence of previous, latent infections becoming active again (Dookie *et al.,* 2018).

Other immunocompromised conditions present, including immunosuppressive treatments like long-term corticosteroids and anti-TNF (tumor necrosis factor) drugs. Pulmonary conditions are chronic, and mostly influenced by consumption of tobacco products. One of the main diseases of poverty is TB, which has a greater probability of developing into an active disease when there is malnutrition. Additionally, diabetes has a higher probability of developing into active TB and having less successful treatment results. Alcohol use (more than 40 grams daily), misuse of intravenous drugs, indoor contaminant, inhaling significant quantities of crystalline silica dust leads to the long-term lung disease (silicosis). Chronic kidney disease, gastrectomy or intestinal bypass surgery and severe malnutrition and malabsorption (inability of the small intestine to take up nutrients) (Falzon *et al.,* 2017).

Additionally, the increasing risk of drug resistance TB is associated with low socioeconomic status (such as employment, education, income, poor nutrition), alcoholism, smoking, immigrant status, co-infection with other diseases (HIV/AIDS, diabetes, fungal infection). Moreover, the presence of a TB patient with an undisclosed status within the house play as an are additional risk factors for drug-resistant TB transmission (Singla, 2017; Hameed *et al.,* 2018; WHO, 2018).

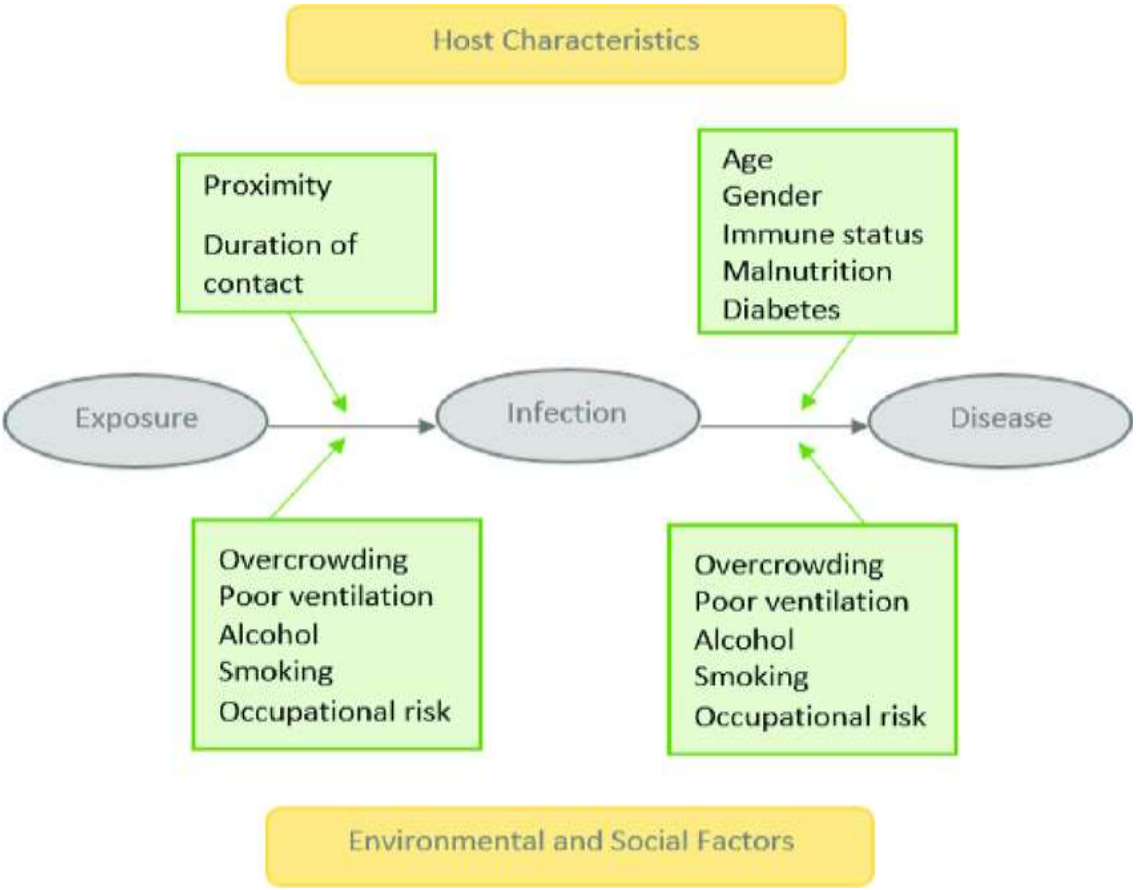


**Figure 2.5:** The risk factors for TB infection and disease (Davies, 2005).

### 2.7.1 HIV (Human Immunodeficiency Virus)

HIV screening is necessary regardless of a person's physical make-up when they are diagnosed with tuberculosis. A significant feature of HIV infection that increases the host's risk of developing active tuberculosis is the chronic activation of T cells and the progressive reduction of CD4+ T lymphocytes. When CD4+ T-cells are decreased in HIV patients with latent TB infection, the steady state and morphology of TB granulomas in the lung are disrupted, which accelerates the progression of infection to disease by a factor of 20 (Geldmacher *et al.,* 2012). According to recent data, patients with tuberculosis (TB) and diabetes mellitus were found to be at an increased risk of treatment failure, relapse, and death if they had clinical findings such as cavities, lower lung field lesions, and acid-fast bacilli (AFB) smear positivity (Carreira *et al.,* 2012; Workneh *et al.,* 2017). The WHO research states that HIV and tuberculosis infections were impacted by the increased incidence of diabetes mellitus in sub-Saharan African countries (WHO, 2016).

### 2.7.2 Elderly individuals

Given that cellular immunosuppression is linked to the likelihood of developing TB disease, compared to HIV-negative people, HIV infection is closely associated with a greater incidence and mortality rate (WHO, 2016). Older adults living with HIV may comprise a diverse group evenly divided between individuals ageing with HIV and those who acquired HIV at an older age (Guaraldi *et al.,* 2015). An increase in older adults living with HIV is rising along with the proportion of senior persons, which is growing annually at a higher rate than the overall population (Mcnicoll, 2012; Smit *et al.,* 2015). In 2016, 20% of new HIV diagnoses in France were for people 50 years of age or older, with 73% of those diagnoses falling into the range of 50 years of age. But those between the ages of 60 and 69 made up to 23%, and 4% those beyond 70 years (Tran *et al.,* 2018). The cotreatment of TB with antiretroviral therapy and its ideal timing of initiation, drug-drug interactions, drug tolerability, and the prevention and treatment of tuberculosis-associated immune reconstitution syndrome are just a few of the factors that must be taken into account when managing TB in elderly HIV individuals (Meintjes *et al.,* 2019).

### 2.7.3 Stigma associated with TB

The public has inadequate knowledge of TB and has many misconceptions about the disease, according to investigations which has been carried out around the globe to investigate the level of knowledge, attitude, and stigma around TB infection (Chinenye, 2015). Overall, attitudes and understanding on TB differ between nations, ranging from highly stigmatized to supportive perspectives regarding the disease and its patients. Sufficient understanding and optimistic attitudes about TB are necessary to enhance the general population's health-seeking behaviours concerning this infection (Chang and Cataldo, 2014; Agho *et al.,* 2014).

According to Duko *et al.,* (2019), TB is a highly stigmatized disease that is found in a variety of social settings, including the family, workplace, and community. According to Samal (2016), the stigma is a condition that prevents an individual from receiving complete social acceptance, thus, transforming such individuals from being regular into being the contaminated ones. The public health strategy for early detection and treatment is severely impacted by stigma. Furthermore, because of the stigma associated with TB, patients choose to hide their diagnosis from colleagues and family members, which

makes it more difficult to trace contacts and identify new cases (Ngamvithayapong *et al.,* 2019; Pinar and Hidiroglu, 2021).

Active TB disease may occur from TB contacts, with the majority of cases being identified three months or less following the diagnosis of the index patient. It was previously believed that knowledge, attitude, and stigma (KAS) linked to TB among contacts was associated with early case detection and health-seeking behaviour. Consequently, quick action can be taken to effectively cure the illness and enhance the prognosis in general (Shin *et al.,* 2023). Furthermore, stigmatization and knowledge about TB are linked to healthcare-seeking behaviour (Ali *et al.,* 2019). Insufficient awareness about TB can result in various problems and poorer health outcomes. It can also delay the behaviour in seeking medical attention, which can lead to noncompliance, treatment failure, and eventually, death (Gelaw, 2016). Therefore, it's critical to have accurate understanding and information on TB in order to raise public awareness of the disease.

## 2.8 TRANSMISSION

*M. tuberculosis* circulates in airborne droplet nuclei, which have a diameter of 1 to 5 microns. Infectious droplets are released to the atmosphere when people with pulmonary or laryngeal TB illness cough, sneeze, yell, or sing. These small particles may float in the air for many hours, depending on the surroundings. MTB spreads by airborne rather than surface-to-surface contact. When a person inhales *M. tuberculosis*-carrying droplet nuclei, the nuclei travel via the mouth or nasal passages, upper respiratory tract, and bronchi to reach the lung's alveoli (Gaharwar *et al.,* 2016).

## 2.9 PATHOGENESIS OF TUBERCULOSIS

TB infection may develop in both pulmonary and extrapulmonary sites of an individual (Sharma and Mohan, 2005).

### 2.9.1 Risk of Latent TB infection (LTBI) developing into TB Disease

Although TB disease can strike anyone with LTBI, certain persons are more susceptible than others. Because HIV infection weakens the immune system, it is the biggest risk factor for TB disease progression in people with LTBI. For those who are not receiving

highly active treatment for HIV and have both *M. tuberculosis* and HIV infection, the chance of acquiring TB disease is 7% to 10% annually; for those who have only *M. tuberculosis* infection, the risk is 10% throughout the course of their lifetime. Youngsters under the age of five are also more vulnerable to LTBI developing into TB illness (CDC, 2011).

### 2.9.2 TB Disease

In certain individuals, the immune system is overpowered by the tubercle bacilli, that leads to the progression of LTBI into TB disease. Individuals suffering from tuberculosis are typically contagious and have the potential to infect others. Any time from shortly after to many years later, the transition from LTBI to TB disease might happen. For an AFB (acid-fast bacilli) smear and culture, bodily fluid or tissue from the illness site needs to be taken. The diagnosis of tuberculosis disease is verified by a positive culture for *M. tuberculosis* (Modlin and Bloom, 2013).

### 2.9.3 Pulmonary TB

Pulmonary TB is the term for the type of tuberculosis that most frequently affects the lungs. Patients with pulmonary tuberculosis typically exhibit an abnormal chest radiograph, a cough, and possible contagiousness. Even while lung infections account for the bulk of TB cases, the disease can spread to practically any anatomical region (McMurray, 2001).

### 2.9.4 Extrapulmonary TB

In addition to the lungs, additional organs affected by extrapulmonary tuberculosis include the larynx, lymph nodes, pleura, brain, kidneys, bones, and joints. Pulmonary TB frequently coexists with extrapulmonary TB illness in HIV-positive individuals. Extrapulmonary TB diseased patients are typically not contagious unless they also have one of the following conditions:

- pulmonary disease in addition to extrapulmonary disease.
- extrapulmonary disease in the oral cavity or larynx; or
- extrapulmonary disease with an open abscess or lesion that has a high concentration of organisms, particularly if the abscess or lesion drains extensively or if the drainage fluid is aerosolized.

Because the effusion fluid compresses the lung, people with TB pleural effusions may have underlying pulmonary TB that is hidden on chest radiographs. Until pulmonary tuberculosis is ruled out, these individuals ought to be treated as contagious (Golden and Vikram, 2005; Lee, 2015; Sharma *et al.,* 2021).

### 2.9.5 Disseminated TB

An immediate progression of primary TB (TB through inhaling aerosols from an infected person's cough or sneeze. Primary TB refers to the lung infection that results from it.). Dispersed over the entire body. Multiple tiny tubercles (miliary tuberculosis) may develop. Usually fatal, usually seen in small children or those with disabilities. Minimal to nonexistent hypersensitivity. gradual systemic illness and demise (Wang *et al.,* 2007).

### 2.9.6 Miliary TB

When tubercle bacilli enters the bloodstream and spread to every area of the body, they multiply and cause disease in numerous locations, leading to mild cases of TB. This is a rare yet dangerous condition. The term "millet seed" describes the appearance of dispersed millet seeds across the lung on radiographs. It most commonly affects highly immunocompromised individuals as well as newborns and kids under the age of five. Miliary TB can be found in one organ, such as the brain, many organs, or the entire body. The illness is marked by a high concentration of tuberculosis bacilli, yet it can be readily overlooked. If left untreated, the condition can be fatal. Patients with miliary TB may have meningeal involvement in as many as 25% of cases (Sharma and Mohan, 2017).

### 2.9.7 Central Nervous System

Tuberculous meningitis is the term for tuberculous disease that develops in the tissue around the brain or spinal cord. Imaging examinations frequently reveal the base of the brain affected by tuberculous meningitis. Headache, lowered consciousness, and stiff neck are some of the symptoms. The length of sickness prior to diagnosis varies and is partially correlated with the existence or non-existence of additional sites of involvement. Patients with meningitis frequently have miliary TB and have abnormalities on a chest radiograph that are consistent with either past or present TB (Be *et al.,* 2009).

## 2.10 DIAGNOSIS

*Mycobacterium tuberculosis* complex *bacilli* isolated from body fluids are used to diagnose active TB. Any patient who is thought to have active TB poses a transmission risk to the public health system and should be isolated with airborne protections. Initial testing for pulmonary tuberculosis comprises a chest X-ray and sputum analysis. Acid-Fast Bacilli smear (AFB smear), mycobacterial culture, and nucleic acid amplification testing (NAAT) are all used in the examination of sputum. Occasionally, the inability to produce sputum can be a problem; in this case, nebulized hypertonic saline can be utilized to induce sputum (Luthra *et al.,* 2015).

### 2.10.1 AFB (acid-fast bacilli)

The CDC suggests collecting three sputum samples, at least one of which should be taken in the morning. Each specimen raises the test's sensitivity (Mase *et al.,* 2007). The sensitivity is increased by 12% using the morning sample (Steingart *et al.,* 2006). Centrifugation or sedimentation may increase the sensitivity of sputum smears (Lewinsohn *et al.,* 2017). The space between samples should be at least eight hours. The minimum amount of sample recommended by the ATS (American Thoracic Society) is 3ml, although 5–10ml of sputum is ideal. The technically straightforward and quick AFB smear cannot distinguish between *Mycobacterium tuberculosis* and nontuberculous mycobacteria (Chihota *et al.,* 2010).

### 2.10.2 Nucleic Acid Amplification Testing (NAAT) and Genetic testing

A new generation of diagnostic methods for TB includes nuclear amplification and gene-based testing. By using DNA-based molecular methods, these tests make it possible to identify the bacteria or bacterium particles. These methods provide expedited diagnosis with high accuracy and are quicker. Instead of waiting days or weeks for a conventional culture, confirmation of the TB infection might be achieved in a matter of hours. These tests are particularly crucial for immunocompromised individuals since they often get false-negative findings. DR-MTB and GeneXpert are two molecular-based diagnostics that enable the detection of multidrug-resistant tuberculosis infections. Regardless of the findings of the AFB smear, a positive NAAT test on a single sputum sample is considered adequate for the diagnosis of Active Tuberculosis. NAAT positive may be utilized as a presumptive indicator of TB in cases when the AFB smear is negative but there is a

moderate to high suspicion of active TB. Thus, pulmonary TB cannot be ruled out by NAAT (Jilani *et al.,* 2018).

### 2.10.3 Sputum Mycobacterial Culture

For diagnosis, mycobacterial culture is the gold standard. Both solid and liquid media should be used for mycobacterial culture. The gold standard for bacterial detection is liquid media culture, which can identify extremely low bacterial loads. Testing for drug susceptibility requires culture (Chihota *et al.,* 2010). Solid medium costs less, but it takes longer for the organism to develop. Although liquid medium is more sensitive and costly, it may start growing organisms after 10 to 14 days. Culture may distinguish between MTB and NTM (O'Grady *et al.,* 2011).

- Solid medium called Remel Lowenstein-Jensen (L-J) medium is recommended for use in high-quality processes for the isolation and culture of *Mycobacterium* species.
- A glycerated egg-potato medium is called L-J Medium. The glycerol and egg combination provide the mycobacteria with the fatty acids and protein they need to function. Malachite-green dye is included as a pH indicator and an inhibitor of microbes other than mycobacteria.
- Antibiotics are added to L-J Medium to suppress contaminating microorganisms as well. Gram-positive bacteria are inhibited by lincomycin and penicillin G, while certain Gram-negative bacteria seen in clinical specimens are inhibited by nalidixic acid. Moulds and yeast that are saprophytic are inhibited by cycloheximide.
- Over the conventional L-J Medium, ribonucleic acid enhances the proportion of positive cultures.

### 2.10.4 Drug susceptibility testing (DST)

Phenotypic and genotypic testing are two ways for detecting drug susceptibility (Nelson *et al.,* 1998). Genotypic testing is more rapid than phenotypic testing. A culture-based approach for distinguishing MTB from NTM and drug susceptibility to rifampicin and isoniazid is the microscopic observation of drug susceptibility (MODS) assay. Another approach for determining antibiotics susceptibility is polymerase chain reaction (PCR).

There are numerous commercial test kits available to detect antibiotics susceptibility, which is important in establishing therapy course and duration (Lewinsohn *et al.,* 2017).

### 2.10.5 Allplex™ (Seegene)

Seegene is the owner of two exceptional PCR technologies: TOCE and dual priming oligonucleotide (DPO). DPO is a vital instrument that enables the creation of assays with remarkably high specificity by preventing the extension of non-specifically primed templates. Assays for the detection of point mutations and multiplexed molecular assays are two examples of molecular diagnostic systems that can effectively integrate the power and usefulness of DPO technology. A new real-time readout method based on melting temperature analysis is called TOCE. Thus far, melting temperature analysis has been limited by a number of intrinsic constraints, including limited probe design, limited capacity for widespread and effective multiplexing, and high probe temperature sensitivity as a result of sequence variation at the probe site. By offering improved multiplexing capabilities, more probe design freedom, a readout that is independent of target sequence variation, and cross-platform compatibility, TOCE technology gets around these drawbacks. Multiple point mutations can be detected simultaneously and with great specificity in real time by combining the DPO and TOCE technologies. A multiplex real-time PCR assay called Allplex™ MTB/MDR/XDRe Detection enables the simultaneous amplification and identification of *Mycobacterium tuberculosis* target sequences (Allplex™, Seegene, Seoul, South Korea).

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### 2.10.6 MassARRAY

The MassARRAY system, a DNA time-of-flight mass spectroscopy tool, was developed in San Diego, California by Sequenom, Inc. MassARRAY is based on multiplex PCR and uses a single-base extension method that is comparable to the Sanger method. The difference in nucleotide masses—adenine, guanine, cytosine, and thymine—is used to identify the kind of mutation. Dideoxynucleotides (ddNTPs) are employed to prolong a single base after the primer. DNA time-of-flight mass spectroscopy is a highly sensitive and adaptable high-throughput approach for detecting mutations (Murray, 1996; Edwards *et al.,* 2005; Tost and Gut, 2006; Yuan *et al.,* 2011). Because of this, the majority of TB drug resistance gene loci can be found in 1–2 reaction systems with a theoretically similar level of accuracy as the Sanger method.

### 2.10.7 Line probe assay (LPA)

Compared to culture-based DST, which takes two to six weeks, LPAs are accurate and only take five hours (WHO, 2021). By reverse hybridizing DNA on a visually readable test strip, the LPA approach targets DNA sequences linked to resistance to first-line (RIF and INH) and some second-line TB medications (fluoroquinolones and second-line injectable therapies) (Mäkinen *et al.,* 2006). The WHO recommends GenoType MTBDRplus and GenoType MTBDRsl (Hain LifeScience GmbH, Germany) as LPAs for drug resistance testing in sputum smear-positive samples (WHO, 2020). By identifying mutations in the rifampicin resistance region of the rpoB gene (codon 505 to 533), the isoniazid resistance region of the inhA promoter (−16 to −8 nucleotides upstream of inhA), and katG (codon 315) in sputum smear-positive samples or indirect culture isolates, the GenoType MTBDRplus (FL-LPA) assay detects Mtb complex and drug resistance.95–98 RIF and INH resistance detection exhibited 96.7% and 98.8% sensitivity and 90.2% and 99.2% specificity in sputum smear-positive samples. For the detection of RIF on culture isolates, 99–103 MTBDRplus exhibits 100% specificity and 95.1% (95% CI 92.2% to 98.1%) sensitivity. According to (Tomasicchio *et al.,* 2016), the INH resistance detection sensitivity and specificity were 96.1% (93.5% to 98.7%) and 90.8% to 100%, respectively.

Sputum smear-positive specimens can be promptly diagnosed and treated by the GenoType MTBDRplus; however, for smear-negative instances, it is dependent on culture isolates from respiratory tract samples. The open-tube format amplicon-producing MTBDRplus assay necessitates many steps to minimize amplicon cross-contamination, a minimum of three DNA extraction rooms, stringent standard operating procedures, and an experienced operator. Hybridized bands from these tests must be interpreted by skilled readers; semi-automation is an additional expense (de Vos *et al.,* 2018). The Genotype® MTBDRsl assay (SL-LPA) version 2.0 was published in 2015 and identifies mutations associated with SLID and FQ resistance. The MTBDRsl assay looks for SLID resistance in the rrs and eis genes (positions 1401, 1402, and 1484), quinolone resistance in the gyrA and gyrB genes (codons 85-96, 536-541), and eis gene (−37 to -2 nucleotides upstream). DNA from Mtb culture or a processed sputum sample can be used for the assay. For FQ resistance identification by direct testing, MTBDRsl provides 86.2% sensitivity and 98.6% specificity; for SLID resistance detection, it gives 87.0% and 99.5% (WHO, 2016). The diagnostic accuracy of the Genotype® MTBDRsl assay was examined in multiple trials, yielding comparable outcomes. LPAs can be performed quickly, simply,

and easily understood (either manually or mechanically). Utilizing LPAs necessitates expensive equipment typically found in reference labs and a sophisticated laboratory infrastructure (WHO, 2020). These limitations are further increased by the considerable amount of unclear data and LPA target coverage to frequent high-confidence mutations (Nandlal *et al.,* 2022).

## 2.11 THE MAJOR SECOND-LINE ANTITUBERCULOSIS DRUGS, MOLECULAR MECHANISMS OF ACTION AND DRUG RESISTANCE

Molecular-based techniques have become an alternate means of diagnosing TB and determining the resistance status of the bacteria. This is because mutations impacting the function and/or expression of chromosome- encoded targets have been found to be associated with resistance to anti-tuberculosis drugs (Aubry *et al.,* 2006).

### 2.11.1 Mutations associated with FQs

Point mutations in the *gyrA* and *gyrB* genes, which encode the two subunits of the DNA topoisomerase gyrase, are the primary mechanism of resistance to FQs. The Quinolone Resistance Determining Region (QRDR) in the *gyrA* gene and the *gyrB* gene experience less common mutations that give resistance to FQs (Zhang and Yew, 2009). Codons 88 and 91 and 90 and 94 are seldom mutated in the *gyrA* gene, but codons 472 and 510 are primarily impacted in the *gyrB* gene (Kumar *et al.,* 2018).

FQs, such as: ofloxacin (OFX), which is frequently used to treat MDR-TB. SNPs in the 16S rRNA gene (*rrs*) are linked to resistances to amikacin (*rrs*), kanamycin (*rrs,eis*), and capreomycin (*rrs,tlyA*), particularly in the region between nucleotides 1400 and 1500 (Alangaden *et al.,* 1998; Wilbrink *et al.,* 2007). The drugs ethionimide encodes for the gene *ethA*, and bedaquiline for the gene *rv0678* (Coll *et al.,* 2018). Anywhere in the *tlyA* gene, which encodes 2-O-methyltransferase, is thought to be another site of mutation that mediates resistance to CAP (Maus *et al.,* 2005; Johansen *et al.,* 2006). According to the scant information that is currently available, nucleotide alteration in *rrs* and amino acid substitution within *gyrA's* QRDR may be responsible for up to 87% and 70%, respectively, of KM/AMK and FQ resistance globally (Zhang and Yew, 2009).

## 2.12 DRUG RESISTANCE MECHANISMS IN *Mycobacterium tuberculosis* TB

Drug resistance is a significant obstacle to the treatment of tuberculosis and presents a problem for worldwide public health and medicines. Drug-resistant isolates of MTB are growing despite the effectiveness of anti-TB medications. Compensatory evolution (an effect size at a locus will develop differently from and be negatively linked with effect sizes at other loci), epistasis (interaction between genes that are not alleles, particularly the inhibition of one such gene's impact by another), clonal interference, cell envelope impermeability, efflux pumps, drug degradation and modification, target mimicry, and phenotypic drug tolerance are a few mechanisms that aid in the evolution of drug resistance in MTB (Saeedi and Hajoj, 2017; Rojas *et al.,* 2019). Both intrinsic (high levels of naturally existing antibiotic resistance) and extrinsic (recently acquired mutations) antibiotic resistance may contribute to the failure of TB treatments.

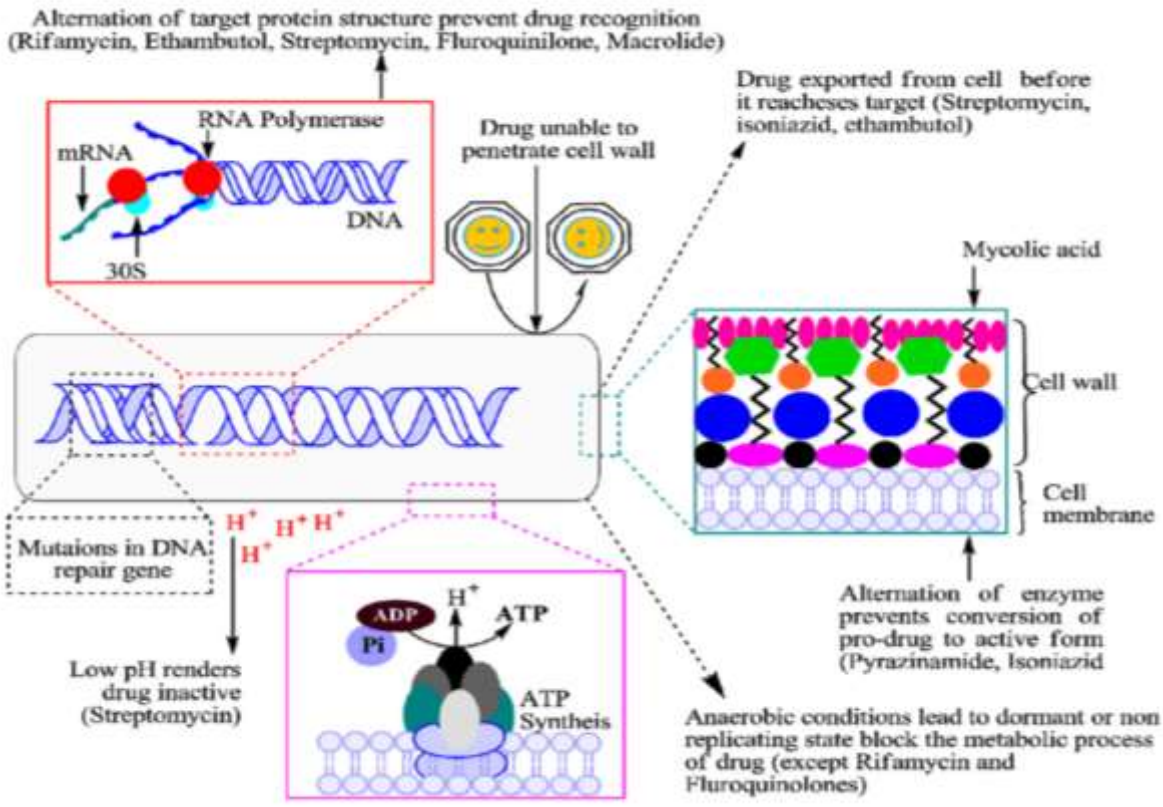



**Figure 2.6:** An illustration of the *Mycobacterium tuberculosis* drug-resistant mechanism (Swain *et al.,* 2020).

For instance, MTB's genome has an enzyme that codes for *β-lactamase*, making it intrinsically resistant to the β-lactam class of antibiotics (Pinto and Menzies, 2011; Shim and Jo 2013; Muller *et al.,* 2013; Nguyen, 2016). Long-term TB therapy often results in patient noncompliance with medication regimens and a fast development of Mtb from mono-drug resistance (resistance to anyone TB treatment) to MDR and XDR. Understanding the mechanisms of antibiotic resistance is the first step in combating drug resistance in *Mycobacterium* (Nguyen, 2016; Kurz *et al.,* 2016).

### 2.12.1 Drug resistant mechanisms of MTB

According to Darwin evolution theory, drug resistant Mtb strains currently proliferate by evading anti-TB treatments through the adoption of genetic changes and other mechanisms in addition to selecting environmental pressure as shown in Figure 2.6. Drug-resistant MTB strains have evolved as a result of long-term, continuous combination medication therapy as well as environmental factors. These bacteria eventually develop resistance to the current anti-TB medications (Mitchison *et al.,* 2008; Palomino and Martin 2014; Zhang and Yew 2015). Genetic changes or mutations in the MTB genome can occasionally result from a variety of environmental variables. These changes not only lessen the efficiency of the treatment being given, but also increase the likelihood that the MTB will survive in any harsh environment (Palomino and Martin, 2014).

This survival encourages the multidrug-resistant phenotypes of MTB and symbolizes the lethal exposures to bactericidal drugs through radical-induced mutagenesis. Reactive oxygen and free radicals can occasionally cause detrimental cell mutagenesis and the emergence of treatment resistance in mycobacterial cells when they are unable to completely eliminate the cell (Palomino and Martin 2014; Zhang and Yew, 2015). Majority of TB infections arise from the bacterium being engulfed by dendritic cells or alveolar macrophages. Here, the bacilli escape the mechanism of death and continue to proliferate by avoiding phagosome-lysosome membrane fusion (Siroy *et al.,* 2008; Burian *et al.,* 2012).

Complementary macrophages and other immune cells are typically confined to granulomas, which are specialized locations. MTB bacilli are actively replicating in the granuloma, in addition to the non-replicating persistent/dormant form of MTB in that

particular place. This is due to the environmental conditions, which include nitric oxide production, nutritional restriction, anorexia/hypoxia, and other factors (Siroy *et al.,* 2008; Kashyap *et al.,* 2018).

### 2.12.2 Intrinsic resistant mechanisms in MTB

MTB resists both old and new medications as shown on Figure 2.6. MTB has intrinsic and acquired drug-resistant mechanisms that passively neutralize anti-TB drugs/regimens (Zheng *et al.,* 2013). Mtb relied on poor cell wall permeability to resist macrolides (MTB anti-drugs used in the management and treatment of Mtb infection). Mtb acquires macrolide resistance due to reduced cell wall permeability via *emr37* expression. *Emr37* is a genetic factor that methylates 23S rRNA-binding site (Cardoso *et al.,* 2007; Zhang and Yew, 2015). A Mtb cell permeability signaling barrier reduced clarithromycin's MIC (minimum inhibitory concentration) eight- to four-fold for natural resistance (Palmino *et al.,* 2014). MTB's intrinsic drug resistance has led to a novel cell wall structure that makes anti-TB chemotherapy ineffective (Viveirosa *et al.,* 2012). *MspA* genetic factors, a porin-associated component in the MTB cell wall that lowers drug permeability, have been linked to inherent resistance to hydrophilic antimycobacterial activity in MTB -*Rv1698* (Siroy *et al.,* 2008; Viveirosa *et al.,* 2012).

After interacting with *SigA*, the auto-regulator transcriptional activator *WhiB7* controls intrinsic drug resistance through the multi-drug transporter tap, which effluxes (over expression of efflux pumps) mycobacterial tetracycline, *TET, STR*, and *PAS* (Burian *et al.,* 2012; Kashyap *et al.,* 2018). *WhiB7*, a tiny protein of 122 amino acids and an iron-sulphur group, regulates antibiotic resistance genes-like, *eis*, *erm37,* and tap and oxireductive reagents like dithiothreitol and diamide (Burian *et al.,* 2012). The eukaryotic-like protein kinase G (*PknG*), a virulence factor, regulates the redox-homeostatic system to let MTB survive in host macrophages. *Lsr2*, a transcription factor associated with nucleoid, regulates oxygen levels for Mtb persistence in host cells by regulating iniBAC, a promoter involved in cell wall biosynthesis inhibition, and *EfpA*, a transport protein involved in efflux mechanisms (Viveirosa *et al.,* 2012). The MDR efflux pump is also regulated by the chromosomal protein Mar-regulon or MarA, reducing TET susceptibility. NADH and reflux pump-associated transcription factors affect antibiotic sensitivity and cellular redox state (Burian *et al.,* 2012; Viveirosa *et al.,* 2012; Bartek *et al.,* 2014; Kashyap *et al.,* 2018).

Thus, stress-responsive sigma factors (*SigA, SigF*), transcriptional proteins (*MarA, SoxR*, and *Rob*), and specific oxidative stress are related with anti-TB medication resistance (Dericck and Morris, 2007; Siroy *et al.,* 2008). Proteomic bioinformatic methods have linked MTB outer membrane proteins to inherent antibiotic resistance. In addition to MTB permeability barriers, host physiological modifications can manage antibiotic resistance (Siroy *et al.,* 2008; Nguyen *et al.,* 2008). Understanding intrinsic resistance is important in MTB drug development because few medicines are available to treat these horrific strains and targeting intrinsic resistance-associated genetic factors could control them (Lomovskaya and Bostian, 2006; Smith *et al.,* 2016).

### 2.12.3 Acquired drug resistance

Drug resistance in MTB typically develops as a result of chromosomal changes in genes that encode drug targets or drug-activating enzymes in response to antibiotic selection pressure (Culyba et al. 2021; Wintersdorff et al. 2016). Drug-resistant mutants develop because of prolonged drug exposure, nonadherence with medication, and long-term treatment regimens. thus, one of the main factors affecting mutations linked to resistance is medication concentration (Luthra *et al.,* 2018).

Horizontal transfer of plasmids and transposons (a section of a chromosome that is capable of transposition, particularly one that contains bacterial DNA and can move as a whole between phage, plasmid, and chromosomal DNA when the host DNA lacks a complementary sequence) promotes drug resistance develop. Drug resistance is acquired through chromosomal or gene mutation or extra-chromosomal or gene transfer (Smith *et al.,* 2016; Mitchison and Davies, 2009). No horizontal transfer of drug resistance genes has been documented in MTB, although most of them are due to selective pressure-induced chromosomal changes. Drug resistance against anti-TB regimens harms microorganisms physiologically. Thus, drug selection has a strong correlation to mutation rate, which is the basic rationale behind the blended anti-TB regimen in every dose. Growth and pathogenicity transmission between hosts determine microorganism fitness costs (Morris *et al.,* 2005; Lynch, 2013).

The fitness cost of certain chromosomal changes is directly proportional to antibiotic resistance; however, MTB drug resistance and fitness cost data are few. The best-known antibiotic resistance mechanism is chromosomal mutation (Morris *et al.,* 2005; Smith *et*

*al.,* 2016). Nearly 40%–94% of INH-resistant MTB clinical isolates had the most common mutation, *S315T* on the catalase-peroxidase enzyme (*katG*). Mutations can reduce *katG's* capacity to convert INH into iso-nicotinic acid, a precursor to the INH-NAD adduct in mycolic acid production (Lui *et al.,* 2012; Palomino and Martin, 2014).

## 2.13 TREATMENT AND DRUG RESISTANCE MECHANISM OF ACTION OF TB DRUGS

Table 2.2 Shows drugs used for the treatment of TB, MDR-TB and XDR-TB with regards to their targeted genes and mode of action (Da Silva, 2011; Hoagland *et al.,* 2016; Rendon *et al.,* 2016; WHO, 2016; Gygli et al. 2017; Nasiruddin et al. 2017; Tiber et. al. 2017; Hameed *et al.,* 2018).

**Table 2.2:** Shows drugs used for the treatment of TB, MDR-TB and XDR-TB with regards to their targeted genes and mode of action.

| TREATMENT | DRUG NAME | ANTI-*M.TB* ACTIVITY | GENES TARGETED | MODE OF ACTION |
|---|---|---|---|---|
| **FIRST-LINE DRUGS (ORALLY ADMINISTRATED)** | Rifampicin (RIF) | Kills or slows down the growth of *M.tb* (bactericidal) | *rpoB* - encodes β-subunit of RNA polymerase<br><br>*rpoA* - encodes α-subunit of RNA polymerase<br><br>*rpoC* - encodes β' subunit of RNA polymerase | Binds to *rpoB* gene and inhibits RNA synthesis, blocking protein synthesis<br>. |
| | Isoniazid (INH) | Kills or slows down the growth of *M.tb* (bactericidal) | *katG* - encodes catalase peroxidase)<br><br>inhA - encodes NADH-specific enoyl-acyl carrier protein (ACP) reductase<br><br>kasA - encodes b-ketoacyl ACP synthase<br><br>Ndh - encodes NADH dehydrogenase II<br><br>ahpC encodes Alkyl hydroperoxidase FabG - encodes 3-Oxoacyl (acyl carrier protein) | Mycolic acid production is inhibited by pro-drug activation by *katG*, followed by targeting of *inhA* and *kasA* by activated INH.<br><br>Mycolic acid production in *Mtb* requires binding of activated INH adduct to *InhA,* a NADH-dependent enoyl-ACP reductase.<br>It has been suggested that *ahpC* lessens the additional stress that organic peroxides place on *Mtb* strains. |

| | | | | |
|---|---|---|---|---|
| | Pyrazinamide (PZA) | Kills or slows down the growth of *M.tb* (bactericidal) | *pncA*- encodes pyrazinamidase<br>*rpsA*- encodes 30S ribosomal protein S1 *panD* encodes aspartate decarboxylase.<br>*clpC1* - encodes ATP-dependent *Clp* protease ATP-binding subunit.<br>*gps*\| - encodes bifunctional protein.<br>polyribonucleotide nucleotidy\|transferase (pnpase) | Pro-drug activation by *pncA* results in the formation of pyrazinoic acid, which lowers pH and inactivates essential fatty acid synthase.<br>The active form of PZA, pyrazinoic acid (POA), binds to *rpsA* to prevent trans-translation by interfering with the tRNA binding.<br>Through the suppression of panD activity, POA prevents the production of pantothenate or β-alanine (a precursor for coenzyme A).<br>Through the suppression of the crucial for all energy metabolisms coenzyme A (CoA) molecule, POA upsets the energy homeostasis. |
| | Ethambutol (EMB) | Prevents *M.tb* from multiplying without destroying it (bacteriostatic) | Three homologous, membrane-associated arabinosyltransferases are encoded by the genes *embC-embA-embB.*<br>*rmlD* - encodes dTDP-4-dehydrorhamnose reductase, while *embR* encodes a transcriptional regulatory protein. | interactions with three homologous, membrane-associated arabinosyltransferases (*embC, embA, and embB*) to alter the production of lipids and cell walls by targeting the synthesis and polymerization of the cell wall component arabinan.<br>Additionally controls the expression of proteins made by INH-inducible genes, including the *iniA* and ACP proteins. |
| **SECOND-LINE DRUGS (FLUOROQUINOLONE, ORALLY ADMINISTRATED)** | Levofloxacin (LFX) | Kills or slows down the growth of *M.tb* (bactericidal) | Topoisomerase II (DNA gyrase, in particular the DNA gyrase subunit A-encoding gene *gyrA*) | Targeting DNA replication by inhibiting DNA gyrase. |
| | Ofloxacin (OFX) / Ciprofloxacin (CIP) | Kills or slows down the growth of *M.tb* (bactericidal) | Topoisomerase II (DNA gyrase) | Targeting DNA replication by inhibiting DNA gyrase. |
| | Moxifloxacin (MFX) / Gatifloxacin (GFX) | Kills or slows down the growth of *M.tb* (bactericidal) | Topoisomerase II (DNA gyrase) | Inhibition of DNA gyrase, which prevents DNA replication.<br>Drug inhibits the dsDNA from being resealed. Breaks in the DNA cause complexes to be cleaved.<br>Complex cleaves prevent bacterial growth.<br>The complexes DNA breaks are released at higher drug concentrations, which is followed by chromosome fragmentation and cell death. |
| **SECOND-LINE INJECTABLE AGENTS (AMINOGLYCOSIDES)** | Kanamycin (KAN) / Amikacin (AMK) | Kills or slows down the growth of *M.tb* (bactericidal) | *rrs*- encodes 16S rRNA.<br>*whiB7* - encodes transcriptional regulatory protein. | Inhibits peptide chain synthesis, which prevents protein synthesis. |

| | | | | |
|---|---|---|---|---|
| | Capreomycin (CAP) | Kills or slows down the growth of *M.tb* (bactericidal) | 16S rRNA is encoded by *rrs*. 16S/23S rRNA (cytidine-2'-0): *tlyA*-methyltransferase aminoglycoside acetyltransferase for gene *eis*. Transcriptional regulating protein is encoded by *whiB7.* | Inhibition of protein synthesis |
| | Streptomycin (SM) | Kills or slows down the growth of *M.tb* (bactericidal) | Encoding ribosomal protein is *rpsL* gene. 16S rRNA is encoded by *rrs.* Gene *gidB*, encoding 16S rRNA methyltransferase. | 30S ribosomal subunit is the target. Binding to the ribosomal protein and 16S rRNA results in incorrect mRNA reading, which in turn causes defective protein synthesis or protein synthesis suppression. |
| **OTHER SECOND-LINE AGENTS** | Ethionamide (ETH) | Kills or slows down the growth of *M.tb* (bactericidal) | *ethA*- encodes flavin monooxygenase. *inhA* - encodes enoyl-acyl ACP reductase. *Ndh* - encodes NADH dehydrogenase II. *mshA* - encodes glycosyl transferase. *ethR* - encodes transcriptional repressor protein. *kasA* - encodes β-ketoacyl ACP synthase | Inhibition of mycolic acid synthesis |
| | Cycloserine (CS) | Keeps *M.tb* in the stationary phase of growth (bacteriostatic) / Kills or slows down the growth of *M.tb* (bactericidal), depending on the concentration on site of infection | *alr* - encoding for alanine racemase. *ddl* - encodes D-alanine-o-alanine ligase. *cycA* – encodes for D/L-serine. *ald* – encodes D-glycine or D-alanine or also D-cycloserine proton symporter (proteins that move two molecules through a membrane at once and in the same direction). | Inhibition of peptidoglycan synthesis. |
| | Terizidone (TRD) | Keeps *M.tb* in the stationary phase of growth (bacteriostatic) | *alr* - encoding for alanine racemase. *ddl* – encodes for D-alanine-D-alanine ligase | By inhibiting L-alanine racemase and D-alanine ligase and impairing the formation of peptidoglycan, it prevents the synthesis of cell walls. |
| | Linezolid (LZD) | Kills or slows down the growth of *M.tb* (bactericidal) | *rrl* – encoding for 23S rRNA. *rplC* – encodes for 50S ribosomal protein | Inhibition of protein synthesis. |
| | Pyrazinamide (PZA), Ethambutol | All 3 drugs kills or slows down the growth of | *rrl* – encoding for 23S rRNA. *rplC* – encodes for 50S ribosomal protein | Inhibition of protein synthesis. |

| | | | | |
|---|---|---|---|---|
| | (EMB) and a high dose of Isoniazid | *M.tb* (bactericidal) | | |
| | Bedaquiline (BDQ) | Kills or slows down the growth of *M.tb* (bactericidal) | *atpE*—encoding ATP synthase subunit C *Rv0678 (mmpR)* -encoding transcriptional repressor. of drug efflux pump Mmpl5 | Inhibition of ATP homeostasis |
| | Delamanid | Kills or slows down the growth of *M.tb* (bactericidal) | Five genes for coenzyme F420: *Rv0407* (fgd1). NADP encodingDependent *fbiA/B/C* glucose-6-phosphate dehydrogenase—encodes the F420 *Rv3547* (*Ddn*) two-electron transfer cofactor. — cofactor F420 deazaflavin encodesdependency-dependent nitroreductase | Inhibition of protein synthesis and inhibits mycolic acid. |
| | Amoxicillin β-lactam antibiotic with clavulanate β-lactamase inhibitor. | Kills or slows down the growth of *M.tb* (bactericidal) | *blaC* – encoding for β-lactamase. *ponA* – encodes for proteins that binds to penicillin. *Pbp* - encodes for proteins that binds to penicillin. | Inhibits cell wall synthesis thus peptidoglycan |
| | Thiacetazone (TAC) | Kills or slows down the growth of *M.tb* (bactericidal) | *hadAB* and *hadBC* – encodes for the FAS-II dehydratase complex consisting of *hadABC* genes | Prevents the cyclopropanation of mycolic acids in cell walls |

## 2.14 PREVENTATIVE MEASURES AND MONITORING STRATEGIES

Routine diagnosis, early detection of drugs resistance, and the start of an efficient treatment plan are all necessary for the effective management of TB (WHO, 2019). Combination therapy with antibiotics is one of the most successful methods for TB control. However, to date, the emergence and transmission of multidrug- and extensively drug-resistant tuberculosis (MDR/XDR-TB) strains of MTBC (Mycobacterium tuberculosis complex) on newly diagnosed and previously treated cases remains a problematic condition for the worldwide TB preventive and control programme and one of the main drivers driving the TB epidemic (WHO, 2016). To stop and manage the spread of TB, early case detection and treatment of MDR/XDR-TB cases are crucial (Zhang and Yew, 2015).

The most crucial step in preventing the spread of drug-resistant TB is to take all TB medications precisely as directed by your doctor. There should be no missed doses or early treatment termination. By ensuring rapid and accurate TB diagnosis, adequate infection control in TB treatment facilities, judicious and necessary drug use for treatment, patient adherence to drug regimen, and social awareness of TB control and drug regimen, it is possible to stop the spread of drug-resistant TB (Pinto and Menzies, 2011).

Avoid close contact with anybody who has contagious TB, particularly in places with inadequate ventilation. Outside in the open air, there is very little chance of contracting TB. Patients with TB should be urged to practice excellent cough hygiene, such as covering their mouths with a handkerchief or, in the beginning phases of therapy, using a surgical mask, particularly in enclosed spaces with limited ventilation (CDC, 2016).

Every year on March 24, World Tuberculosis Day is observed globally. This yearly event honours the discovery of *Mycobacterium tuberculosis* by Robert Koch in 1882. World TB Day raises awareness of the disease's impact on one's health as well as its social and economic ramifications. To restrict the spread of TB and reduce the disease's impact on global health, it is critical that everyone be aware of the signs of the disease and seek medical assistance if they suspect of having TB (CDC, 2023).

## 2.15 SUMMARY OF LITERATURE REVIEW

Before Koch discovered *Mycobacterium* in 1882, TB was considered a vampire disease. Although TB usually affects the lungs, it may affect other bodily parts. TB symptoms may be linked to organ damage. However, fever, chills, night sweats, loss of appetite, weight loss, and weariness are common symptoms of pulmonary and extra pulmonary TB. The airborne nature of TB makes it very contagious. It can be prevented, treated, and cured (Gehre *et al.,* 2016).

Tuberculosis affects all nations (Singh *et al.,* 2020). A third of the world's population, 2 billion, is infected with *Mycobacterium tuberculosis* (WHO, 2020). Active TB is treated with many medicines. medication resistance, which occurs when the bacterium survives medication therapy, thereby increasing the burden of active TB disease (Hameed *et al.,* 2018).

MDR, Pre-XDR, and XDR TB have emerged since 1950 due to resistance to the first-line TB treatment. Multidrug-resistant TB occurs when an individual does not respond to isoniazid and rifampicin, the two most potent anti-TB drugs. Pre-XDR TB is resistant to Rifampicin (RIF) and Isoniazid (INH) and either a fluoroquinolone (FQ) or one of the three second-line injectables (AMK, CAP, or KAN), but not both. MDR-TB with resistance to fluoroquinolone (FQs) and at least one of the three injectable aminoglycosides (CAP, AMK, KAN) (Lienhardt *et al.,* 2012) OR mycobacterium resistant to INH, RIF, a FQ, Bedaquiline, or Linezolid (CDC, 2022).

HIV is the most prevalent risk factor for active tuberculosis (20–30 times higher in HIV-positive people) (Habib *et al.,* 2018). TB primarily affects the homeless, old, malnourished, inebriated men (alcoholics), minorities, immigrants, prisoners, and congested dwellers. It could be nosocomial. One-fourth to one-third of current TB infections may indicate recent transmission. Thus, most current instances result from latent infections reactivating (Dookie *et al.,* 2018).

There are other immunocompromised conditions, such as long-term corticosteroids and anti-TNF medications. Chronic pulmonary diseases, smoking. Malnutrition increases the risk of TB becoming active, one of the primary diseases of poverty. Diabetes increases the risk of active TB and poor treatment outcomes. Long-term lung illness is caused by excessive alcohol consumption (over 40 grams per day), intravenous drug abuse, indoor contaminants, and crystalline silica dust inhalation. Chronic kidney disease, gastrectomy, intestinal bypass, severe malnutrition, and malabsorption (Falzon *et al.,* 2017).

Numerous global research on TB knowledge, attitude, and stigma have found that the public has many misconceptions regarding the disease. TB attitudes and understanding vary by country, from stigmatized to supportive of the disease and its patients. Understanding and optimism about TB are needed to improve public health-seeking (Chinenye, 2015).

A major global concern is the prevalence rate of MTB and resistance mechanisms against continuous therapy. The main causes of the MDR, pre-XDR and XDR MTB strains' fast growth include the lack of viable treatment candidates, patient awareness, and unsanitary behaviours. The main elements linked to drug resistance include an

understanding and analysis of compensatory evolution, clonal interference, decreased cell wall permeability, overexpression of efflux pumps, target modification, and target mimicry modulation. In order to create new anti-TB regimens, it is imperative to counterattack the potent molecular mechanism and intricate pathway that informs drug resistance in MTB. Ideally, the integration of novel genetic insights into drug-resistant pathways in tuberculosis (TB) would provide a fresh approach to combination drug discovery, bolstering the development of potent anti-TB medications (Swain *et al.,* 2020).

# CHAPTER 3
# MATERIALS AND METHODS

## 3.1 ETHICAL CONSIDERATION

This research project was approved by the Research Ethics committee of the University of Venda (SMNS/20/MBY/13). Approval to access the health care facilities was obtained from the Limpopo provincial Department of Health (LP_ 2021-11-001). Permission to gain access to the specific hospitals and clinics was obtained from the respective health care managers. A written informed consent was obtained from all the participants in the study. This study is part of the South African Medical Research Council (SAMRC) project. Before enrolling into the study, all participants were given a consent form to sign. Participants' rights were considered, and they had a right to withdraw at any moment without consequences.

## 3.2 STUDY SITE

The study was carried out in Vhembe district, Limpopo province, South Africa (Figure 3.1). There are about 1.4 million people living in the Vhembe district of Limpopo province, with 121 clinics and 8 hospitals in the area. Selected Clinics (Magwedzha, Phipidi, Lwamondo, Malamulele, William Eadie, Mphambo, Xigalo, & Mavambe) and hospitals (Tshilidzini, and Donald Fraizer) were visited, and the project presented to staff and managers, prior arrangements to meet with the TB patients.

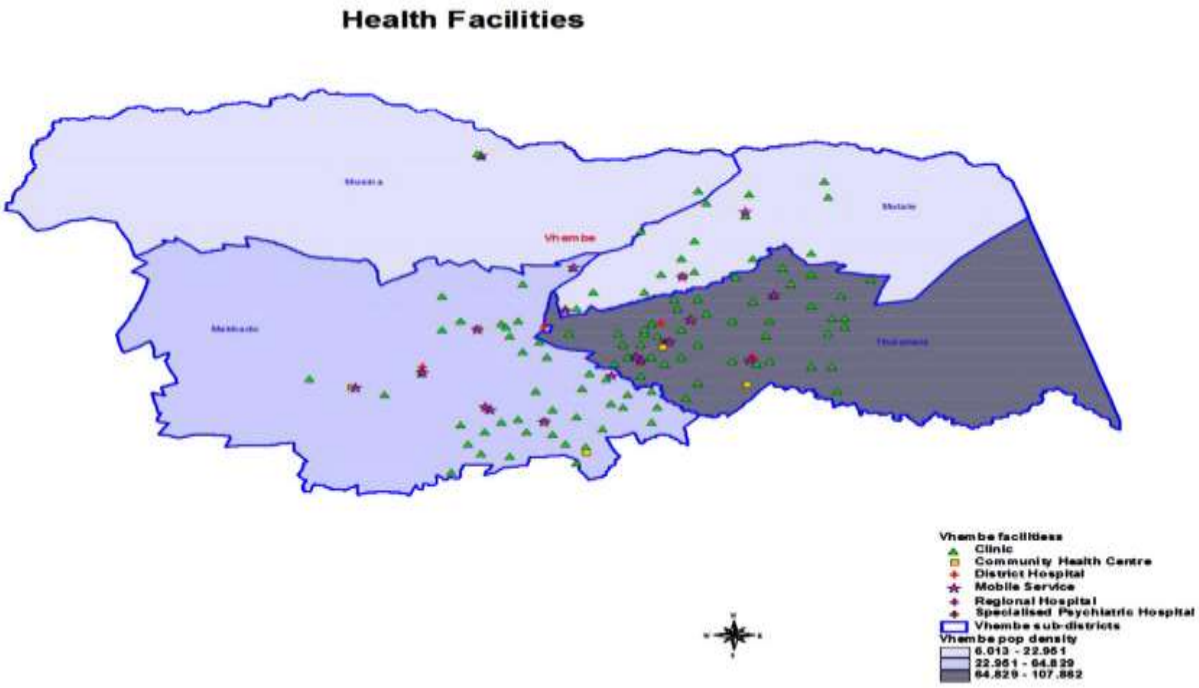



**Figure 3.1:** A map showing the study site where the study was conducted in Vhembe region, Limpopo province, South Africa (Vhuromu *et al.,* 2018).

### 3.3 SAMPLE COLLECTION

A total of 50 sputum samples were collected from TB positive patients that were aged 18 years and above. When collecting sputum specimens, patients were provided clear instructions. Patients took samples outside in a private spot away from other people. Patients were not allowed to collect samples in confined spaces such as toilets. They were asked to collect samples as follow: to rinse their mouths with water before collection of sputum, without brushing their teeth. Patients had to cough hard to expectorate sputum into the container. A sputum sample of 3-5 mL was collected from each patient into A 100 mL collection bottle containing 5 mL of NaOH (Sodium hydroxide, Sigma, St. Louis, MI, USA) for sample decontamination. Collected samples were kept in a cooler box with ice pack (2-8 °C) and transported to the Microbiology Laboratory at the University of Venda for further analysis.

### 3.4 PRE-TREATMENT OF SPUTUM SAMPLES

Samples were incubated for 15 minutes at room temperature before pre-treatment was done. Afterwards, 1.5 mL of the sample was transferred to a new sterile tube and

centrifuged (Thermo Scientific Heraeus Pico 17 Benchtop, Waltham, MA, USA) at 15,000 x g (13,000 rpm) for 5 minutes. The supernatant was discarded, and 1 mL of 1X PBS (Sigma) solution was added and mixed well. Centrifugation at 15,000 x g (13,000 rpm) for 5 minutes took place and the supernatant was discarded with a pipette. 1 mL of 1X PBS solution was added and mixed well. Followed by centrifugation at 15,000 x g (13,000 rpm) for 5 minutes and the supernatant was discarded with a pipette. Thereafter the resulting pellet was preserved at -20°C for further analysis.

### 3.5 NUCLEIC ACID EXTRACTION FROM SPUTUM SAMPLES

The Allplex™ MTB/XDRe (Allplex™, Seegene, Seoul, South Korea) kit was used for the DNA extraction and the manufacturer's instructions were followed. Before DNA extraction, the prepared sediments were removed from -20°C and allowed to thaw. The sediments were then mixed with 1 mL of sterile water, centrifuged for 5 minutes at 15,000 x g (13,000 rpm), and the supernatant was discarded using a pipette. Before adding 100 mL of DNA Extraction Solution (included in the kit), 10 μL of MTB/DRe internal control (IC, included in the kit, allows one to confirm DNA extraction procedure and also identify PCR inhibition) was added and vortexed for 30 seconds. The tubes were then locked using a cap-lock and boiled for 20 minutes on a heat block. Afterwards, centrifuged at 15,000 x g (13,000 rpm) for 5 minutes. Lastly, 5 μL of the supernatant was used as PCR (polymerase chain reaction) template (Allplex™, Seegene, Seoul, South Korea).

Target sequences of *Mycobacterium tuberculosis* included: 7 fluoroquinolone (FQ) resistance- causing mutations [gyrA A90V(GTG), S91P(CCG), D94A(GCC), D94G(GGC), D94H(CAC), D94N(AAC), D94Y(TAC)], 6 injectable drug resistance-causing mutations [rrs 1401(G), 1402(T), 1484(T), eis promoter -37(T), -14(T), -10(A)], (Allplex™, Seegene, Seoul, South Korea).

### 3.6 SEQUENCING OF DRUG RESISTANCE TB USING MASSARRAY ASSAY TECHNIQUE (XDR-TB)

The protocol by Yang et al. (2023) was followed. Briefly, MTB and the locations of MTB resistance gene mutations were identified using MassArray. Table 3.1 displays the anti-tuberculosis medications together with the gene resistance loci that correspond to them.

There were two PCR reaction systems running at once. HPLC H2O (0.8 µL), 10X PCR buffer (20 mM MgCl2 0.5 µL, 25 mM MgCl2 0.4 µL), 25 mM dNTP Mix (0.1 µL), 0.5 µM Primer Mix (1 µL), PCR Enzyme (0.2 µL), and 2 µL template DNA were all included in the reaction system, which had a final volume of 5 µ. The reaction programme was 72°C for 5 minutes, hold at 4°C; 95°C for 30 seconds; 60°C for 30 seconds; 72°C for 60 seconds; and so on. Put two microliters of shrimp alkaline phosphatase (SAP) in each well, and then let it sit at 37°C for forty minutes, 85°C for five minutes, and 4°C for holding. Fill each well with 2 µL of the iPLEX extension mix (Agena bioscience, San Diego, CA, USA; nanopure water 0.62 µL, iPLEX buffer 0.2 µL, iPLEX termination mix 0.2 µL, extend primer mix 0.94 µL, and iPLEX enzyme 0.04 µL). The reaction programme was as follows: 72°C for three minutes, hold at 4°C; 95°C for five seconds; 52°C for five seconds, 80°C for five cycles); and so on for forty cycles.

Subsequently, the production was executed at Inqaba Biotechnical Industries (Pty) Ltd utilising a high-throughput MassARRAY technology. Typer 4.0 and plate manager 1.0 software were utilised for data analysis. The test results were categorized as having no alleles, low probability, aggressive, moderate, and conservative quality.

**Table 3.1:** Primers used for sequencing (Evans and Segal, 2010)

| Drugs | Genes | Primers | Annealing temperature (°C) | Size (bp) |
|---|---|---|---|---|
| **Fluoroquinolones (FQs)** | ***gyrA* and *gyrB*** | **F:CCGGATCGAACCGGTTGAC R:GTTAGGGATGAAATCGACTG** | **57.2** | **427** |
| **Ethionimide** | ***ethA*** | **F:GTGAGATGTTGGGTTAAGTCC R: TGGTGCTCCTTAGAAAGGAG** | **55.3** | **481** |
| **Bedaquiline** | ***rv0678*** | **F:ACGTTGGATGTATCGATTCCAGTGGA CGG R: TTCAACCAGTCGATCCCAGTGGAACG G** | **46.9** | **431** |
| **Amikacin** | ***Rrs*** | **F:ACGTTGGATGTATCGATTCCAGTGGA CGG R: TTCAACCAGTCGATCCCAGTGGAACG G** | **50.2** | **456** |
| **Capreomycin** | ***rrs* and *tlyA*** | **F: ACGTTGGATGTGTTCGTCCATACGAC CTC R:ACGTTGGATGCGTTGACAGATACGTC AGG** | **60.3** | **463** |

| | | | | |
|---|---|---|---|---|
| **Kanamycin** | ***rrs* and *eis*** | **F:ACGTTGGATGAATAGTTGGACATGTAGCCG<br>R: TCGGCGACTCGGGCCA** | **42.6** | **501** |
| **Ethambultol** | ***emb*** | **F:CCGGATCGAACCGGTTGAC<br>R: TGGTGCTCCTTAGAAAGGAG** | **50.1** | **521** |

## 3.7 DATA ANALYSIS

Excel spreadsheet was used to record the acquired data. The group of TB patients was described using descriptive statistical techniques. R-studio was used, a statistical analysis software package, as defined by (Kronthaler and Zöllner, 2021), to investigate the impact of risk factors linked to tuberculosis on treatment outcomes among patients. Using the chi-squared method, association between TB recruited patients with regards to educational level, employment status, religion, dusty environment, wearing protective mask, mode of transport, family members with TB diagnoses, sharing a room, type of infection [HIV, Hypertension, Diabetes, Ulcer, Asthma, *Nontuberculous mycobacteria* (NTM), *Mycobacterium tuberculosis* (MTB), NTM and MTB co-infection and drug resistance] were compared.

# CHAPTER 4
# RESULTS & DISCUSSION

This section highlights the findings of the objectives set to determine drug resistant tuberculosis amongst TB patients in the Vhembe district, Limpopo, South Africa.

## 4.1. RESULTS OF THE STUDY

### 4.1.1 Demographic characteristics of study participants

A total of 50 patients enrolled in the study (March-August 2023), all sputum samples were collected from TB positive patients that were aged 18 years and above, from healthcare facilities in the Vhembe region. Patients were surveyed using a detailed questionnaire, in order to find out risk factors that are correlated with XDR-TB.

As shown in Table 4.1 majority were males (60% [n=30]) of the participants enrolled in the study, while 40% of the enrolled were females (40% [n=20]). Statistical analysis yielded significant associations for the enrolled TB candidates, i.e., those of ages between 30-40 ($p$ = 0.0002) and 40-50 ($p$ = 0.20) years. From the enrolled patients, level of education was significantly associated with TB risk infection, as most of the participant had reached secondary level education ($p$ = 0.009) and individuals that did not go to school, no education ($p$ = 0.02). This implies that participants might have not acquired sufficient knowledge about TB. Unemployment amongst study population was 66% this was highly significant ($p$ = 0.001). The main religion was Christianity 76% and was highly significant ($p$ = 0.0003) to TB enrolled patients.

**Table 4.1:** Socio demographic data of recruited TB patients

| VARIABLES | N=50 (100%) | P-VALUE |
|---|---|---|
| **GENDER** | | |
| **MALE** | 30 (60%) | - |
| **FEMALE** | 20 (40%) | - |
| **AGE GROUP (YEARS)** | | |
| **<20** | 1 (2%) | 1.0 |
| **20-30** | 3 (6%) | 0.12 |
| **30-40** | 11 (22%) | 0.0002 |
| **40-50** | 15 (30%) | 0.20 |
| **50-60** | 8 (16%) | 0.92 |
| **≥60** | 12 (24%) | 0.40 |
| **EDUCATIONAL LEVEL** | | |
| **NO EDUCATION** | 5 (10%) | 0.02 |
| **PRIMARY LEVEL** | 10 (20%) | 0.05 |
| **SECONDARY LEVEL** | 27 (54%) | 0.009 |
| **TERTIARY LEVEL** | 8 (16%) | 0.92 |
| **EMPLOYMENT STATUS** | | |
| **SELF EMPLOYED** | 3 (6%) | 1.0 |
| **EMPLOYED** | 12 (24%) | 0.40 |
| **UNEMPLOYED** | 33 (66%) | 0.001 |
| **STUDENTS** | 2 (4%) | 0.03 |
| **RELIGION** | | |
| **AFRICAN RELIGION** | 6 (12%) | 1.0 |
| **CHRISTIANITY** | 38 (76%) | 0.0003 |
| **ATHEIST** | 2 (4%) | 1.0 |
| **OTHER** | 4 (8%) | 1.0 |

Below, Figure 4.1 shows the age group (years) of recruited TB patients based on their gender, wherein majority of males were within age groups of [30-40] (16%) (young adults) and [50-60] (16%) (middle-aged adults) years. Among the patients, elderly males were 14%, that were of 60 years and above. Among the patients, twelve percent (12%) males were senior adults [40-50] and minority 2% of males were below the age of 20 years (young teen adult). Majority 14% of females were senior adults under the age category of [40-50]. Young adult females were 12% between the age [30-40] years. Among the patients, elderly females were 8%, that is of 60 years and above. Six percent (6%) females were young adults aged between [20-30].

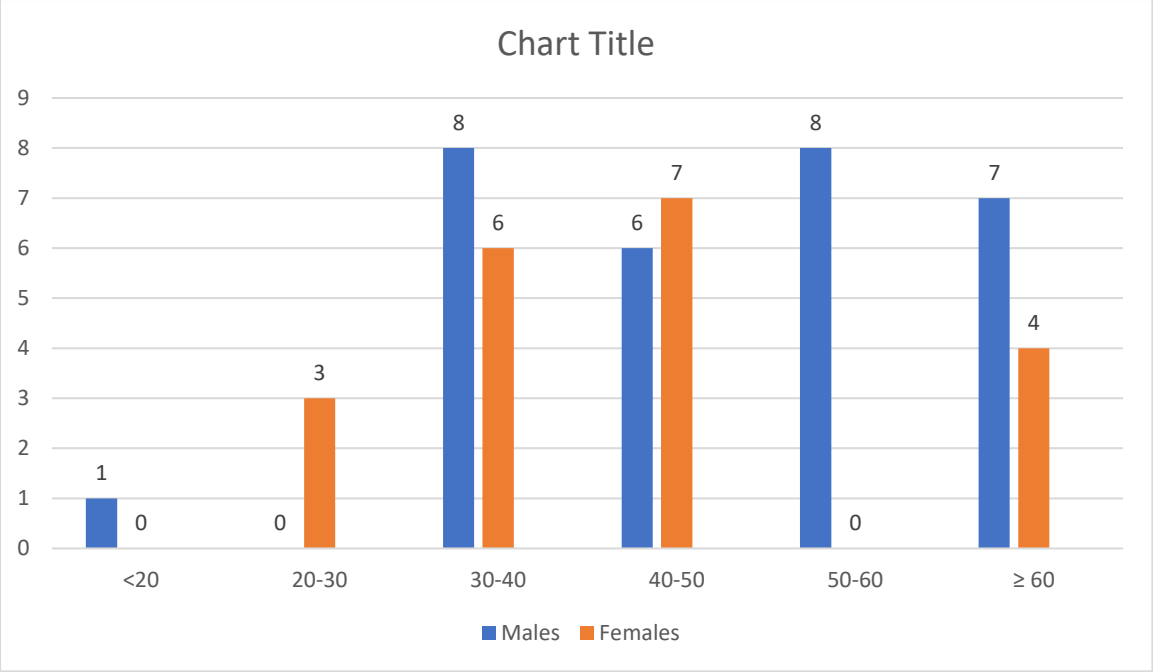



**Figure 4.1:** Age group of TB recruited patients based on gender.

Figure 4.2 presents educational level of participating patients, 10% never went to school, 20% dropped out at primary level (went to school from Grade 1-7), 54% dropped out at secondary level (went to school from Grade 8-12) and 16% pursue their education to tertiary level.

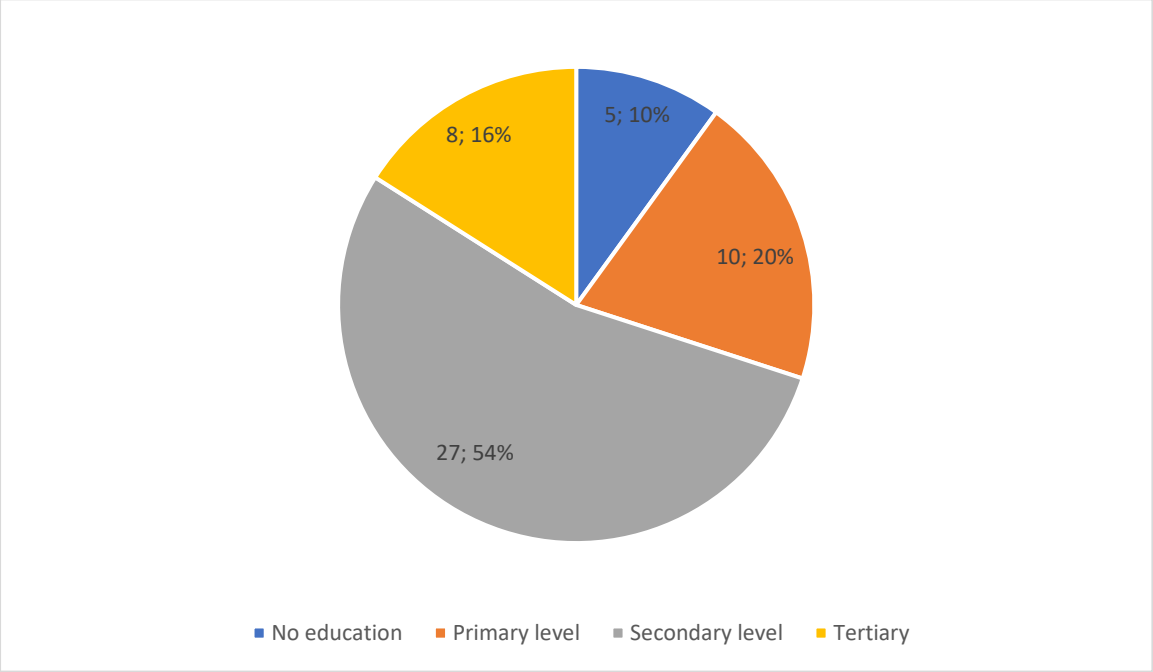



**Figure 4.2:** Educational level of TB recruited patients.

Figure 4.3 presents a summary of socio-economic status of participating patients, wherein majority (66%) were unemployed, 24% were employed, 6% were self-employed and 4% were students as per the collected data.

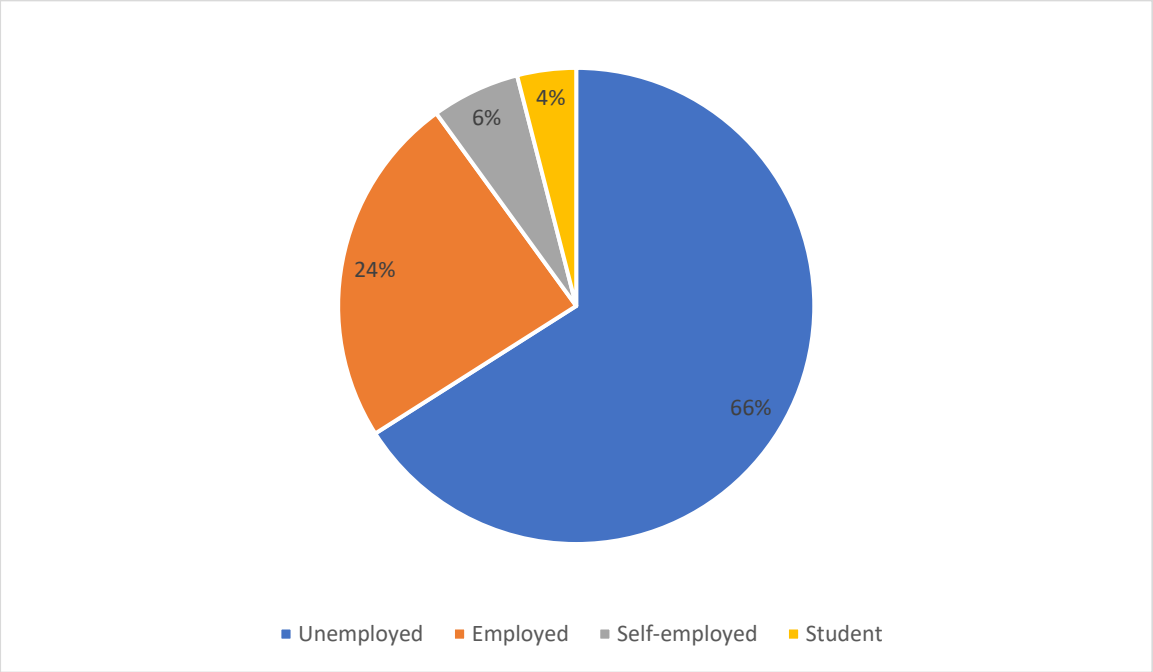



**Figure 4.3:** Employment status of participating patients

Figure 4.4 presents religious believe with regards to participating patients wherein majority believed in Christianity 76%, 12% were believing in African religion, 4% were Atheist and 8% other.

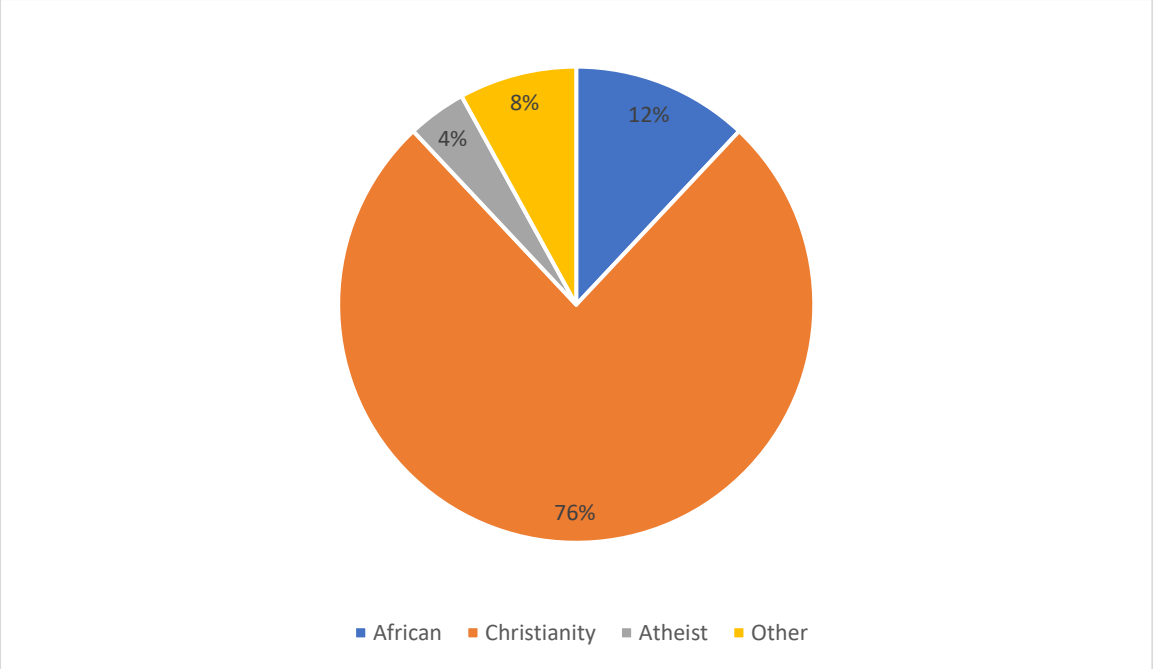



**Figure 4.4:** Religious believe based on participating patients.

Table 4.2 indicates that majority of participants were South African (92%) and minority were migrants (4%). Statistical analysis yielded significant associations. Amongst recruited TB patients most (56%) revealed that they worked in a dusty environment ($p$ = 0.006) and was the most significant with regards to TB recruited patients. Only 28% accounted for wearing a face mask consistently and 54% wore sometimes thus still have potential to transmit TB. Wearing a safety mask sometimes was significantly associated with TB ($p$ = 0.031), these individuals wearing face mask sometimes find the mask uncomfortable, resulting to non-compliance and transmission of disease. Mode of transport was significantly associated with TB, most (66%) participants travelled using public transport ($p$ = 0.001) public transports are generally known to be crowded, poorly ventilated thus more people might be prone to contracting the airborne pathogen and thus contracting TB. About 54% lived with 5-9 family members and 46% lived with 0-4 members. 46% shared a room and sharing of rooms was significantly associated with TB ($p$ = 0.02), consequently household members are more at risk for latent tuberculosis infection due to prolonged airborne contact with active TB patients. Approximately 22% of family members had a history of diagnosed TB.

**Table 4.2:** Environmental factors of participating patients

| VARIABLES | n=50 YES (%) | P-VALUE |
|---|---|---|
| **PLACE OF RESIDENCE** | | |
| **NATIVE** | 46 (92%) | - |
| **MIGRANTS** | 4 (8%) | - |
| **IS IT DUSTY?** | | |
| **YES** | 28 (56%) | 0.006 |
| **SOMETIMES** | 7 (14%) | |
| **DO YOU WEAR PROTECTIVE MASK?** | | |
| **YES** | 14 (28%) | 1.0 |
| **SOMETIMES** | 27 (54%) | 0.031 |
| **DO YOU WORK IN AN OPEN SPACE?** | | |
| **YES** | 43 (86%) | 6.05 |
| **MODE OF TRANSPORT** | | |
| **PUBLIC TRANSPORT** | 33 (66%) | 0.001 |
| **WALKING** | 13 (26%) | 0.32 |
| **OWN CAR** | 4 (8%) | 1.0 |
| **HOW MANY FAMILY MEMBERS DO YOU LIVE WITH?** | | |
| **[0-4]** | 23 (46%) | 0.027 |
| **[5-9]** | 27 (54%) | 0.009 |
| **ARE YOU SHARING A ROOM?** | | |
| **YES** | 23 (46%) | 0.02 |
| **SOMETIMES** | 4 (8%) | |
| **IS THERE A FAMILY MEMBER WHO HAS BEEN DIAGNOSED WITH TB?** | | |
| **YES** | 11 (22%) | - |
| **WHO IN THE FAMILY HAD BEEN DIAGNOSED WITH TB?** | | |
| **N/A** | 37 (74%) | - |
| **NO** | 3 (6%) | - |
| **NOT SURE** | 1 (2%) | - |
| **FATHER** | 2 (4%) | - |
| **MOTHER** | 3 (6%) | - |
| **MOTHER AND BROTHER** | 1 (2%) | - |
| **WIFE AND CHILD** | 1 (2%) | - |
| **SISTER** | 1 (2%) | - |
| **SIBLING** | 1 (2%) | - |

Symbol (-) means p value could not be determined.

### 4.1.2. Cultural factors, social behaviour and attitudes (Stigma among TB patients)

Table 4.3 shows healthcare-seeking behaviour and responses among individuals diagnosed with tuberculosis. The study reveals that a minority of participants (4%) sought consultation from traditional healers during their illness, with about 1% relying on

traditional healers for medication. A majority of patients (74%) sought medical care at healthcare centres within less than 3 weeks of initial stages of illness, while 26% did so after 3 weeks of disease onwards. Tuberculosis vaccination coverage was observed in 76% of the participants. Minority (8%) had a positive reaction to TB skin test. Upon receiving a positive tuberculosis diagnosis, majority (92%) of participants disclosed their condition within a week to their families, with 94% of the families providing support. Interestingly, 44% of the respondents disclosed their tuberculosis status to colleagues and community members, but only 38% of them received support from the community. Only 26% were previously treated for active/latent TB.

**Table 4.3:** Understanding Knowledge, Social Behaviour, Cultural factors and Stigma among TB patients

| VARIABLES | n=50<br>YES (%) |
|---|---|
| HAVE YOU EVER CONSULTED A TRADITIONAL HEALER? | Yes 2 (4%) |
| DID THE TRADITIONAL HEALER GIVE YOU A TB REMEDY? | Yes 1 (2%) |
| AFTER HOW LONG DID IT TAKE YOU GO TO GO TO THE HEALTH-CARE CENTRE FOR CONSULTATION? | |
| ≤ 3 WEEKS | 37 (74%) |
| ≥ 3 WEEKS | 13 (26%) |
| VACCINE TO PREVENT TB? | 38 (76%) |
| POSITIVE OR REACTIVE TO TB SKIN TEST? | Yes 4 (8%) |
| SHARING TB STATUS WITH FAMILY? | Yes 46 (92%) |
| TIME TAKEN TO SHARE STATUS WITH FAMILY? | |
| 1-7 DAYS | 42 (84%) |
| 2 WEEKS | 1 (2%) |
| ≥ 2 WEEKS | 1 (2%) |
| SUPPORT FROM FAMILY? | Yes 47 (94%) |
| SHARING TB STATUS WITH COLLEAGUES, COMMUNITY MEMBERS OR FRIENDS? | Yes 22 (44%) |
| SUPPORT TO CONSULT HEALTH-CARE CENTRES? | Yes 19 (38%) |
| HAVE YOU EVER BEEN TREATED FOR EITHER LATENT OR ACTIVE TB TREATMENT? | Yes 13 (26%) |

Host-related risk factors and co-morbidities were evaluated amongst the TB patients that were aged 20 to ≥60, a significant co-occurrence of HIV infection (66%) was observed, along with lower prevalence rates of hypertension (6%), asthma (4%), diabetes (4%) and ulcer (2%) as shown in Table 4.4. The study evaluated the association between HIV and TB ($p$ = 0.130), association between Hypertension and TB ($p$ = 7.581), Asthma and TB ($p$ = 2.747), Diabetes and TB ($p$ = 2.747) and Ulcer and TB ($p$ = 1.612). Statistical analysis

had no significance on these associations, although emphasis on the importance of these factors should be considered while managing tuberculosis in patients.

**Table 4.4:** TB specific factors and co-morbidities among TB patients

| CO-MORBIDITY (CHRONIC ILLNESSES) | n=50 | P-VALUE |
|---|---|---|
| HIV | Yes 33 (66%) | 0.130 |
| HYPERTENSION | Yes 3 (6%) | 7.581 |
| ASTHMA | Yes 2 (4%) | 2.747 |
| DIABETES | Yes 2 (4%) | 2.747 |
| ULCER | Yes 1 (2%) | 1.612 |

### 4.1.3. Prevalence of drug resistant tuberculosis among patients

In total 50 patients enrolled in the study, prevalence of *Mycobacterium tuberculosis* (MTB), Multi-drug resistance tuberculosis (MDR-TB) and Extensively drug resistance (XDR-TB) were assessed from early morning sputum samples, that were subjected to Allplex™ [multiplex real-time PCR (polymerase chain reaction)].

Table 4.5 below shows infection status of recruited TB patients whereby a significant occurrence of 58% NTM (Non-tuberculosis mycobacteria) was recorded, along with lower prevalence rates of MTB 4% (*Mycobacterium tuberculosis,* susceptible TB) and 28% of MTB+NTM (co-infection) was recorded. Statistical analysis yielded a significant association, whereby majority were infected with NTM ($p$ = 0.005) and was of most significant amongst TB recruited patients. There was no significant association between MTB+NTM (co-infection) and TB ($p$ = 0.257) and MTB and TB ($p$ = 1.0).

**Table 4.5:** Infection status of recruited TB patients

| **VARIABLES** | **N=50** | **P-VALUE** |
|---|---|---|
| **TYPE OF INFECTION** | | |
| **NTM** | 29 (58%) | 0.005 |
| **MTB AND NTM** | 14 (28%) | 0.257 |
| **MTB** | 2 (4%) | 1.0 |

Furthermore, due to simultaneous amplification and detection of target sequences of *Mycobacterium tuberculosis* with Allplex™ assay, from MTB (susceptible TB) (4% [n=2]) and of MTB and NTM (co-infection) (28% [n=14]), Allplex™ assay yielded mutations associated with drug resistance, that is (4% [n=2]) of RIF-R+INH-R+FQ-R (Rifampicin resistance + Isoniazid resistance+ Fluoroquinolone resistance =Pre-XDR). Statistical analysis yielded no significant association between drug resistance TB and TB recruited patients. Overall, 4% (2 cases) of drug resistance (pre-extensively drug resistance) was noted, as shown in Table 4.6.

**Table 4.6:** Mutations detected in TB recruited patients.

| VARIABLES | N=50 | P-VALUE |
|---|---|---|
| **TYPE OF INFECTION** | | |
| **MTB AND NTM** | 14 (28%) | 0.257 |
| **MTB** | 2 (4%) | 1.0 |
| **RESISTANCE** | | |
| **FQ-R** | 1 (2%) | 1.0 |
| **RIF-R+INH-R+FQ-R** | 1(2%) | 1.0 |

Mutations were prevalent in male participants and not females as shown in Table 4.7 below. In the current study, drug resistance was prevalent in two male patients. Wherein demographic characteristics possibly be risk factors associated with TB, MDR, pre-XDR TB and consequently XDR-TB.

**Table 4.7:**Demographic characteristics associated with Pre-XDR TB patients

| RISK FACTORS | | |
|---|---|---|
| **GENDER** | Patient 1 (FQ-R) | Patient 2 (RIF-R+INH-R+FQ-R) |
| **AGE** | 43 | 49 |
| **EDUCATIONAL LEVEL** | Secondary level | Primary level |
| **OCCUPATION** | Unemployed | Unemployed |
| **RELIGION** | Christianity | Other |
| **PLACE OF RESIDENCE** | Native | Native |
| **IS IT DUSTY?** | Yes | Yes |
| **DO YOU WEAR PROTECTIVE FACE MASK?** | Sometimes | No |
| **DO YOU WORK IN AN OPEN SPACE?** | No | No |
| **MODE OF TRANSPORT?** | Walking | Public transport |
| **HOW MANY FAMILY MEMBERS DO YOU LIVE WITH?** | 5-9 | 0-4 |
| **ARE YOU SHARING A ROOM?** | No | No |
| **FAMILY MEMBER PREVIOUSLY DIAGNOSED WITH TB?** | No | Yes |
| **WHO IN THE FAMILY WAS PREVIOUSLY DIAGNOSED WITH TB?** | N/A | Sibling |
| **CONSULTED A TRADITIONAL HEALER?** | No | No |
| **TIME TAKEN TO CONSULT HEALTH-CARE FACILITIES?** | ≥ 3 weeks | 1-3 weeks |
| **VACCINE TO PREVENT TB?** | No | Yes |
| **TUBERCULIN SKIN TEST?** | No | No |
| **SHARING TB STATUS WITH FAMILY?** | Yes | Yes |
| **TIME TAKEN TO SHARE TB STATUS WITH FAMILY?** | 1-7 days | 1-7 days |
| **SUPPORT FROM FAMILY?** | Yes | Yes |
| **SHARING TB STATUS WITH COLLEAGUES, COMMUNITY MEMBERS OR FRIENDS?** | No | N/A |
| **SUPPORT TO CONSULT HEALTH CARE CENTERS?** | N/A | N/A |
| **PREVIOUSLY TREATED FOR ACTIVE OR LATENT TB?** | No | No |
| **CO-MORBIDITIES?** | None | HIV |

Figure 4.6 shows a summary highlighting the infection status of males and females TB recruited patients, wherein HIV was predominately detected in females (34%). However, most detection of NTM, a coinfection of NTM and Mtb was seen on males than on female participants. MTB as a single pathogen was detected on one individual per gender. Of note, resistance was reported only on male participant.

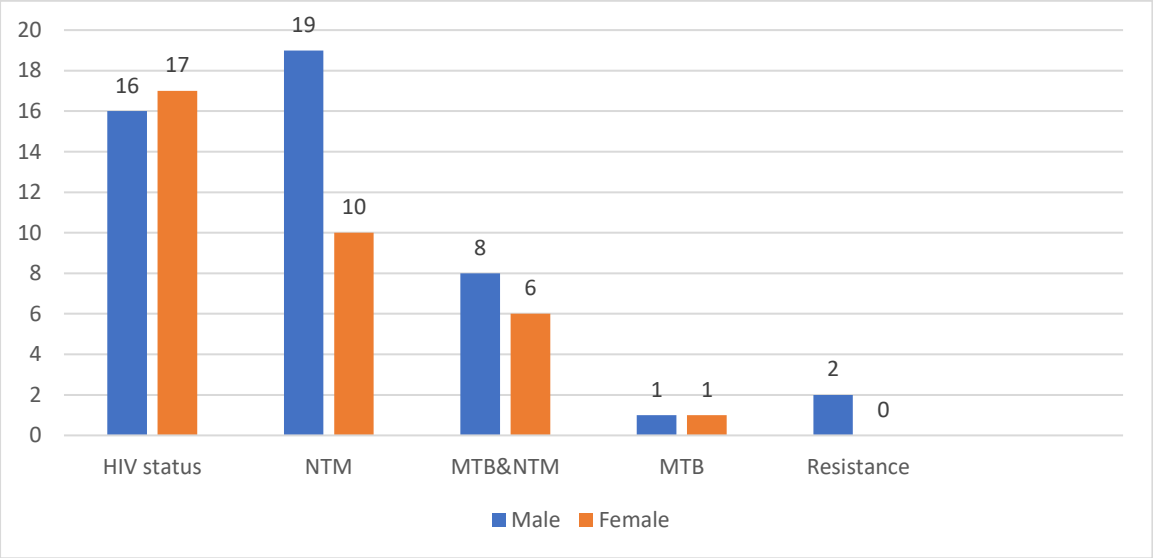



**Figure 4.5:** Infection status on the enrolled participants

### 4.1.4 Prevalence of genetic mutations [single-nucleotide polymorphisms (SNPs)]

Of the further analysed samples, 14 (28%) of MTB and NTM and 2 (4%) of MTB isolates were subjected to MassArray (Agena). Drug-resistance strains were observed in 2 males (4%) pre-XDR (FQ-R and RIF-R+INH-R+FQ-R). Common mutations were detected in both patients, wherein patient 1 had single mutation [Adenine (A)], while patient 2 had 7 mutations [that is, 2x Adenine (A), 4x Thymine (T), 1x Guanine (G)].

**Table 4.8:** Genotypic mutations in both patients (SNPs).

| SNPS ANALYSED | PATIENT 1 (FQ-R) | PATIENT 2 (RIF-R+INH-R+FQ-R) |
|---|---|---|
| ***EMB_4247429*** | - | - |
| ***INHA_1674481*** | - | A |
| ***PNCA_2288964*** | - | T |
| ***RPO_B_761110*** | - | T |
| ***RPOB_761155*** | - | G |
| ***RPSL_781687*** | - | T |
| ***RRS_1472359*** | - | T |
| ***RRS_1473167*** | A | A |

## 4.2 DISCUSSION

Several risk factors play a crucial role in one contracting TB (Omotowo *et al.,* 2012). To effectively combat TB and prevent transmission it is essential to understand the underlining risk factors that are associated with TB (Workneh *et al.,* 2017; Narasimhan *et al.,* 2013). Similarly, the current study also investigated numerous risk factors in order to determine the prevalence of TB in the Vhembe region Limpopo, South Africa.

All age groups and both sexes are affected by TB, however adult males bear the greatest burden as they account for 56% of all cases in 2019 while adult women account for 32% of cases. When it comes to the age distribution of TB cases, those between the ages of 25 and 54 make up the group with the highest percentage of TB cases (WHO, 2019). Similarly, majority of male participants in this study were within age groups of 20 to 60 and above (young adults and elderly) (Table 4.1/ Figure 4.2), this is in line with many studies carried out in Ethiopia, where majority of cases were discovered in this age group (Weyer *et al.,* 2007; Sharma *et al.,* 2011). It was reported by (Stead, 1998 and Orme, 1998) that, lesions that have remained latent for several decades are responsible for most tuberculosis cases in the elderly. The emergence of these lesions can be attributed to senescence-related immune system modifications, specifically the reduction in the capacity to reactivate previously acquired immunity, as well as other variables.

In the current study most, participants were males (Table 4.1/ Figure 4.1) but participants' gender had no association to the development of tuberculosis. Nonetheless, numerous studies found that there was a higher risk of tuberculosis development in males (Hirpa *et al.,* 2013; Law *et al.,* 2008). In a study conducted by (Long *et al.,* 1999) men were considered to be more susceptible than women to contract tuberculosis (TB) because, men were exposed to greater risk factors both at work and during their free time. Consequently, women did not seek out TB services even when they had symptoms of the disease. This could imply that a significant number of females are likely to have limited access to sources of information about tuberculosis because of their extensive involvement in farming activities and everyday chores at home.

Educational level is one of the risk factors that influence the occurrence of tuberculosis disease, as a lack of education leads in a lack of understanding about the disease's transmission and danger (Sastroasmoro *et al.,* 2014). As a result, one's level of schooling

influences one's understanding of TB-related issues. Someone with sufficient knowledge will attempt to reduce the risk of TB disease transmission and exposure (Sastroasmoro *et al.,* 2014). In this study, educational level of most participants was till secondary level, 54% grade 8-12) (Table 4.1/ Figure 4.1), this implies that most participants had known of TB but did not have sufficient knowledge on how to prevent the spread or combat the disease and 10% had no education. Thus, inadequate education levels were linked to limited TB awareness and delays in diagnosis and access to TB services as previously documented (Bates *et al.,* 2004; Johansson *et al.,* 2004; Long *et al.,* 2001).

South Africa's unemployment rate is high (32.79%) and it is of significant concern (Francis and Webster, 2019). Majority of participants in this study were unemployed (66%) (Table 4.1/ Figure 4.4), and unemployment is one of the social risk factors that might contribute to the development of this illness (Korzeniewska, 2007). Although it is not mentioned explicitly in many research, a lack of money can contribute to societal impoverishment and, in turn, the pathological issues brought on by poor living situations (Tadolini and Migliori, 2012; Djibuti *et al.,* 2014). In this study 76% participants were Christians and 12% believed in African Religion (Table 4.1/ Figure 4.4). Some Christians assume to believe that prayer can solve any issue, thus, stopping to take their prescribed medication in the hopes that praying will help them get better from their illnesses. This influences the rate at which the illness progresses and raises the risk of TB (Levinsohn, 2008). Moreover, some individuals (in this study) adhering to African customs disclosed that they had consulted traditional healers or consumed medicinal plants as a means of treating tuberculosis. It is possible that these actions could conflict with the drugs prescribed by medical practitioners to treat tuberculosis (Cramm *et al.,* 2010).

Migrants were only 8% (Table 4.2) as recorded in this study, this can be crucial on cases of potential carriers of TB from their country of origin as previously suggested (Zimmerman *et al.,* 2011). A study conducted by (Boudville *et al.,* 2020) stated that, social barriers like language, culture, stigma, and unwelcoming health facilities also play a role in the high rate of tuberculosis among migrants. A high number of native individuals had contracted TB (92%) (Table 4.2), and it has been reported that most patients' health-care centres are far, and the distance plays a negative role on reporting of such infected patients due to lack of financial support for transportation. In contrast, to avoid meetings with acquaintances, most of the TB patients preferred to receive their medications or seek

counsel at healthcare facilities distance from their home. Similarly in a study conducted by Chang and Cataldo (2014) in India, it was found that older patients prefer government facilities and younger patients prefer private health care facilities. Wherein young people may do this to avoid community recognition at home.

Most respondents in this study worked in a dusty environment 56% (Table 4.2), similarly in a study conducted by Alavi and colleagues (2013), who investigated the impact of soil, dust on public health, and reported that inhaling dust-filled air can cause a long-term lung pathological alteration. The hazardous effect of silica, cotton, wool, weaving mills, and coal mines is a known problem as it impacts on making one prone to respiratory diseases such as TB (Alavi et al., 2013). Only 28% of respondents in this study wore a protective mask, wearing a protective mask significantly reduce spread of TB (Dharmadhikari *et al.,* 2013). While 18% of participants that did not wear masks and 54% wore a protective mask sometimes (Figure 4.2). These poses a serious threat in spreading airborne pathogens including MTB. Additionally, the irritation of wearing a mask may be a factor in non-compliance. World Health Organisation's report has highlighted the critical importance of face masks in reducing the spread of infectious diseases (WHO, 2009). It is important to note wearing a mask alone is not enough to prevent TB entirely, other preventive measures, such as proper ventilation, early diagnosis, and treatment, are also crucial in controlling TB (WHO, 2019).

In this study, collected data showed that a high number of participants used public transportations 66%, while 26% preferred or were obliged to walked (Figure 4.2). According to Andrew and colleagues (2017), utilising public transport can raise one's risk of contracting tuberculosis since it allows people of different backgrounds to come into contact with one another and spread the disease and public transports are generally known to be crowded, poorly ventilated thus more people will be prone to contracting the airborne pathogen TB (Sultan *et al.,* 2010; Horna *et al.,* 2011), also while walking one may contract this airborne pathogen, though chances are very less.

Twenty-three percent (23%) of participants in this study shared a bedroom (Figure 4.2) this is included as a risk factor for latent TB (Eom *et al.,* 2018), due to prolonged airborne contact with active TB patients, household members are most at risk for latent tuberculosis infection (Fox *et al.,* 2013). Additionally, majority of participants in this study

did not have family members that were diagnosed with TB, but 22% (Figure 4.2) did have family members that were diagnosed with TB. This supports a study conducted by (Hur *et al.,* 2013) who reported that living in close quarters with an infected family member increases the chances of exposure to the TB bacteria. Hence to find and identify cases of latent TB infection as well as risk factors for latent TB infection, it is crucial to track down the contacts of family members who live with active TB patients, of which might assist to stop and limit the spread of active tuberculosis cases in the future (Reichler *et al.,* 2018).

The findings of this study suggests that most participants did not believe in traditional healers, as 88% did not consult traditional healers (Table 4.3). According to Bates *et al.,* (2004), it was discovered that the idea that TB is incurable caused stigma to persist, which kept people from seeking help. It was also discovered that the near symptom similarities between TB and AIDS contributed to stigma, making patients reluctant to seek TB treatment out of concern that they may get HIV. In the Eastern Cape province, South Africa 95% of people concealed their TB status out of fear of discrimination and stigmatization, and 63% postponed seeking a proper diagnosis from medical professionals out of concern that they would be discovered to be HIV positive (Møller *et al.,* 2011).

According to these research, stigma and perceptions play a role in a person's decision to seek TB diagnosis and other relevant therapies. Only 2% (Table 4.3) of participants in this study got remedy from traditional healer, which suggests that traditional beliefs also play a role, according to some participants. Another study reported that TB could be cured by herbal medications delivered by traditional healers (Greenway *et al.,* 2011). According to a research conducted in Botswana's Kweneng district, access to health-care facilities was impacted by traditional beliefs that TB was caused by either God or ancestor spirits (Steen and Mazonde, 1999). In some cases, traditional beliefs linking TB to witchcraft and sorcery were shown to foster secrecy surrounding the disease's diagnosis, which in turn limited treatment adherence and early diagnosis (Bates *et al.,* 2004).

One of the main factors contributing to the spread of TB has been shown to be delayed diagnosis and treatment (Storla *et al.,* 2008; Uys *et al.,* 2009). However, 74% (Table 4.3) of participants in this study consulted health-care centre in ≤3 weeks, indicating that

participants were concerned about their well-being. On the other hand, 26% (Table 4.3) delayed seeking medical attention even though South Africans are highly aware of TB manifestation or signs (Makgopa and Madiba, 2021). Individuals who exhibit signs of the disease frequently neglect seeking medical attention; a research conducted in Mpumalanga revealed a median delay of four weeks for conventional care-seeking (Pronyk *et al.,* 2004). When tested more than 30 days after symptoms started, almost one-third of patients with active TB initially sought therapy from informal sources, such as traditional healers (Makgopa and Madiba 2021). There are several reasons why this care was delayed, such as not considering TB to be the cause of their symptoms, thinking the clinic was too far away, or not thinking their symptoms weren't serious enough to require medical attention (Makgopa and Madiba 2021; Chiposi *et al.,* 2021). There is also the influence of traditional beliefs; in another study, most participants said that traditional healers could administer herbal drugs that may cure TB (Kigozi *et al.,* 2018).

Tuberculosis vaccine was administered by 76% (Table 4.3) of participants in this study, and TB vaccine Bacille Calmette-Guérin (BCG) is one of the most widely provided vaccines worldwide. The findings indicate that the BCG vaccine's protective effects could begin to wane as kids get older. As a result, adults, and kids over 10 should get a booster shot, as well as a new vaccination later on, since the booster shot may not be as effective at providing immunity against TB as it was in the past. New immunisations are unfortunately required because the effectiveness of a BCG booster is limited (Orujyan et., 2022). Even though 24% (Table 4.3) of participants reported to have not been vaccinated, however, it is still mandatory to be vaccinated as it does give one protection to TB to some extent.

It is suggested that sharing a patient's TB diagnosis with family members may affect their adherence (Gebremariam *et al.,* 2016). From this study, 92% (Table 4.3) of participants disclosed their TB status to their family, wherein 84% (Table 4.3) told their family within 1-7 days, with a positive outcome of moral support. Additionally, moral support may encourage early implementation of suitable infection control measures and facilitate the household contacts' access to diagnostic, therapeutic, and preventative TB services. However, stigmatization surrounding TB, the link between TB and HIV infection, the belief that TB is incurable, and misconceptions regarding the cause of TB may make disclosure of TB more difficult (Cremers *et al.,* 2015). Eight (8%) did not disclose their TB status to

their family, with 4% of respondents not having support from their family. Of the enrolled participants, 42% did not disclose their TB status to friends and colleagues (Table 4.3). There have been previous studies on the negative effects of disclosure, including patient neglect, seclusion, loss of social support and divorce (Lee *et al.,* 2015).

People with weakened immune systems are more susceptible to TB since the disease is an opportunistic infection and makes an individual's immune system less capable of fighting off infections. HIV patients are thought to be more susceptible to infection because of their weakened immune systems (Mitchell *et al.,* 2012). The findings of this study revealed that HIV appears as a significant risk factor among the host-related factors considered in the current study. HIV emerged as an important risk factor, with a particularly high prevalence of 66% in comparison to other host-related factors such as: hypertension (6%), ulcer (2%), diabetes (4%), and asthma (4%) (Table 4.4). It has been reported in other previous studies (Chung *et al.,* 2014; Seegert *et al.,* 2017), that countries with high rates of hypertension and tuberculosis share a significant amount of comorbidity. Compared to those without TB, patients with TB have a higher percentage of hypertension.

The infections caused by NTM has been progressively becoming more prevalent throughout the world, and in recent years, it has emerged as a public health issue (Kendall *et al.,* 2013; Prevots *et al.,* 2015; Kee *et al.,* 2017). In the current study a significant number of participants (58%) were infected with NTM (Table 4.5), this may be because NTM lung infection has multiple effects on the host, similar to other lung aetiologies. Clinical signs and symptoms may resemble those of other lung infections, including chronic lung illnesses, atypical bacterial infections, and mycobacterial tuberculosis (Kang *et al.,* 2015). Additionally, it is reported that isolating NTM from human samples can be difficult, and that a positive sputum test could be misleading. Accordingly, NTM species from the environment that colonise the airway are suspected to possibly contaminate specimens, resulting in false positive with a low positive predictive value. Therefore, current guidelines demand three early morning sputum specimens on three different days to accurately diagnose NTM lung infection (Griffith, 2007). In a study conducted by Mirabal and Ferrer (2022), Nuclei-acid amplification testing (NAAT) was suggested as the most accurate test in identification of mycobacterial strains.

The prevalence of drug-resistance was 4%, wherein male patients (age group of [40-50] years) were pre-XDR cases (Table 4.7/ Figure 4.6). This possibly arose from either an individual's infection with a resistant strain or from inappropriate treatment that caused the evolution of the resistant strain (WHO, 2010). Alternatively, due to inconsistent medication intake of Rifampicin and Isoniazid, leading to a MDR-TB stage (WHO, 2018). The condition is also known as secondary MDR-TB when inadequate therapy results in the selection of spontaneously resistant bacteria, thus drug resistance is acquired (Loddenkemper *et al.,* 2002). It can be suspected that these patients might be infected with susceptible TB and MDR-TB, as the *Mycobacterium* is developing resistance to the host leading resistance to FQs (ofloxacin, moxifloxacin and levofloxacin) leading to pre-XDR. Banerjee et al (2008), alluded that when assessing the prevalence of MDR and XDR in a particular population, an important but often overlooked category is “pre-XDR”, that is defined as TB caused by strains of *M. tuberculosis* that meet the criteria for multidrug-resistant tuberculosis (MDR-TB) and are also resistant to any fluoroquinolones (Viney *et al.,* 2021).

Anti-tuberculosis drugs target specific regions of the MTB bacterial genome (Shetye *et al.,* 2020), chemically targeting MTB DNA replication, protein synthesis, cell wall biosynthesis, energy metabolism, and proteolysis (breakdown of proteins into amino acids, due to enzymatic action) these are some of the crucial modes of action of anti-tb drugs (Blower *et al.,* 2016). In this study, mutations on the targeted SNPs associated with the drug resistance genotypes were not identified (Table 4.6). However, the presence of resistance without associated changes in *Emb_4247429, inhA_1674481*, *pncA_2288964, Rpo_B_761110, RpoB_761155, Rpsl_781687, Rrs_1472359* and *Rrs_1473167* gene may possibly be that other genes or factors could be attributed for such resistances (Jugheli *et al.,* 2009; Lagutkin *et al.,* 2022).

Molecular diagnostic approaches that detect mutations in genes linked to anti-TB drug resistance are more efficient and effective. Gene alterations in resistance-determining areas of resistant Mtb isolates can quickly identify anti-TB medication resistance. This approach helps identify resistance and study resistance mechanisms, enabling the development of drug-resistant tuberculosis management options. Understanding the molecular mechanisms of treatment resistance in Mtb is essential for developing better diagnostic techniques. More research is needed to identify gene alterations that cause

drug-resistant Mtb, especially MDR-TB. This research will help local TB control efforts and inform MDR-TB prevention methods (Marahatta *et al.,* 2011).

# CHAPTER 5
# CONCLUSION AND RECOMMENDATIONS

## 5.1 CONCLUSION

The main objective of the study was to determine the prevalence of drug resistant tuberculosis amongst TB patients in the Vhembe district. This was achieved by resolving a set of secondary objectives and answering 2 specific questions.

First objective: To correlate the risk factors associated with drug resistance TB using a survey. The results obtained indicate that gender plays an important role (male). Lack of education (insufficient knowledge on TB) is the main factor leading to poverty. Active age group [35-55] plays a major role.

Second objective: To assess the presence of drug resistance using Allplex™ Multiplex PCR protocol. To avoid any unnecessary spending the samples were first subjected to Anyplex™ in order to determine the presence of TB and then TB susceptible samples were further subjected to Allplex™ in order to determine drug-resistant TB. Out of the TB samples analysed 16 were found to be positive for TB. Furthermore, only 2 (4%) were found to be drug resistant (pre-XDR).

Third objective: To evaluate resistance to second-line injectable anti-TB drugs targeting the mutation status of genotypes conferring resistance for INH, RIF, EMB, PZA, FQs, all of that are linked to resistance to second-line drugs (SLDs); using MassARRAY (Agena) technique. In this approach, patient 1 harboured only 1 common mutation while in Patient 2, had several (7 mutations) mutations.

The study included 2 questions to be answered. The questions included were “What are the risk factors commonly associated with XDR-TB” and “What is the extent of XDR-TB amongst patients”. These questions were answered in objective 1 and 2 respectively.

In conclusion, the findings provided important new information about the characteristics and prevalence of tuberculosis as well as its associated risk factors amongst patients of Vhembe region. Less active TB patients indicates that the region has a high rate of treatment success. The identification of drug resistance highlights the necessity for continued pattern monitoring and the application of suitable treatment approaches in order to mitigate the spread of drug-resistant strains. To draw firm conclusions about

drug-resistant tuberculosis risk factors in patients in the Vhembe region, further information is still required.

## 5.2 LIMITATIONS

Smoking, alcohol use, marital status, source of drinking water (NTM), symptoms patients experience (weight loss, coughing blood etc.) and house-hold income, aspects that affect the patient treatment outcome were not recorded. Improving tracking and monitoring of patients transferred to other health facilities may improve the exclusion of incomplete medical records. Transportation and timing to meet with patients was always a hindrance, which played a role in collecting sufficient samples.

## 5.3 RECOMMENDATIONS

The results of the study indicate that more research with a larger sample size in the study region should be conducted to determine the incidence of drug-resistant TB. Random selection of the samples should not be used since this will restrict the scope of the investigation. In order to accomplish the End TB strategy, the study suggests that the best way to prioritise intervention measures that will affect the projected future burden of drug-resistant tuberculosis is to use provincial DR-TB surveillance data. Consideration should be given to raising community knowledge of DR-TB, encouraging health-seeking behaviour through suggestive signs and symptoms, and actively seeking out active cases of tuberculosis. Time delays in DR-TB diagnosis and treatment initiation may be minimised by providing healthcare facilities with quick diagnostic tests and increasing the ability of healthcare professionals to diagnose the disease. The province of Limpopo may see even higher treatment success rates with certain efforts including early diagnosis, therapy should be taken during initial stages of disease, and patient treatment monitoring.

# REFERENCES

Adane, A.A., Alene, K.A., Koye, D.N. and Zeleke, B.M., 2013. Non-adherence to anti-tuberculosis treatment and determinant factors among patients with tuberculosis in northwest Ethiopia. *PloS one*, *8*(11), p.e78791.

Agho, K.E., Hall, J. and Ewald, B., 2014. Determinants of the knowledge of and attitude towards tuberculosis in Nigeria. *Journal of health, population, and nutrition*, *32*(3), p.520.

Al-Saeedi, M. and Al-Hajoj, S., 2017. Diversity and evolution of drug resistance mechanisms in Mycobacterium tuberculosis. *Infection and drug resistance*, pp.333-342.

Alangaden, G.J., Kreiswirth, B.N., Aouad, A., Khetarpal, M., Igno, F.R., Moghazeh, S.L., Manavathu, E.K. and Lerner, S.A., 1998. Mechanism of resistance to amikacin and kanamycin in Mycobacterium tuberculosis. *Antimicrobial agents and chemotherapy*, *42*(5), pp.1295-1297.

Alavi-Naini, R., Moghtaderi, A., Metanat, M., Mohammadi, M. and Zabetian, M., 2013. Factors associated with mortality in tuberculosis patients. *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences*, *18*(1), p.52.

Ali, S.M., Anjum, N., Ishaq, M., Naureen, F., Noor, A., Rashid, A., Abbas, S.M. and Viney, K., 2019. Community knowledge about tuberculosis and perception about tuberculosis-associated stigma in Pakistan. *societies*, *9*(1), p.9.

Andrews, J.R., Nemes, E., Tameris, M., Landry, B.S., Mahomed, H., McClain, J.B., Fletcher, H.A., Hanekom, W.A., Wood, R., McShane, H. and Scriba, T.J., 2017. Serial QuantiFERON testing and tuberculosis disease risk among young children: an observational cohort study. *The lancet Respiratory medicine*, *5*(4), pp.282-290.

Arnold, A., Witney, A.A., Vergnano, S., Roche, A., Cosgrove, C.A., Houston, A., Gould, K.A., Hinds, J., Riley, P., Macallan, D. and Butcher, P.D., 2016. XDR-TB transmission in London: case management and contact tracing investigation assisted by early whole genome sequencing. *Journal of Infection*, *73*(3), pp.210-218.

Aubry, A., Veziris, N., Cambau, E., Truffot-Pernot, C., Jarlier, V. and Fisher, L.M., 2006. Novel gyrase mutations in quinolone-resistant and-hypersusceptible clinical isolates of Mycobacterium tuberculosis: functional analysis of mutant enzymes. *Antimicrobial Agents and Chemotherapy*, *50*(1), pp.104-112.

Banerjee, R., Allen, J., Westenhouse, J., Oh, P., Elms, W., Desmond, E., Nitta, A., Royce, S. and Flood, J., 2008. Extensively drug-resistant tuberculosis in California, 1993–2006. *Clinical Infectious Diseases*, *47*(4), pp.450-457.

Barlow, K.E., Tiger, J.H., Slocum, S.K. and Miller, S.J., 2013. Comparing acquisition of exchange-based and signed mands with children with autism. *The Analysis of Verbal Behavior*, *29*, pp.59-69.

Bartek, I.L., Woolhiser, L.K., Baughn, A.D., Basaraba, R.J., Jacobs Jr, W.R., Lenaerts, A.J. and Voskuil, M.I., 2014. Mycobacterium tuberculosis Lsr2 is a global transcriptional regulator required for adaptation to changing oxygen levels and virulence. *MBio*, *5*(3), pp.10-1128.

Bates, I., Fenton, C., Gruber, J., Lalloo, D., Lara, A.M., Squire, S.B., Theobald, S., Thomson, R. and Tolhurst, R., 2004. Vulnerability to malaria, tuberculosis, and HIV/AIDS infection and disease. Part 1: determinants operating at individual and household level. *The Lancet infectious diseases*, *4*(5), pp.267-277.

Be, N.A., Kim, K.S., Bishai, W.R. and Jain, S.K., 2009. Pathogenesis of central nervous system tuberculosis. *Current molecular medicine*, *9*(2), pp.94-99.

Blair, J.M., Webber, M.A., Baylay, A.J., Ogbolu, D.O. and Piddock, L.J., 2015. Molecular mechanisms of antibiotic resistance. *Nature reviews microbiology*, *13*(1), pp.42-51.

Blower, T.R., Williamson, B.H., Kerns, R.J. and Berger, J.M., 2016. Crystal structure and stability of gyrase–fluoroquinolone cleaved complexes from Mycobacterium tuberculosis. *Proceedings of the National Academy of Sciences*, *113*(7), pp.1706-1713.

Boudville, D.A., Joshi, R. and Rijkers, G.T., 2020. Migration and tuberculosis in Europe. *Journal of clinical tuberculosis and other mycobacterial diseases*, *18*, p.100143.

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

Burian, J., Ramon-Garcia, S., Howes, C.G. and Thompson, C.J., 2012. WhiB7, a transcriptional activator that coordinates physiology with intrinsic drug resistance in Mycobacterium tuberculosis. *Expert review of anti-infective therapy*, *10*(9), pp.1037-1047.

Cardoso, R.F., Cardoso, M.A., Leite, C.Q.F., Sato, D.N., Mamizuka, E.M., Hirata, R.D.C., Mello, F.F.D. and Hirata, M.H., 2007. Characterization of ndh gene of isoniazid resistant and susceptible Mycobacterium tuberculosis isolates from Brazil. *Memórias do Instituto Oswaldo Cruz*, *102*, pp.59-61.

Carreira, S., Costeira, J., Gomes, C., André, J.M. and Diogo, N., 2012. Impact of diabetes on the presenting features of tuberculosis in hospitalized patients. *Revista Portuguesa de Pneumologia (English Edition)*, *18*(5), pp.239-243.

Centers for Disease Control and Prevention., 2011. The Difference Between Latent TB Infection and TB Disease. Accessed from https://www.cdc.gov/tb/statistics/ltbi.htm [Accessed date 13 March 2022].

Centers for Disease Control and Prevention., 2014. The Difference Between Latent TB Infection and TB Disease. Accessed from https://www.cdc.gov/tb/publications/factsheets/general/ltbiandactivetb.htm#:~:text=When%20a%20person%20with%20infectious,with%20TB%20bacteria%20becomes%20sick. [Accessed 16 September 2022].

Centers for Disease Control and Prevention., 2022. Dear Colleague Letters, Surveillance definitions for extensively drug resistant (XDR) and pre-XDR tuberculosis. Accessed from https://www.cdc.gov/tb/publications/letters/2022/surv-def-xdr.html [Accessed date 5 December 2023].

Centers for Disease Control and Prevention., 2023. Provisional CDC Guidance for the Use of Pretomanid as part of a Regimen [Bedaquiline, Pretomanid, and Linezolid (BPaL)] to Treat Drug-Resistant Tuberculosis Disease. Accessed from https://www.cdc.gov/tb/topic/drtb/bpal/default.htm [Accessed date 12 December 2023].

Chang, S.H. and Cataldo, J., 2014. A systematic review of global cultural variations in knowledge, attitudes and health responses to tuberculosis stigma. *The International Journal of Tuberculosis and Lung Disease*, *18*(2), pp.168-173.

Chihota, V.N., Ginindza, S., McCarthy, K., Grant, A.D., Churchyard, G. and Fielding, K., 2010. Missed opportunities for TB investigation in primary care clinics in South Africa: experience from the XTEND trial. *PLoS One*, *10*(9), p.e0138149.

Chinenye, N.M., 2015. Evaluation of knowledge, attitude and practices of TB diagnosed patients in Rwanda towards TB infection. Case of TB diagnosed patients in Kigali urban and rural health facilities. *International Journal of Scientific and Research Publications*, *5*(8), pp.1-19.

Chiposi, L., Cele, L.P. and Mokgatle, M., 2021. Prevalence of delay in seeking tuberculosis care and the health care seeking behaviour profile of tuberculous patients in a rural district of KwaZulu Natal, South Africa. *Pan African Medical Journal*, *39*(1).

Chowdhury, K., Ahmad, R., Sinha, S., Dutta, S. and Haque, M., 2023. Multidrug-Resistant TB (MDR-TB) and Extensively Drug-Resistant TB (XDR-TB) Among Children: Where We Stand Now. *Cureus*, *15*(2).

Chung, W.S., Lin, C.L., Hung, C.T., Chu, Y.H., Sung, F.C., Kao, C.H. and Yeh, J.J., 2014. Tuberculosis increases the subsequent risk of acute coronary syndrome: a nationwide population-based cohort study. *The International journal of tuberculosis and lung disease*, *18*(1), pp.79-83.

Clay, H., Volkman, H.E. and Ramakrishnan, L., 2008. Tumor necrosis factor signaling mediates resistance to mycobacteria by inhibiting bacterial growth and macrophage death. *Immunity*, *29*(2), pp.283-294.

Cohen, J., 2006. Extensively drug-resistant TB gets foothold in South Africa.

Cohen, K.A., Abeel, T., Manson McGuire, A., Desjardins, C.A., Munsamy, V., Shea, T.P., Walker, B.J., Bantubani, N., Almeida, D.V., Alvarado, L. and Chapman, S.B., 2015. Evolution of extensively drug-resistant tuberculosis over four decades: whole genome sequencing and dating analysis of Mycobacterium tuberculosis isolates from KwaZulu-Natal. *PLoS medicine*, *12*(9), p.e1001880.

Coll, Y., Niehaus, K.E., Walker, T.M., Iqbal, Z., Walker, A.S., Wilson, D.J., Peto, T.E., Crook, D.W., Smith, E.G., Zhu, T. and Clifton, D.A., 2018. Machine learning for classifying tuberculosis drug-resistance from DNA sequencing data. *Bioinformatics*, *34*(10), pp.1666-1671.

Cowan, J., De Schacht, C., Mutaquiha, C., Faria, F., Castro, G., Manaca, N. and Manhica, I. 2019. Barriers to access and adherence to tuberculosis services, as perceived by patients: A qualitative study in Mozambique. *PloS one*, *14*(7), p.e0219470.

Cramm, J.M., Finkenflügel, H.J., Møller, V. and Nieboer, A.P., 2010. TB treatment initiation and adherence in a South African community influenced more by perceptions than by knowledge of tuberculosis. *BMC public health*, *10*(1), pp.1-8.

Cremers, A.L., de Laat, M.M., Kapata, N., Gerrets, R., Klipstein-Grobusch, K. and Grobusch, M.P., 2015. Assessing the consequences of stigma for tuberculosis patients in urban Zambia. *PloS one*, *10*(3), p.e0119861.

Culyba, M.J. and Van Tyne, D., 2021. Bacterial evolution during human infection: adapt and live or adapt and die. *PLoS Pathogens*, *17*(9), p.e1009872.

da Silva, P.E.A., Von Groll, A., Martin, A. and Palomino, J.C., 2011. Efflux as a mechanism for drug resistance in Mycobacterium tuberculosis. *FEMS Immunology & Medical Microbiology*, *63*(1), pp.1-9.

Davies, P.D.O., 2005. Risk factors for tuberculosis. *Monaldi archives for chest disease*, *63*(1).

de Vos, M., Derendinger, B., Streicher, E.M., Dolby, T., Scott, L.A., Steinhobel, A.D., Warren, R.M. and Theron, G., 2018. Non-actionable Results, Accuracy, and Effect of First-and Second-line Line Probe Assays for Diagnosing Drug-Resistant Tuberculosis,

Including on Smear-Negative Specimens, in a High-Volume Laboratory. *Clinical Infectious Diseases*, *76*(3), pp.e920-e929.

Dericck, S.C. and Morris, S.L., 2007. The ESAT6 protein of Mycobacterium tuberculosis induces apoptosis of macrophages by activating caspase expression. *Cellular microbiology*, *9*(6), pp.1547-1555.

Desai, A.N. and Hurtado, R., 2021. Nontuberculous mycobacterial infections. *JAMA*, *325*(15), pp.1574-1574.

Dharmadhikari, A., Smith, J., Nardell, E., Churchyard, G. and Keshavjee, S., 2013. Aspiring to zero tuberculosis deaths among southern Africa's miners: is there a way forward?. *International Journal of Health Services*, *43*(4), pp.651-664.

Dietrich, J. and Doherty, T.M., 2009. Interaction of Mycobacterium tuberculosis with the host: consequences for vaccine development. *Apmis*, *117*(5-6), pp.440-457.

Dixit, A., Freschi, L., Vargas, R., Calderon, R., Sacchettini, J., Drobniewski, F., Galea, J.T., Contreras, C., Yataco, R., Zhang, Z. and Lecca, L., 2019. Whole genome sequencing identifies bacterial factors affecting transmission of multidrug-resistant tuberculosis in a high-prevalence setting. *Scientific Reports*, *9*(1), p.5602.

Djibuti, M., Mirvelashvili, E., Makharashvili, N. and Magee, M.J., 2014. Household income and poor treatment outcome among patients with tuberculosis in Georgia: a cohort study. *BMC public health*, *14*, pp.1-7.

Dodd, P.J., Looker, C., Plumb, I.D., Bond, V., Schaap, A., Shanaube, K., Muyoyeta, M., Vynnycky, E., Godfrey-Faussett, P., Corbett, E.L. and Beyers, N., 2016. Age-and sex-specific social contact patterns and incidence of Mycobacterium tuberculosis infection. *American journal of epidemiology*, *183*(2), pp.156-166.

Dookie, N., Sturm, A.W. and Moodley, P., 2016. Mechanisms of first-line antimicrobial resistance in multi-drug and extensively drug resistant strains of Mycobacterium tuberculosis in KwaZulu-Natal, South Africa. *BMC infectious diseases*, *16*, pp.1-8.

Dooley, K.E., Rosenkranz, S.L., Conradie, F., Moran, L., Hafner, R., von Groote-Bidlingmaier, F., Lama, J.R., Shenje, J., De Los Rios, J., Comins, K. and Morganroth, J.,

2021. QT effects of bedaquiline, delamanid, or both in patients with rifampicin-resistant tuberculosis: a phase 2, open-label, randomised, controlled trial. *The Lancet Infectious Diseases*, *21*(7), pp.975-983.

Duko, B., Bedaso, A., Ayano, G. and Yohannis, Z., 2019. Perceived stigma and associated factors among patient with tuberculosis, Wolaita Sodo, Ethiopia: cross-sectional study. *Tuberculosis research and treatment*, *2019*.

Edwards, J.R., Ruparel, H. and Ju, J., 2005. Mass-spectrometry DNA sequencing. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, *573*(1-2), pp.3-12.

Eom, J.S., 2018. The prevalence and risk factors of latent tuberculosis infection among health care workers working in a tertiary hospital in South Korea. *Tuberculosis and respiratory diseases*, *81*(4), pp.274-280.

Falzon, F.A., Salim, M.H., du Cros, P., Casas, E.C., Khamraev, A., Sikhondze, W., Benedetti, A., Bastos, M., Lan, Z., Jaramillo, E. and Khan, D., 2017. Effectiveness and safety of standardised shorter regimens for multidrug-resistant tuberculosis: individual patient data and aggregate data meta-analyses. *European Respiratory Journal*, *50*(1).

Fedrizzi, T., Meehan, C.J., Grottola, A., Giacobazzi, E., Fregni Serpini, G., Tagliazucchi, S., Fabio, A., Bettua, C., Bertorelli, R., De Sanctis, V. and Rumpianesi, F., 2017. Genomic characterization of nontuberculous mycobacteria. *Scientific reports*, *7*(1), p.45258.

Ferguson, J.S., Weis, J.J., Martin, J.L. and Schlesinger, L.S., 2004. Complement protein C3 binding to Mycobacterium tuberculosis is initiated by the classical pathway in human bronchoalveolar lavage fluid. *Infection and immunity*, *72*(5), pp.2564-2573.

Fillol, M., Shitikov, E., Kolchenko, S., Mokrousov, I., Bespyatykh, J., Ischenko, D., Ilina, E. and Govorun, V., 2017. Evolutionary pathway analysis and unified classification of East Asian lineage of Mycobacterium tuberculosis. *Scientific reports*, *7*(1), p.9227.

Ford, N., Matteelli, A., Shubber, Z., Hermans, S., Meintjes, G., Grinsztejn, B., Waldrop, G., Kranzer, K., Doherty, M. and Getahun, H., 2016. TB as a cause of hospitalization and in-hospital mortality among people living with HIV worldwide: a systematic review and meta-analysis. *Journal of the International AIDS Society*, *19*(1), p.20714.

Forrellad, M.A., Klepp, L.I., Gioffré, A., Sabio y Garcia, J., Morbidoni, H.R., Santangelo, M.D.L.P., Cataldi, A.A. and Bigi, F., 2013. Virulence factors of the Mycobacterium tuberculosis complex. *Virulence*, *4*(1), pp.3-66.

Fox, G.J., Barry, S.E., Britton, W.J. and Marks, G.B., 2013. Contact investigation for tuberculosis: a systematic review and meta-analysis. *European Respiratory Journal*, *41*(1), pp.140-156.

Francis, H.W. and Webster, J.E., 2019. Adverse outcome pathway for aminoglycoside ototoxicity in drug-resistant tuberculosis treatment. *Archives of toxicology*, *93*, pp.1385-1399.

Franzblau, S.G., DeGroote, M.A., Cho, S.H., Andries, K., Nuermberger, E., Orme, I.M., Mdluli, K., Angulo-Barturen, I., Dick, T., Dartois, V. and Lenaerts, A.J., 2012. Comprehensive analysis of methods used for the evaluation of compounds against Mycobacterium tuberculosis. *Tuberculosis*, *92*(6), pp.453-488.

Gaharwar, U.S., Meena, R. and Rajamani, P., 2016. Iron oxide nanoparticles induced cytotoxicity, oxidative stress and DNA damage in lymphocytes. *Journal of Applied Toxicology*, *37*(10), pp.1232-1244.

Gandhi, N.R., Migliori, G.B., Sotgiu, G., Falzon, D., DeRiemer, K., Centis, R., Hollm-Delgado, M.G., Palmero, D., Pérez-Guzmán, C., Vargas, M.H. and D'Ambrosio, L., 2013. Drug resistance beyond extensively drug-resistant tuberculosis: individual patient data meta-analysis. *European Respiratory Journal*, *42*(1), pp.169-179.

Gandhi, N.R., Moll, A., Sturm, A.W., Pawinski, R., Govender, T., Lalloo, U., Zeller, K., Andrews, J. and Friedland, G., 2006. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *The Lancet*, *368*(9547), pp.1575-1580.

Gebremariam, G., Asmamaw, G., Hussen, M., Hailemariam, M.Z., Asegu, D., Astatkie, A. and Amsalu, A.G., 2016. Impact of HIV status on treatment outcome of tuberculosis patients registered at Arsi Negele Health Center, Southern Ethiopia: a six year retrospective study. *PloS one*, *11*(4), p.e0153239.

Gehre, F., Otu, J., Kendall, L., Forson, A., Kwara, A., Kudzawu, S., Kehinde, A.O., Adebiyi, O., Salako, K., Baldeh, I. and Jallow, A., 2016. The emerging threat of pre-extensively drug-resistant tuberculosis in West Africa: preparing for large-scale tuberculosis research and drug resistance surveillance. *BMC medicine*, *14*(1), pp.1-12.

Gelaw, S.M., 2016. Socioeconomic factors associated with knowledge on tuberculosis among adults in Ethiopia. *Tuberculosis Research and Treatment*, *2016*.

Geldmacher, A., Heinrich, N. and Rachow, C., 2012. Early identification of progressive TB disease using host biomarkers. *EBioMedicine*, *2*(2), pp.107-108.

Glickman, M.S. and Jacobs, W.R., 2001. Microbial pathogenesis of Mycobacterium tuberculosis: dawn of a discipline. *Cell*, *104*(4), pp.477-485.

Golden, M.P. and Vikram, H.R., 2005. Extrapulmonary tuberculosis: an overview. *American family physician*, *72*(9), pp.1761-1768.

Gorna, A.E., Bowater, R.P. and Dziadek, J., 2010. DNA repair systems and the pathogenesis of Mycobacterium tuberculosis: varying activities at different stages of infection. *Clinical science*, *119*(5), pp.187-202.

Greenaway, C., Sandoe, A., Vissandjee, B., Kitai, I., Gruner, D., Wobeser, W., Pottie, K., Ueffing, E., Menzies, D. and Schwartzman, K., 2011. Tuberculosis: evidence review for newly arriving immigrants and refugees. *Cmaj*, *183*(12), pp.E939-E951.

Griffith, D.E., 2007. Therapy of nontuberculous mycobacterial disease. *Current opinion in infectious diseases*, *20*(2), pp.198-203.

Guaraldi, G., Zona, S., Brothers, T.D., Carli, F., Stentarelli, C., Dolci, G., Santoro, A., Beghetto, B., Menozzi, M., Mussini, C. and Falutz, J., 2015. Aging with HIV vs. HIV seroconversion at older age: a diverse population with distinct comorbidity profiles. *PloS one*, *10*(4), p.e0118531.

Gupta, R.K., Turner, C.T., Venturini, C., Esmail, H., Rangaka, M.X., Copas, A., Lipman, M., Abubakar, I. and Noursadeghi, M., 2020. Concise whole blood transcriptional signatures for incipient tuberculosis: a systematic review and patient-level pooled meta-analysis. *The Lancet Respiratory Medicine*, *8*(4), pp.395-406.

Gursimrat, K.S., 2011. Tuberculosis: Current Situation, Challenges and Over-view of Its Control Programs in India. Faculty of Medicine, Lund University, Lund, Sweden.

Gygli, S.M., Borrell, S., Trauner, A. and Gagneux, S., 2017. Antimicrobial resistance in Mycobacterium tuberculosis: mechanistic and evolutionary perspectives. *FEMS microbiology reviews*, *41*(3), pp.354-373.

Habib, S.S., Zaidi, S.M.A., Jamal, W.Z., Azeemi, K.S., Khan, S., Khowaja, S., Domki, A.K., Khan, A. and Khan, F.A., 2018. Gender-based differences in community-wide screening for pulmonary tuberculosis in Karachi, Pakistan: an observational study of 311 732 individuals undergoing screening. *Thorax*.

Hameed, H.A., Islam, M.M., Chhotaray, C., Wang, C., Liu, Y., Tan, Y., Li, X., Tan, S., Delorme, V., Yew, W.W. and Liu, J., 2018. Molecular targets related drug resistance mechanisms in MDR-, XDR-, and TDR-Mycobacterium tuberculosis strains. *Frontiers in cellular and infection microbiology*, *8*, p.114.

Harichander, S., Wiafe, E., Mensah, K.B., Bangalee, V. and Oosthuizen, F., 2022. The incidence of TB and MDR-TB in pediatrics and therapeutic options: a systematic review. *Systematic reviews*, *11*(1), p.157.

Hayward, S., Harding, R.M., McShane, H. and Tanner, R., 2018. Factors influencing the higher incidence of tuberculosis among migrants and ethnic minorities in the UK. *F1000Research*, *7*, p.461.

Hayward, S.E., Rustage, K., Nellums, L.B., van der Werf, M.J., Noori, T., Boccia, D., Friedland, J.S. and Hargreaves, S., 2018. Extrapulmonary tuberculosis among migrants in Europe, 1995 to 2017. *Clinical Microbiology and Infection*, *27*(9), pp.1347-e1.

Hirpa, S., Medhin, G., Girma, B., Melese, M., Mekonen, A., Suarez, P. and Ameni, G., 2013. Determinants of multidrug-resistant tuberculosis in patients who underwent first-line treatment in Addis Ababa: a case control study. *BMC public health*, *13*, pp.1-9.

Hoagland, D.T., Liu, J., Lee, R.B. and Lee, R.E., 2016. New agents for the treatment of drug-resistant Mycobacterium tuberculosis. *Advanced drug delivery reviews*, *102*, pp.55-72.

Horna, D.R., Munayco, C.V., Grijalva, C.G., Cayla, J.A., Campos, O., Ch, K.A. and Suarez, L.A., 2012. Factors associated with the non-completion of conventional anti-tuberculosis treatment in Peru. *Archivos de Bronconeumología (English Edition)*, *48*(5), pp.150-155.

Https://www.gov.za/documents/annual-reports/department-health-annual-report-20172018-27-Sep-2018 [Accessed 12 December 2023].

Hur, Y.G., Kang, Y.A., Jang, S.H., Hong, J.Y., Kim, A., Lee, S.A., Kim, Y. and Cho, S.N., 2015. Adjunctive biomarkers for improving diagnosis of tuberculosis and monitoring therapeutic effects. *Journal of Infection*, *70*(4), pp.346-355.

Isaboke, J.N., 2016. Risk Factors for Tuberculosis and Multidrug-Resistant Tuberculosis Complications among Foreign-Born Persons in Houston, Texas.

Jackson, C., Southern, J., Gupta, R.K., Drobniewski, F., Lalvani, A., Lipman, M., Mangtani, P. and Abubakar, I., 2020. Effectiveness of BCG vaccination against Mycobacterium tuberculosis infection in adults: a cross-sectional analysis of a UK-based cohort. *The Journal of Infectious Diseases*, *221*(1), pp.146-155.

Jenkins, H.E., Tolman, A.W., Yuen, C.M., Parr, J.B., Keshavjee, S., Pérez-Vélez, C.M., Pagano, M., Becerra, M.C. and Cohen, T., 2014. Incidence of multidrug-resistant tuberculosis disease in children: systematic review and global estimates. *The Lancet*, *383*(9928), pp.1572-1579.

Jilani, T.N., Avula, A., Gondal, Z. and Siddiqui, A.H., 2018. Active tuberculosis.

Johansen, S., Muma, J., Munyeme, M., Mbulo, G., Muwonge, A., Shamputa, I.C., Djønne, B., Godfroid, J. and Johansen, T.B., 2014. Isolation and molecular characterization of Mycobacterium tuberculosis from humans and cattle in Namwala District, Zambia. *Ecohealth*, *11*, pp.564-570.

Johansson, N., Çevikbaş, A. and Duman, C., 2004. The effects of rifampicin and fluoroquinolones on tubercle bacilli within human macrophages. *International journal of antimicrobial agents*, *23*(1), pp.84-87.

Jugheli, L., Bzekalava, N., de Rijk, P., Fissette, K., Portaels, F. and Rigouts, L., 2009. High level of cross-resistance between kanamycin, amikacin, and capreomycin among Mycobacterium tuberculosis isolates from Georgia and a close relation with mutations in the rrs gene. *Antimicrobial agents and chemotherapy*, *53*(12), pp.5064-5068.

Kamerbeek, J., Tadesse, M., Abebe, G., Bekele, A., Bezabih, M., de Rijk, P., Meehan, C.J., de Jong, B.C. and Rigouts, L., 2017. The predominance of Ethiopian specific Mycobacterium tuberculosis families and minimal contribution of Mycobacterium bovis in tuberculous lymphadenitis patients in Southwest Ethiopia. *Infection, Genetics and Evolution*, *55*, pp.251-259.

Kang, H.K., Park, H.Y., Kim, D., Jeong, B.H., Jeon, K., Cho, J.H., Kim, H.K., Choi, Y.S., Kim, J. and Koh, W.J., 2015. Treatment outcomes of adjuvant resectional surgery for nontuberculous mycobacterial lung disease. *BMC infectious diseases*, *15*(1), pp.1-9.

Kashyap, B., Dewan, P., Hyanki, P. and Singh, N.P., 2018. Clinical spectrum of pediatric tuberculosis: a microbiological correlation from a tertiary care center. *Journal of Tropical Pediatrics*, *65*(2), pp.130-138.

Kee, S.J. and Suh, S.P., 2017. Increasing burden of nontuberculous mycobacteria in Korea. *Journal of Korean Medical Science*, *32*(8), pp.1215-1216.

Kendall, B.A. and Winthrop, K.L., 2013, February. Update on the epidemiology of pulmonary nontuberculous mycobacterial infections. In *Seminars in respiratory and critical care medicine*(Vol. 34, No. 01, pp. 087-094). Thieme Medical Publishers.

Kigozi, G., Engelbrecht, M., Heunis, C. and Janse van Rensburg, A., 2018. Household contact non-attendance of clinical evaluation for tuberculosis: a pilot study in a high burden district in South Africa. *BMC infectious diseases*, *18*(1), pp.1-8.

Kirirabwa, N.S., Kimuli, D., Nanziri, C., Sama, D., Ntudhu, S., Okello, D.A., Byaruhanga, R., Lukoye, D. and Kasozi, S., 2019. A four-year trend in pulmonary bacteriologically confirmed tuberculosis case detection in Kampala-Uganda. *BMC pulmonary medicine*, *19*, pp.1-8.

Korf, H., Vander Beken, S., Romano, M., Steffensen, K.R., Stijlemans, B., Gustafsson, J.Å., Grooten, J. and Huygen, K., 2009. Liver X receptors contribute to the protective immune response against Mycobacterium tuberculosis in mice. *The Journal of clinical investigation*, *119*(6), pp.1626-1637.

Korzeniewska-Koseła, M., Kuś, J., Lewandowska, K. and Siemion-Szcześniak, I., 2007. Tuberculosis in homeless persons in Poland. *Przegl Epidemiol*, *69*(3), pp.445-51.

Kronthaler, F. and Zöllner, S., 2021. Data analysis with RStudio. *An Easygoing Introduction*, pp.7-131.

Kumar, N.P., Fukutani, K.F., Shruthi, B.S., Alves, T., Silveira-Mattos, P.S., Rocha, M.S., West, K., Natarajan, M., Viswanathan, V., Babu, S. and Andrade, B.B., 2018. Persistent inflammation during anti-tuberculosis treatment with diabetes comorbidity. *Elife*, *8*, p.e46477.

Kurz, S.G., Furin, J.J. and Bark, C.M., 2016. Drug-resistant tuberculosis: challenges and progress. *Infectious Disease Clinics*, *30*(2), pp.509-522.

Lagutkin, D., Panova, A., Vinokurov, A., Gracheva, A., Samoilova, A. and Vasilyeva, I., 2022. Genome-Wide study of drug resistant Mycobacterium tuberculosis and its intra-host evolution during treatment. *Microorganisms*, *10*(7), p.1440.

Law, W.S., Yew, W.W., Chiu Leung, C., Kam, K.M., Tam, C.M., Chan, C.K. and Leung, C.C., 2008. Risk factors for multidrug-resistant tuberculosis in Hong Kong. *The international journal of tuberculosis and lung disease*, *12*(9), pp.1065-1070.

Lee, H.Y., Lee, J., Lee, Y.S., Kim, M.Y., Lee, H.K., Lee, Y.M., Shin, J.H. and Ko, Y., 2015. Drug-resistance pattern of Mycobacterium tuberculosis strains from patients with pulmonary and extrapulmonary tuberculosis during 2006 to 2013 in a Korean tertiary medical center. *The Korean Journal of Internal Medicine*, *30*(3), p.325.

Lee, J.Y., 2015. Diagnosis and treatment of extrapulmonary tuberculosis. *Tuberculosis and respiratory diseases*, *78*(2), pp.47-55.

Levinsohn., 2008. Cross-national prevalence and risk factors for suicidal ideation, plans and attempts. *The British journal of psychiatry*, *192*(2), pp.98-105.

Lewinsohn, D.M., Leonard, M.K., LoBue, P.A., Cohn, D.L., Daley, C.L., Desmond, E., Keane, J., Lewinsohn, D.A., Loeffler, A.M., Mazurek, G.H. and O'Brien, R.J., 2017. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention clinical practice guidelines: diagnosis of tuberculosis in adults and children. *Clinical Infectious Diseases*, *64*(2), pp.e1-e33.

Lienhardt, C., Glaziou, P., Uplekar, M., Lönnroth, K., Getahun, H. and Raviglione, M., 2012. Global tuberculosis control: lessons learnt and future prospects. *Nature Reviews Microbiology*, *10*(6), pp.407-416.

Liu, Y., Wu, Y.H., Ban, Y., Wang, H. and Cheng, M.M., 2020. Rethinking computer-aided tuberculosis diagnosis. In *Proceedings of the IEEE/CVF conference on computer vision and pattern recognition* (pp. 2646-2655).

Loddenkemper, R., Sagebiel, D. and Brendel, A., 2002. Strategies against multidrug-resistant tuberculosis. *European respiratory journal*, *20*(36 suppl), pp.66s-77s.

Lomovskaya, O., Zgurskaya, H.I., Bostian, K.A. and Lewis, K., 2008. Multidrug efflux pumps: structure, mechanism, and inhibition. *Bacterial resistance to antimicrobials*, pp.45-69.

Long, N.H., Johansson, E., Diwan, V.K. and Winkvist, A., 1999. Different tuberculosis in men and women: beliefs from focus groups in Vietnam. *Social Science & Medicine*, *49*(6), pp.815-822.

Long, R. and Gardam, M., 2001. Tumour necrosis factor-α inhibitors and the reactivation of latent tuberculosis infection. *Cmaj*, *168*(9), pp.1153-1156.

Lui, H., Li, D., Zhao, L., Fleming, J., Lin, N., Wang, T., Liu, Z., Li, C., Galwey, N., Deng, J. and Zhou, Y., 2013. Genome sequencing of 161 Mycobacterium tuberculosis isolates from China identifies genes and intergenic regions associated with drug resistance. *Nature genetics*, *45*(10), pp.1255-1260.

Luthra, S., Rominski, A. and Sander, P., 2015. The role of antibiotic-target-modifying and antibiotic-modifying enzymes in Mycobacterium abscessus drug resistance. *Frontiers in microbiology*, *9*, p.2179.

Lynch, J.B., 2013. Multidrug-resistant tuberculosis. *Medical Clinics*, *97*(4), pp.553-579.

Maguga-Phasha, N.T.C., Munyai, N.S., Mashinya, F., Makgatho, M.E. and Mbajiorgu, E.F., 2017. Genetic diversity and distribution of Mycobacterium tuberculosis genotypes in Limpopo, South Africa. *BMC Infectious Diseases*, *17*, pp.1-8.

Makgopa, S. and Madiba, S., 2021. Tuberculosis knowledge and delayed health care seeking among new diagnosed tuberculosis patients in primary health facilities in an urban district, south africa. *Health services insights*, *14*, p.11786329211054035.

Mäkinen, J., Marttila, H.J., Marjamäki, M., Viljanen, M.K. and Soini, H., 2006. Comparison of two commercially available DNA line probe assays for detection of multidrug-resistant Mycobacterium tuberculosis. *Journal of clinical microbiology*, *44*(2), pp.350-352.

Mäkinen, M.M., Badawy, M.S.E., Taleb, M.H. and Menofy, N.G.E., 2006. Tuberculosis Diagnosis and Detection of Drug Resistance: A Comprehensive Updated Review. *Journal of Pure & Applied Microbiology*, *17*(4).

Malik, A.A., Gandhi, N.R., Marcy, O., Walters, E., Tejiokem, M., Chau, G.D., Omer, S.B., Lash, T.L., Becerra, M.C., Njuguna, I.N. and LaCourse, S.M., 2022, November. Development of a clinical prediction score including monocyte-to-lymphocyte ratio to inform tuberculosis treatment among children with HIV: a multicountry study. In *Open Forum Infectious Diseases* (Vol. 9, No. 11, p. ofac548). US: Oxford University Press.

Maputle, S.M., Tshitangano, T.G and Netshikweta, L.M., 2013. Availability of tuberculosis infection control plans at rural hospitals of Vhembe district, Limpopo Province of South Africa. *African Journal of Primary Health Care and Family Medicine*, *5*(1), pp.1-6.

Marahatta, S.B., Gautam, S., Dhital, S., Pote, N., Jha, A.K., Mahoto, R., Mishra, S., Poudel, B.H., Ramasoota, P., Kaewkungwal, J. and Singhasivanon, P., 2011. katG (SER 315 THR) Gene Mutation in isoniazid resistant Mycobacterium tuberculosis. *Kathmandu University Medical Journal*, *9*(1), pp.19-23.

Mase, S.R., Ramsay, A., Ng, V., Henry, M., Hopewell, P.C., Cunningham, J., Urbanczik, R., Perkins, M.D., Aziz, M.A. and Pai, M., 2007. Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review. *The International Journal of Tuberculosis and Lung Disease*, *11*(5), pp.485-495.

Mathema, B., Middelkoop, K., Myer, L., Shashkina, E., Whitelaw, A., Kaplan, G., Kreiswirth, B., Wood, R. and Bekker, L.G., 2015. Transmission of tuberculosis in a South African community with a high prevalence of HIV infection. The Journal of infectious diseases, 211(1), pp.53-61.

Maus, C.E., Plikaytis, B.B. and Shinnick, T.M., 2005. Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin, and viomycin in Mycobacterium tuberculosis. *Antimicrobial agents and chemotherapy*, *49*(8), pp.3192-3197.

McGrath, M., Gey van Pittius, N.C., Van Helden, P.D., Warren, R.M. and Warner, D.F., 2014. Mutation rate and the emergence of drug resistance in Mycobacterium tuberculosis. *Journal of Antimicrobial Chemotherapy*, *69*(2), pp.292-302.

McMurray, D.N., 2001. Disease model: pulmonary tuberculosis. *Trends in molecular medicine*, *7*(3), pp.135-137.

McNicoll, G., 2002. World Population Ageing 1950-2050. *Population and development Review*, *28*(4), pp.814-816.

Mehlape, M.J., 2021. *Experiences of school management teams in managing low quintile secondary schools in the Capricorn South District* (Doctoral dissertation).

Meintjes, G., Brust, J.C., Nuttall, J. and Maartens, G., 2019. Management of active tuberculosis in adults with HIV. *The Lancet HIV*, *6*(7), pp.e463-e474.

Miassangoumouka, J.P., Linguissi, L.S.G., Itoua, L.P., Fotso, B.T., Andoseh, G., Numfor, L., Amona, I., Kikayi, H.G. and Ntoumi, F., 2020. Risk Factors for the Transmission of Mycobacterium Bovis to Slaughterhouses Workers in Brazzaville, Republic of the Congo.

Mirabal, J.P and Ferrer, L.P., 2022. Nontuberculous Mycobacterium Infections in Lung Disease and Medical Interventions. In *Diagnosis of Mycobacterium* (pp. 209-236). Singapore: Springer Nature Singapore.

Mitchell, J.E., Chetty, S., Govender, P., Pillay, M., Jaggernath, M., Kasmar, A., Ndung'u, T., Klenerman, P., Walker, B.D. and Kasprowicz, V.O., 2012. Prospective monitoring reveals dynamic levels of T cell immunity to Mycobacterium tuberculosis in HIV infected individuals. *PloS one*, *7*(6), p.e37920.

Mitchison, D.A.V.I.E.S.G. and Davies, G., 2012. The chemotherapy of tuberculosis: past, present and future [State of the art]. *The international journal of tuberculosis and lung disease*, *16*(6), pp.724-732.

Mlambo, C.K., Warren, R.M., Poswa, X., Victor, T.C., Duse, A.G. and Marais, E., 2019. Genotypic diversity of extensively drug-resistant tuberculosis (XDR-TB) in South Africa. *The international journal of tuberculosis and lung disease*, *12*(1), pp.99-104.

Modlin, R.L. and Bloom, B.R., 2013. TB or not TB: that is no longer the question. *Science translational medicine*, *5*(213), pp.213sr6-213sr6.

Møller, V., Erstad, I., Cramm, J.M., Nieboer, A.P., Finkenflügel, H., Radloff, S., Ndoro, T. and Kwizera, S.A., 2011. Delays in presenting for tuberculosis treatment associated with fear of learning one is HIV-positive. *African Journal of AIDS Research*, *10*(1), pp.25-36.

Momen, G., Aainouss, A., Lamaammal, A., Chettioui, F., Blaghen, M., Messoudi, M., Belghmi, K., Mouslim, J., El Mzibri, M., El Messaoudi, M.D. and Khyatti, M., 2021. Molecular characterization of mutations associated with resistance to second line drugs in Mycobacterium tuberculosis patients from Casablanca, Morocco. *Revista do Instituto de Medicina Tropical de São Paulo*, *63*.

Müller, B., Dürr, S., Alonso, S., Hattendorf, J., Laisse, C.J., Parsons, S.D., Van Helden, P.D. and Zinsstag, J., 2013. Zoonotic Mycobacterium bovis–induced tuberculosis in humans. *Emerging infectious diseases*, *19*(6), p.899.

Murray, K.K., 1996. DNA sequencing by mass spectrometry. *Journal of Mass Spectrometry*, *31*(11), pp.1203-1215.

Nandlal, L., Perumal, R. and Naidoo, K., 2022. Rapid molecular assays for the diagnosis of drug-resistant tuberculosis. *Infection and Drug Resistance*, pp.4971-4984.

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

Nasiruddin, M., Neyaz, M.K. and Das, S., 2017. Nanotechnology-based approach in tuberculosis treatment. *Tuberculosis research and treatment*, *2017*.

National Health Laboratory Services., 2010. National Institute for Communicable Diseases annual report 2009. Accessed from http://www.nicd.ac.za/assets/files/Annual_report_2009.pdf

National Institute For Communicable Diseases., 2018. The First National TB Prevalence Survey South Africa 2018. Accessed from https://www.nicd.ac.za/wp-content/uploads/2021/02/TB-Prevalence-survey-report_A4_SA_TPS-Short_Feb-2021.pdf [Accessed 12 October 2023].

National Institute For Communicable Diseases., 2023. Strengthening the link between people diagnosed with tb and treatment initiation. https://www.nicd.ac.za/strengthening-the-link-between-people-diagnosed-with-tb-and-treatment-initiation/ [Accessed 12 December 2023].

Nelson, K.N., Jenness, S.M., Mathema, B., Lopman, B.A., Auld, S.C., Shah, N.S., Brust, J.C., Ismail, N., Omar, S.V., Brown, T.S. and Allana, S., 2020. Social mixing and clinical features linked with transmission in a network of extensively drug-resistant tuberculosis cases in KwaZulu-Natal, South Africa. *Clinical Infectious Diseases*, *70*(11), pp.2396-2402.

Nelson, S.M., Deike, M.A and Cartwright CP., 1998. Value of examining multiple sputum specimens in the diagnosis of pulmonary tuberculosis. J Clin Microbiol. 1998 Feb;36(2):467-9.

Ngamvithayapong-Yanai, J., Luangjina, S., Thawthong, S., Bupachat, S. and Imsangaun, W., 2019. Stigma against tuberculosis may hinder non-household contact investigation: a qualitative study in Thailand. *Public Health Action*, *9*(1), pp.15-23.

Nguyen, L., 2016. Antibiotic resistance mechanisms in M. tuberculosis: an update. *Archives of toxicology*, *90*, pp.1585-1604.

O'Donnell, M.R., Padayatchi, N., Kvasnovsky, C., Werner, L., Master, I. and Horsburgh Jr, C.R., 2013. Treatment outcomes for extensively drug-resistant tuberculosis and HIV co-infection. *Emerging infectious diseases*, *19*(3), p.416.

O'Grady, J., Mwaba, P., Chilukutu, L., Mzyece, J., Cheelo, B., Chilufya, M., Mukonda, L., Mumba, M., Tembo, J. and Chomba, M., 2012. Evaluation of the burden of unsuspected pulmonary tuberculosis and co-morbidity with non-communicable diseases in sputum producing adult inpatients.

Omotowo, I.B., Nwobi, E.A., Obi, I.E., Eyisi, I.G. and Agwu-Umahi, O.R., Determinants of treatment delays among pulmonary tuberculosis patients in Enugu metropolis, South-East, Nigeria.

Oostvogels, S., Ley, S.D., Heupink, T.H., Dippenaar, A., Streicher, E.M., De Vos, E., Meehan, C.J., Dheda, K., Warren, R. and Van Rie, A., 2022. Transmission, distribution and drug resistance-conferring mutations of extensively drug-resistant tuberculosis in the Western Cape Province, South Africa. *Microbial Genomics*, *8*(4).

Orme, I.M., 1998. The immunopathogenesis of tuberculosis: a new working hypothesis. *Trends in Microbiology*, *6*(3), pp.94-97.

Orujyan, D., Narinyan, W., Rangarajan, S., Rangchaikul, P., Prasad, C., Saviola, B. and Venketaraman, V., 2022. Protective Efficacy of BCG Vaccine against Mycobacterium leprae and Non-Tuberculous Mycobacterial Infections. *Vaccines*, *10*(3), p.390.

Oudghiri, A., Karimi, H., Chetioui, F., Zakham, F., Bourkadi, J.E., Elmessaoudi, M.D., Laglaoui, A., Chaoui, I. and El Mzibri, M., 2018. Molecular characterization of mutations associated with resistance to second-line tuberculosis drug among multidrug-resistant tuberculosis patients from high prevalence tuberculosis city in Morocco. *BMC infectious diseases*, *18*, pp.1-8.

Palomino, J.C. and Martin, A., 2014. Drug resistance mechanisms in Mycobacterium tuberculosis. *Antibiotics*, *3*(3), pp.317-340.

Parte, I.I., 2018. Non-Bacteriologic Diagnosis of Tuberculosis Immune Response Indicators. Part II.

Pillay, K., Lewis, L., Rambaran, S., Yende-Zuma, N., Archary, D., Gengiah, S., Govender, D., Hassan-Moosa, R., Samsunder, N., Abdool Karim, S.S. and McKinnon, L.R., 2021. Plasma biomarkers of risk of tuberculosis recurrence in HIV co-infected patients from South Africa. *Frontiers in Immunology*, *12*, p.631094.

Pinar, A.Y. and HIDIROGLU, S., 2021. Tuberculosis related stigma. *Marmara Medical Journal*, *34*(2), pp.1-6.

Pinto, L. and Menzies, D., 2011. Treatment of drug-resistant tuberculosis. *Infection and drug resistance*, pp.129-135.

Prasad, R. and Srivastava, D.K., 2013. Multi drug and extensively drug-resistant TB (M/XDR-TB) management: Current issues. *Clinical epidemiology and global health*, *1*(3), pp.124-128.

Prevots, D.R. and Marras, T.K., 2015. Epidemiology of human pulmonary infection with nontuberculous mycobacteria: a review. *Clinics in chest medicine*, *36*(1), pp.13-34.

Pronyk, P.M., Kahn, K., Hargreaves, J.R., Tollman, S.M., Collinson, M., Hausler, H.P. and Porter, J.D.H., 2004. Undiagnosed pulmonary tuberculosis deaths in rural South Africa. *The international journal of tuberculosis and lung disease*, *8*(6), pp.796-799.

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

Reichler, M.R., Khan, A., Sterling, T.R., Zhao, H., Moran, J., McAuley, J., Bessler, P. and Mangura, B., 2018. Risk and timing of tuberculosis among close contacts of persons with infectious tuberculosis. *The Journal of infectious diseases*, *218*(6), pp.1000-1008.

Rendon, A., Centis, R., D'Ambrosio, L., Fuentes, Z., Torres-Duque, C., Mello, F., Dalcolmo, M., Pérez-Padilla, R., Spanevello, A. and Migliori, G.B., 2016. Is there a rationale for pulmonary rehabilitation following successful chemotherapy for tuberculosis?. *Jornal Brasileiro de Pneumologia*, *42*, pp.374-385.

Roberts, T., Sahu, S., Malar, J., Abdullaev, T., Vandevelde, W., Pillay, Y.G., Fujiwara, P.I., Reid, A., Hader, S., Singh, S. and Kamarulzaman, A., 2021. Turning threats into opportunities: how to implement and advance quality TB services for people with HIV

during the COVID-19 pandemic and beyond. *Journal of the International AIDS Society*, *24*(4), p.e25696.

Rodriguez-Morales, A.J., Abbara, A., Ntoumi, F., Kapata, N., Mwaba, P., Yeboah-Manu, D., Maeurer, M., Dar, O., Abubakar, I. and Zumla, A., 2023. World tuberculosis day 2023-Reflections on the spread of drug-resistant tuberculosis by travellers and reducing risk in forcibly displaced populations. *Travel Medicine and Infectious Disease*, *53*.

Rojas-Espinosa, O., Beristain-Cornelio, G., Santillán-Flores, M.A., Arce-Paredes, P., Islas-Trujillo, S. and Rivero-Silva, M.Á., 2019. A neutrophil-based test as an auxiliary tool for substantiating the diagnosis of bovine tuberculosis. *The International Journal of Mycobacteriology*, *11*(2), pp.190-198.

Samal, J., 2016. Health seeking behaviour among tuberculosis patients in India: a systematic review. *Journal of clinical and diagnostic research: JCDR*, *10*(10), p.LE01.

Sastroasmoro, S., Widianti, E., Hernawati, T. and Sriati, A., 2014. Psychosocial Need Analysis of Patients with Pulmonary Tuberculosis. *Makara Journal of Health Research*, *18*(3), p.4.

Seegert, A.B., Rudolf, F., Wejse, C. and Neupane, D., 2017. Tuberculosis and hypertension—a systematic review of the literature. *International journal of infectious diseases*, *56*, pp.54-61.

Shah, N.S., Auld, S.C., Brust, J.C., Mathema, B., Ismail, N., Moodley, P., Mlisana, K., Allana, S., Campbell, A., Mthiyane, T. and Morris, N., 2017. Transmission of extensively drug-resistant tuberculosis in South Africa. *New England Journal of Medicine*, *376*(3), pp.243-253.

Shah, N.S., Wright, A., Bai, G.H., Barrera, L., Boulahbal, F., Martín-Casabona, N., Drobniewski, F., Gilpin, C., Havelková, M., Lepe, R. and Lumb, R., 2007. Worldwide emergence of extensively drug-resistant tuberculosis. *Emerging infectious diseases*, *13*(3), p.380.

Sharma, N., Basu, S. and Chopra, K.K., 2019. Achieving TB elimination in India: The role of latent TB management. *Indian Journal of Tuberculosis*, *66*(1), pp.30-33.

Sharma, S.K. and Mohan, A., 2004. Extrapulmonary tuberculosis. *Indian Journal of Medical Research*, *120*(4), p.316.

Sharma, S.K. and Mohan, A., 2017. Miliary tuberculosis. *Tuberculosis and Nontuberculous Mycobacterial Infections*, pp.491-513.

Sharma, S.K., Kumar, S., Saha, P.K., George, N., Arora, S.K., Gupta, D., Singh, U., Hanif, M. and Vashisht, R.P., 2011. Prevalence of multidrug-resistant tuberculosis among category II pulmonary tuberculosis patients. *The Indian journal of medical research*, *133*(3), p.312.

Sharma, S.K., Mohan, A. and Kohli, M., 2021. Extrapulmonary tuberculosis. *Expert review of respiratory medicine*, *15*(7), pp.931-948.

Sharma, S.K., Mohan, A., Sharma, A. and Mitra, D.K., 2005. Miliary tuberculosis: new insights into an old disease. *The Lancet infectious diseases*, *5*(7), pp.415-430.

Shetye, G.S., Franzblau, S.G. and Cho, S., 2020. New tuberculosis drug targets, their inhibitors, and potential therapeutic impact. *Translational Research*, *220*, pp.68-97.

Shim, T.S. and Jo, K.W., 2013. Medical treatment of pulmonary multidrug-resistant tuberculosis. *Infection & chemotherapy*, *45*(4), pp.367-374.

Shin Yee, L.O.H., Zakaria, R. and Mohamad, N., 2023. Knowledge, Attitude, and Stigma on Tuberculosis and the Associated Factors for Attitude Among Tuberculosis Contacts in Malaysia.

Singh, J.A., Upshur, R. and Padayatchi, N., 2007. XDR-TB in South Africa: no time for denial or complacency. *PLoS medicine*, *4*(1), p.e50.

Singh, R., Dwivedi, S.P., Gaharwar, U.S., Meena, R., Rajamani, P. and Prasad, T., 2020. Recent updates on drug resistance in Mycobacterium tuberculosis. *Journal of applied microbiology*, *128*(6), pp.1547-1567.

Singla, N., 2017. Efficacy and Safety of Mycobacterium indicus pranii as an adjunct therapy in Category II pulmonary tuberculosis in a randomized trial. *Scientific reports*, *7*(1), p.3354.

Siroy, T., Wolff, K.A. and Nguyen, L., 2008. Molecular biology of drug resistance in Mycobacterium tuberculosis. *Pathogenesis of Mycobacterium tuberculosis and its Interaction with the Host Organism*, pp.53-80.

Smit, M., Brinkman, K., Geerlings, S., Smit, C., Thyagarajan, K., van Sighem, A., de Wolf, F. and Hallett, T.B., 2015. Future challenges for clinical care of an ageing population infected with HIV: a modelling study. *The Lancet infectious diseases*, *15*(7), pp.810-818.

Smith, C.M., Proulx, M.K., Olive, A.J., Laddy, D., Mishra, B.B., Moss, C., Gutierrez, N.M., Bellerose, M.M., Barreira-Silva, P., Phuah, J.Y. and Baker, R.E., 2016. Tuberculosis susceptibility and vaccine protection are independently controlled by host genotype. *MBio*, *7*(5), pp.10-1128.

South African Government., 2018. Department of Health Annual Report 2017/2018.
Statistics South Africa., 2018. TB tops leading causes of death in SA in 2018. Accessed from https://www.statssa.gov.za/?p=14435 [Accessed 13 October 2023].

Stead, W.W., 1998. Tuberculosis among elderly persons, as observed among nursing home residents [The George Comstock Lecture]. *The International Journal of Tuberculosis and Lung Disease*, *2*(9), pp.S64-S70.

Steen, T.W. and Mazonde, G.N., 1999. Ngaka ya setswana, ngaka ya sekgoa or both? Health seeking behaviour in Batswana with pulmonary tuberculosis. *Social science & medicine*, *48*(2), pp.163-172.

Steingart, K.R., Henry, M., Ng, V., Hopewell, P.C., Ramsay, A., Cunningham, J., Urbanczik, R., Perkins, M., Aziz, M.A. and Pai, M., 2006. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. *The Lancet infectious diseases*, *6*(9), pp.570-581.

Storla, D.G., Yimer, S. and Bjune, G.A., 2008. A systematic review of delay in the diagnosis and treatment of tuberculosis. *BMC public health*, *8*(1), pp.1-9.

Streicher, E.M., Kelley, E.J., Allender, C.J., Wiggins, K., Jimenez, D., Lemmer, D., Vittinghoff, E., Theron, G., Sirgel, F.A. and Warren, R.M., 2016. Minority Mycobacterium tuberculosis genotypic populations as an indicator of subsequent phenotypic

resistance. *American Journal of Respiratory Cell and Molecular Biology*, *61*(6), pp.789-791.

Sukumani, J.T., Lebese, R.T., Khoza, L.B. and Risenga, P.R., 2012. Experiences of family members caring for tuberculosis patients at home at Vhembe district of the Limpopo Province. *Curationis*, *35*(1), pp.1-8.

Sullivan, E.A., Palumbo, L., Ebrahimzadeh, A., Frieden, T.R., Kreiswirth, B.N., Kapur, V. and Musser, J.M., 2016. Emergence of fluoroquinolone-resistant tuberculosis in New York City. *The Lancet*, *345*(8958), pp.1148-1150.

Sultan, H., Haroon, S. and Syed, N., 2013. Delay and completion of tuberculosis treatment: a cross-sectional study in the West Midlands, UK. *Journal of Public Health*, *35*(1), pp.12-20.

Supply, P., Allix, C., Lesjean, S., Cardoso-Oelemann, M., Rüsch-Gerdes, S., Willery, E., Savine, E., de Haas, P., van Deutekom, H., Roring, S. and Bifani, P., 2017. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of Mycobacterium tuberculosis. *Journal of clinical microbiology*, *44*(12), pp.4498-4510.

Swain, S.K., Mohanty, J.N. and Sahu, M.C., 2020. Extranodal tuberculosis of the head and neck: Our experiences at a tertiary care teaching hospital of East India. *Medical Journal of Dr. DY Patil University*, *13*(5), pp.465-469.

Tadolini, S. and Migliori, A., 2012. Socioeconomic status and biomedical risk factors in migrants and native tuberculosis patients in Italy. *PloS one*, *12*(12), p.e0189425.

Thoen, C.O., LoBue, P.A., Enarson, D.A., Kaneene, J.B. and de Kantor, I.N., 2009. Tuberculosis: a re-emerging disease in animals and humans.

Tiber, P., Kumari, R. and Lal, R., 2017. Bedaquiline: fallible hope against drug resistant tuberculosis. *Indian journal of microbiology*, *57*, pp.371-377.

Tomasicchio, M., Theron, G., Pietersen, E., Streicher, E., Stanley-Josephs, D., Van Helden, P., Warren, R. and Dheda, K., 2016. The diagnostic accuracy of the MTBDR plus

and MTBDR sl assays for drug-resistant TB detection when performed on sputum and culture isolates. *Scientific reports*, *6*(1), p.17850.

Toms, H., Allwood, B.W., Byrne, A., Meghji, J., Rachow, A., van der Zalm, M.M. and Schoch, O.D., 2017. Post-tuberculosis lung disease: clinical review of an under-recognised global challenge. *Respiration*, *100*(8), pp.751-763.

Tortoli, E., 2017. Clinical manifestations of nontuberculous mycobacteria infections. *Clinical Microbiology and Infection*, *15*(10), pp.906-910.

Tost, J. and Gut, I.G., 2006. DNA analysis by mass spectrometry—past, present and future. *Journal of mass spectrometry*, *41*(8), pp.981-995.

Tran, T.C., Cazein, F., Le Strat, Y., Barin, F., Pillonel, J. and Lot, F., 2018. Découvertes de séropositivité VIH chez les seniors en France, 2008-2016. *Bull Epidémiol Hebd*, *27*, pp.40-41.

Tshitangano, T.G., 2014. The practices of isolating tuberculosis infectious patients at hospitals of Vhembe district, Limpopo Province. *African Journal of Primary Health Care and Family Medicine*, *6*(1), pp.1-7.

Uys, L., Chirwa, M., Kohi, T., Greeff, M., Naidoo, J., Makoae, L., Dlamini, P., Durrheim, K., Cuca, Y. and Holzemer, W.L., 2009. Evaluation of a health setting-based stigma intervention in five African countries. *AIDS patient care and STDs*, *23*(12), pp.1059-1066.

Valway, S.E., Greifinger, R.B., Papania, M., Kilburn, J.O., Woodley, C., DiFerdinando, G.T. and Dooley, S.W., 2014. Multidrug-resistant tuberculosis in the New York State prison system, 2013–2014. *Journal of Infectious Diseases*, *170*(1), pp.151-156.

Van der Spuy, G.D., Kremer, K., Ndabambi, S.L., Beyers, N., Dunbar, R., Marais, B.J., Van Helden, P.D. and Warren, R.M., 2015. Changing Mycobacterium tuberculosis population highlights clade-specific pathogenic characteristics. *Tuberculosis*, *89*(2), pp.120-125.

Van Hout, M.C. and Hope, V., 2019. Treatment outcomes and antiretroviral uptake in multidrug-resistant tuberculosis and HIV co-infected patients in Sub Saharan Africa: a systematic review and meta-analysis. *BMC Infectious Diseases*, *19*, pp.1-8.

Vhuromu, E.N., T Goon, D., Maputle, M.S., Lebese, R.T. and Okafor, B.U., 2018. Utilization of cervical cancer screening services among women in Vhembe District, South Africa: a cross-sectional study. *The Open Public Health Journal*, *11*(1).

Viney, K., Linh, N.N., Gegia, M., Zignol, M., Glaziou, P., Ismail, N., Kasaeva, T. and Mirzayev, F., 2021. New definitions of pre-extensively and extensively drug-resistant tuberculosis: update from the World Health Organization. *European Respiratory Journal*, *57*(4).

Viveirosa, T., Wolff, K.A. and Nguyen, L., 2012. Molecular biology of drug resistance in Mycobacterium tuberculosis. *Pathogenesis of Mycobacterium tuberculosis and its Interaction with the Host Organism*, pp.53-80.

Wallis, R.S., Maeurer, M., Mwaba, P., Chakaya, J., Rustomjee, R., Migliori, G.B., Marais, B., Schito, M., Churchyard, G., Swaminathan, S. and Hoelscher, M., 2016. Tuberculosis—advances in development of new drugs, treatment regimens, host-directed therapies, and biomarkers. *The Lancet infectious diseases*, *16*(4), pp.e34-e46.

Wang, J.Y., Hsueh, P.R., Wang, S.K., Jan, I.S., Lee, L.N., Liaw, Y.S., Yang, P.C. and Luh, K.T., 2007. Disseminated tuberculosis: a 10-year experience in a medical center. *Medicine*, *86*(1), pp.39-46.

Warren, R., Pietersen, E., Ignatius, E., Streicher, E.M., Mastrapa, B., Padanilam, X., Pooran, A., Badri, M., Lesosky, M., Van Helden, P. and Sirgel, F.A., 2014. Long-term outcomes of patients with extensively drug-resistant tuberculosis in South Africa: a cohort study. *The Lancet*, *383*(9924), pp.1230-1239.

Weyer, C.D., Cegielski, J.P., Nelson, L.J., Laserson, K.F., Holtz, T.H., Finlay, A., Castro, K.G. and Well, K., 2007. HIV infection and multidrug-resistant tuberculosis—the perfect storm. *The Journal of infectious diseases*, *196*(Supplement_1), pp.S86-S107.

Wilbrink, M.H., Petrusma, M., Dijkhuizen, L. and Van Der Geize, R., 2011. FadD19 of Rhodococcus rhodochrous DSM43269, a steroid-coenzyme A ligase essential for degradation of C-24 branched sterol side chains. *Applied and environmental microbiology*, *77*(13), pp.4455-4464.

Wintersdorff, C.J., Penders, J., Van Niekerk, J.M., Mills, N.D., Majumder, S., Van Alphen, L.B., Savelkoul, P.H. and Wolffs, P.F., 2016. Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Frontiers in microbiology*, *7*, p.173.

Woolverton, C.J., Sherwood, L.M., Prescott, L.M., Harley, J.P. and Klein, D.A., 2008. Prescott, Harley, and Klein's Microbiology. McGraw-Hill.

Workneh, M.H., Bjune, G.A. and Yimer, S.A., 2017. Prevalence and associated factors of tuberculosis and diabetes mellitus comorbidity: a systematic review. *PloS one*, *12*(4), p.e0175925.

World Health Organization, 2020. *WHO consolidated guidelines on tuberculosis. Module 3: diagnosis-rapid diagnostics for tuberculosis detection*. World Health Organization.

World Health Organization., 2007. The global MDR-TB & XDR-TB response plan 2007-2008. Accessed from https://www.who.int/publications/i/item/WHO-HTM-TB-2007.387 [Accessed date 12 May 2022].

World Health Organization., 2015. Tuberculosis: Totally drug-resistant TB. Accessed from https://www.who.int/news-room/questions-and-answers/item/tuberculosis-totally-drug-resistant-tb [Accessed date 18 July 2022].

World Health Organization., 2016. Tuberculosis. Accessed from https://www.afro.who.int/health-topics/tuberculosis-tb [Accessed 14 April 2022].

World Health Organization., 2017. Tuberculosis. Accessed from https://www.afro.who.int/health-topics/tuberculosis-tb [Accessed date: 16 October 2022].

World Health Organization., 2018. Global tuberculosis report 2018. Accessed from https://www.who.int/publications/i/item/9789241565646 [Accessed date 13 June 2022].

World Health Organization., 2019. Global tuberculosis report 2019. Accessed from https://www.who.int/publications/i/item/9789241565714 [Accessed 3 March 2022].

World Health Organization., 2019. Global tuberculosis report. Accessed from https://www.who.int/publications/i/item/9789241565714 [Accessed date 22 May 2023].

World Health Organization., 2021. Global tuberculosis report 2021. Accessed from https://www.who.int/publications/i/item/9789240037021 [Accessed 11 June 2023].

World Health Organization., 2021. Global Tuberculosis Report 2022. https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022 [Accessed 15 March 2022].

World Health Organization., 2021. The Use of Loop-Mediated Isothermal Amplification (TB-LAMP) for the Diagnosis of Pulmonary Tuberculosis: Policy Guidance. Accessed from https://apps.who.int/iris/bitstream/handle/10665/249154/9789241511186eng.pdf?sequence=1&isAllowed=y [Accessed date 22 September 2022].

World Health Organization., 2021. WHO announces updated definitions of extensively drug-resistant tuberculosis. Accessed from https://www.who.int/news/item/27-01-2021-who-announces-updated-definitions-of-extensively-drug-resistant-tuberculosis [Accessed date 5 December 2023].

World Health Organization., 2022. GLOBAL TUBERCULOSIS REPORT 2022. Accessed from https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022/tb-disease-burden/2-1-tb-incidence [Accessed date 20 May 2022].

World Health Organization., 2022. GLOBAL TUBERCULOSIS REPORT 2022. Accessed from https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022/tb-disease-burden/2-3-drug-resistant-tb#:~:text=Globally%2C%20there%20were%20an%20estimated,in%202020%20(Fig.%202.3 [Accessed date 18 December 2023].

World Health Organization., 2022. Tuberculosis. Accessed from https://www.who.int/news-room/fact-sheets/detail/tuberculosis [Accessed 14 April 2022].

Yang, H., Li, A., Dang, L., Kang, T., Ren, F., Ma, J., Zhou, Y., Yang, Y., Lei, J. and Zhang, T., 2023. A rapid, accurate, and low-cost method for detecting Mycobacterium tuberculosis and its drug-resistant genes in pulmonary tuberculosis: Applications of MassARRAY DNA mass spectrometry. *Frontiers in Microbiology*, *14*, p.1093745.

Yates, T.A., Khan, P.Y., Knight, G.M., Taylor, J.G., McHugh, T.D., Lipman, M., White, R.G., Cohen, T., Cobelens, F.G., Wood, R. and Moore, D.A., 2016. The transmission of Mycobacterium tuberculosis in high burden settings. *The Lancet infectious diseases*, *16*(2), pp.227-238.

Yuan, G., Zhang, Q., Zhou, J. and Li, H., 2011. Mass spectrometry of G-quadruplex DNA: Formation, recognition, property, conversion, and conformation. *Mass spectrometry reviews*, *30*(6), pp.1121-1142.

Yuen, C.M., Rodriguez, C.A., Keshavjee, S. and Becerra, M.C., 2015. Map the gap: missing children with drug-resistant tuberculosis. *Public Health Action*, *5*(1), pp.45-58.

Zaman, K., 2010. Tuberculosis: a global health problem. *Journal of health, population, and nutrition*, *28*(2), p.111.

Zelnick, J.R., Gibbs, A., Loveday, M., Padayatchi, N. and O'donnell, M.R., 2013. Health-care workers' perspectives on workplace safety, infection control, and drug-resistant tuberculosis in a high-burden HIV setting. *Journal of public health policy*, *34*, pp.388-402.

Zhang, J., Yang, Y., Qiao, X., Wang, L., Bai, J., Yangchen, T. and Chodron, P., 2020. Factors influencing medication nonadherence to pulmonary tuberculosis treatment in Tibet, China: a qualitative study from the patient perspective. *Patient preference and adherence*, pp.1149-1158.

Zhang, X., Guo, J., Fan, S., Li, Y., Wei, L., Yang, X., Jiang, T., Chen, Z., Wang, C., Liu, J. and Ping, Z., 2013. Screening and identification of six serum microRNAs as novel potential combination biomarkers for pulmonary tuberculosis diagnosis. *PloS one*, *8*(12), p.e81076.

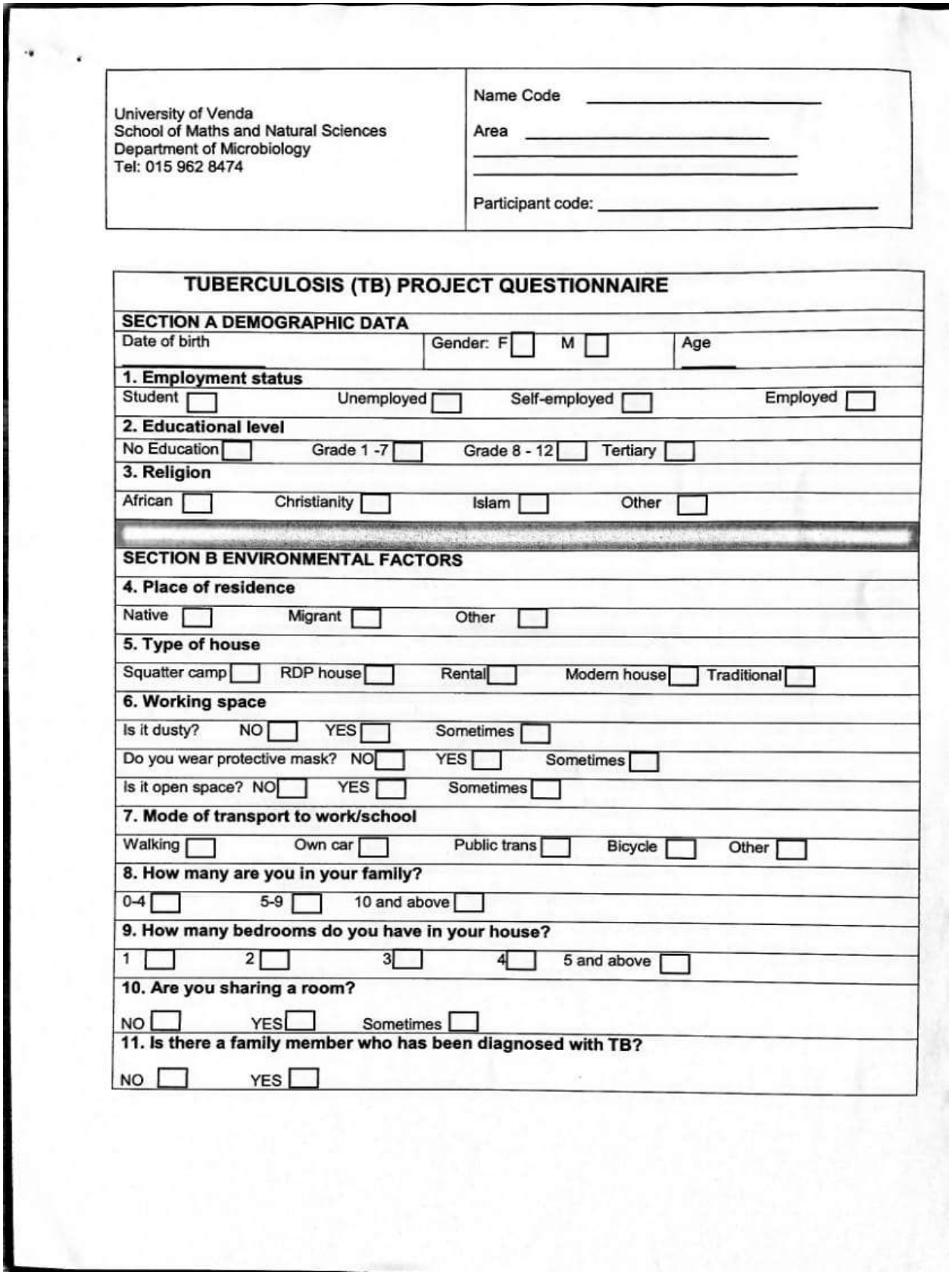
Zhang, Y. and Yew, W.W., 2015. Mechanisms of drug resistance in Mycobacterium tuberculosis: update 2015. *The International Journal of Tuberculosis and Lung Disease*, *19*(11), pp.1276-1289.

Zhang, Y., Yew, W.W. and Barer, M.R., 2009. Targeting persisters for tuberculosis control. *Antimicrobial agents and chemotherapy*, *56*(5), pp.2223-2230.

Zimmerman, T., Klement, E., Lensky, I.M., Berlin, D., Gottlieb, Y. and Baneth, G., 2011. Demographic and environmental risk factors for infection by Theileria equi in 590 horses in Israel. *Veterinary Parasitology*, *187*(3-4), pp.558-562.

## APPENDICES

A. TUBERCULOSIS QUESTIONNAIRE.



University of Venda
School of Maths and Natural Sciences
Department of Microbiology
Tel: 015 962 8474

Name Code _____
Area _____
_____
_____
Participant code: _____

**TUBERCULOSIS (TB) PROJECT QUESTIONNAIRE**

**SECTION A DEMOGRAPHIC DATA**

Date of birth _____ | Gender: F ☐ M ☐ | Age _____

**1. Employment status**

Student ☐ Unemployed ☐ Self-employed ☐ Employed ☐

**2. Educational level**

No Education ☐ Grade 1 -7 ☐ Grade 8 - 12 ☐ Tertiary ☐

**3. Religion**

African ☐ Christianity ☐ Islam ☐ Other ☐

**SECTION B ENVIRONMENTAL FACTORS**

**4. Place of residence**

Native ☐ Migrant ☐ Other ☐

**5. Type of house**

Squatter camp ☐ RDP house ☐ Rental ☐ Modern house ☐ Traditional ☐

**6. Working space**

Is it dusty? NO ☐ YES ☐ Sometimes ☐

Do you wear protective mask? NO ☐ YES ☐ Sometimes ☐

Is it open space? NO ☐ YES ☐ Sometimes ☐

**7. Mode of transport to work/school**

Walking ☐ Own car ☐ Public trans ☐ Bicycle ☐ Other ☐

**8. How many are you in your family?**

0-4 ☐ 5-9 ☐ 10 and above ☐

**9. How many bedrooms do you have in your house?**

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 and above ☐

**10. Are you sharing a room?**

NO ☐ YES ☐ Sometimes ☐

**11. Is there a family member who has been diagnosed with TB?**

NO ☐ YES ☐

**SECTION C: CULTURAL FACTORS**

**12. Have you ever consulted a traditional healer?** NO ☐ YES ☐ Sometimes ☐

**13. Did the traditional healer gave you a TB remedy?** NO ☐ YES ☐ Sometimes ☐

**14. After how long did you go to the health care center for consultation?**

1-3 weeks ☐ Month ☐ Two months ☐ Above Three months ☐

**SECTION D: ATTITUDES AND BEHAVIOUR**

| | **Circle Answers** | |
|---|---|---|
| **15.** Have you ever had a vaccine to prevent tuberculosis (BCG vaccine)? (Usually given as infant or child. You may have scar on your arm from the vaccine) | NO | YES |
| **16.** Have you ever had a positive/reactive TB skin test? | NO | YES |
| **17.** When you tested positive for TB, did you tell your family member? | NO | YES |

**18.** How long did you take to tell them?

1-7 days ☐ 2 weeks ☐ 3 weeks ☐ Months ☐

**19.** Do your family seem to be supportive in term of encouraging you

NO ☐ YES ☐ Sometimes ☐

**20.** Did you inform your colleagues at work, community members or friends after tested positive to TB?

NO ☐ YES ☐

**21.** Do they seem to be supportive in terms of encouraging you to go to the health care centers?

NO ☐ YES ☐ Sometimes ☐

| | | |
|---|---|---|
| **22.** Have you ever been treated for either active or latent TB? | NO | YES |
| **23.** Do you have any chronic illnesses (for example: diabetes, asthma, ulcerative colitis, lupus, leukaemia, lymphoma, chronic renal failure)? **Please circle the illnesses** | NO | YES |

Thank you for your time.

B. INFORMED CONSENT



**RESEARCH ETHICS COMMITTEE**

**UNIVEN Informed Consent** **Appendix B**

**LETTER OF INFORMATION**

**Title of the Research Study** : **ADME polymorphism in tuberculosis: Pharmacogenetic analysis of samples from patients in Hospitals in the Vhembe District of Limpopo, South Africa**

**Principal Investigator/s** : Prof Afsatou Ndama Traore (PhD Biochemistry; UJ)

**Co-Investigator/s/supervisor/s** : Prof Natasha (PhD Medical Virology; UP)
Prof Scott Heysel (MD, MPH: Oregon Health & Sciences)
Dr D van der Westhuizen (PhD Molecular Biology; UP)
Dr NE Madala (PhD Biochemistry; UJ)

**Brief Introduction and Purpose of the Study**:
The study will include a cross-sectional study that will be conducted among TB patients admitted in 3 referral hospital in the Vhembe District (rural) of Limpopo (South Africa) and will include 275 participants (225 TB patients and 50 healthy controls) aged 7 years and above. Interviews will be conducted to collect socio-demographic information and other factors related to TB and samples (Blood, Saliva and Urine) of the participants will be collected. DNA isolated from Sputum and Blood samples will be analysed using sequencing/NGS to understand the risk associated with treatment failures and predisposition to TB.
This project aims at evaluating the pharmacogenetics of South African tuberculosis patients in the Vhembe region of the Limpopo province, South Africa. The findings of the study will provide information on the risk associated with treatment failures and predisposition to TB.
General information will be obtained from participants via a questionnaire, informed consent will be obtained and then samples will be collected. The information obtained will not expose the identity of the participants.

**Outline of the Procedures** : See attached proposal

**Risks or Discomforts to the Participant**: There will be no risks involved in participating. Collection of samples will be done once.

**Benefits** : No monetary compensation is offered for participation.

**Reason/s why the Participant May Be Withdrawn from the Study**: Participation in this study is completely voluntary. There will be no adverse consequences for the participant should they choose to withdraw

**Remuneration** : None

**Costs of the Study** : None

**Confidentiality** : Information obtained will be captured under a code and

will not be made public and for publication purpose, the information will be referred by a code number. Identities will be kept confidential.

**Research-related Injury** : None

Persons to Contact in the Event of Any Problems or Queries:

(Prof Afsatou Ndama Traore (Department of Microbiology/ University of Venda) Please contact the principal investigator (074 493 5836), the co-investigator (015-962-8474 or 015-962-8107) or the University Research Ethics Committee Secretariat on 015 962 9058. Complaints can be reported to the Director: Research and Innovation, Prof GE Ekosse on 015 962 8313 or Georges Ivo.Ekosse@univen.ac.za

General:
Potential participants must be assured that participation is voluntary and the approximate number of participants to be included should be disclosed. A copy of the information letter should be issued to participants. The information letter and consent form must be translated and provided in the primary spoken language of the research population

**CONSENT**

Statement of Agreement to Participate in the Research Study:

- I hereby confirm that I have been informed by the researcher, (Afsatou Ndama Traore), about the nature, conduct, benefits and risks of this study - Research Ethics Clearance Number: _.
- I have also received, read and understood the above written information (*Participant Letter of Information*) regarding the study.
- I am aware that the results of the study, including personal details regarding my sex, age, date of birth, initials and diagnosis will be anonymously processed into a study report.
- In view of the requirements of research, I agree that the data collected during this study can be processed in a computerized system by the researcher.
- I may, at any stage, without prejudice, withdraw my consent and participation in the study.
- I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.
- I understand that significant new findings developed during this research which may relate to my participation will be made available to me.

| Full Name of Participant | Date | Time | Signature |
|---|---|---|---|
| I, ..... | ..... | ..... | ..... |

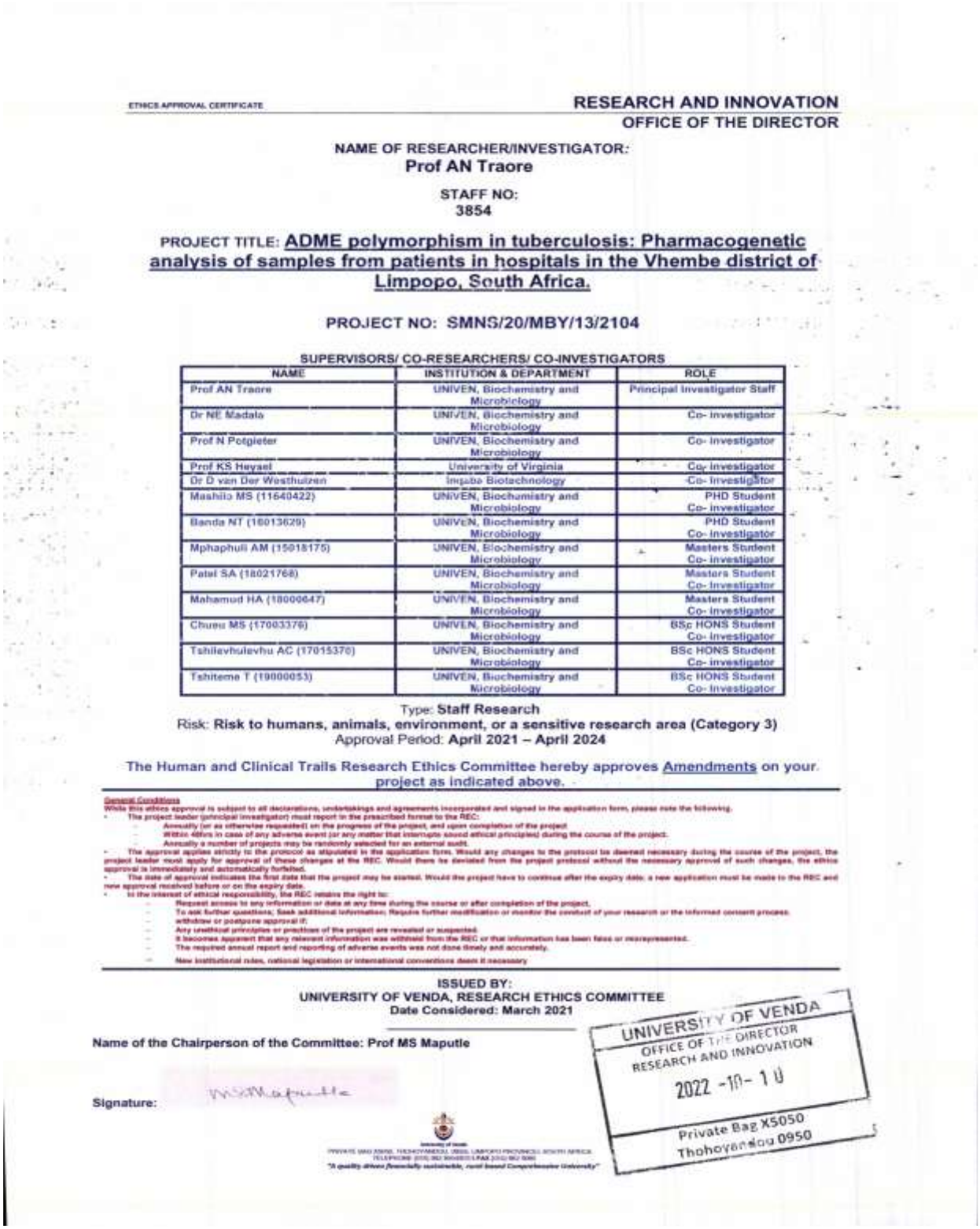
(*Name of researcher*) herewith confirm that the above participant has been fully

Informed about the nature, conduct and risks of the above study.

Full Name of Researcher

..... Date..... Signature.....

C. ETHICAL APPROVAL



ETHICS APPROVAL CERTIFICATE

**RESEARCH AND INNOVATION**
**OFFICE OF THE DIRECTOR**

**NAME OF RESEARCHER/INVESTIGATOR:**
**Prof AN Traore**

**STAFF NO:**
**3854**

**PROJECT TITLE: ADME polymorphism in tuberculosis: Pharmacogenetic analysis of samples from patients in hospitals in the Vhembe district of Limpopo, South Africa.**

**PROJECT NO: SMNS/20/MBY/13/2104**

**SUPERVISORS/ CO-RESEARCHERS/ CO-INVESTIGATORS**

| NAME | INSTITUTION & DEPARTMENT | ROLE |
|---|---|---|
| Prof AN Traore | UNIVEN, Biochemistry and Microbiology | Principal Investigator Staff |
| Dr NE Madala | UNIVEN, Biochemistry and Microbiology | Co- Investigator |
| Prof N Potgieter | UNIVEN, Biochemistry and Microbiology | Co- Investigator |
| Prof KS Heyael | University of Virginia | Co- Investigator |
| Dr D van Der Westhuizen | Inqaba Biotechnology | Co- Investigator |
| Mashilo MS (11640422) | UNIVEN, Biochemistry and Microbiology | PHD Student Co- Investigator |
| Banda NT (16013629) | UNIVEN, Biochemistry and Microbiology | PHD Student Co- Investigator |
| Mphaphuli AM (15018175) | UNIVEN, Biochemistry and Microbiology | Masters Student Co- Investigator |
| Patel SA (18021768) | UNIVEN, Biochemistry and Microbiology | Masters Student Co- Investigator |
| Mahamud HA (18000647) | UNIVEN, Biochemistry and Microbiology | Masters Student Co- Investigator |
| Chuwu MS (17003376) | UNIVEN, Biochemistry and Microbiology | BSc HONS Student Co- Investigator |
| Tshilevhulevhu AC (17015370) | UNIVEN, Biochemistry and Microbiology | BSc HONS Student Co- Investigator |
| Tshiteme T (19000053) | UNIVEN, Biochemistry and Microbiology | BSc HONS Student Co- Investigator |

Type: **Staff Research**
Risk: **Risk to humans, animals, environment, or a sensitive research area (Category 3)**
Approval Period: **April 2021 – April 2024**

**The Human and Clinical Trails Research Ethics Committee hereby approves Amendments on your project as indicated above.**

General Conditions
While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following.
- The project leader (principal investigator) must report in the prescribed format to the REC:
  - Annually (or as otherwise requested) on the progress of the project, and upon completion of the project
  - Within 48hrs in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.
  - Annually a number of projects may be randomly selected for an external audit.
- The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the REC. Would there be deviated from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date, a new application must be made to the REC and new approval received before or on the expiry date.
- In the interest of ethical responsibility, the REC retains the right to:
  - Request access to any information or data at any time during the course or after completion of the project.
  - To ask further questions; Seek additional information; Require further modification or monitor the conduct of your research or the informed consent process.
  - withdraw or postpone approval if:
  - Any unethical principles or practices of the project are revealed or suspected.
  - It becomes apparent that any relevant information was withheld from the REC or that information has been false or misrepresented.
  - The required annual report and reporting of adverse events was not done timely and accurately.
  - New institutional rules, national legislation or international conventions deem it necessary

**ISSUED BY:**
**UNIVERSITY OF VENDA, RESEARCH ETHICS COMMITTEE**
**Date Considered: March 2021**

**Name of the Chairperson of the Committee: Prof MS Maputle**

**Signature:** MSMaputle

UNIVERSITY OF VENDA
OFFICE OF THE DIRECTOR
RESEARCH AND INNOVATION
2022 -10- 10
Private Bag X5050
Thohoyandou 0950

"A quality driven financially sustainable, rural based Comprehensive University"

D. PROVINCIAL DEPARTMENT OF HEALTH CLEARANCE

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF
HEALTH

| | | |
|---|---|---|
| Ref | : | LP_2023-05-013 |
| Enquires | : | Dr Ramalivhana NJ |
| Tel | : | 015-293 6028 |
| Email | : | Phoebe.Mahlokwane@dhsd.limpopo.gov.za |

**Prof Traore AN et al**

**PERMISSION TO CONDUCT RESEARCH IN DEPARTMENTAL FACILITIES**

Your Study Topic as indicated below;

**ADME POLYMORPHISM IN TUBERCULOSIS: PHARMACOGENETIC AND PHARMACOKINETICS ANALYSIS IN TB PATIENTS FROM HEALTHCARE FACILITIES IN THE VHEMBE DISTRICT OF LIMPOPO, SOUTH AFRICA**

1. Permission to extend your research study as per your research proposal is hereby Granted.
2. Kindly note the following:
   a. Present this letter of permission to the Office of District Executive Manager a week before the study is conducted.
   b. In the course of your study, there should be no action that disrupts the routine services or incur any cost on the Department.
   c. After completion of study, it is mandatory that the findings should be submitted to the Department to serve as a resource.
   d. The researcher should be prepared to assist in the interpretation and implementation of the study recommendation where possible.
   e. **The approval is only valid for a 1-year period.**
   f. If the proposal has been amended, a new approval should be sought from the Department of Health
   g. Kindly note that, the Department can withdraw the approval at any time.

Your cooperation will be highly appreciated.

14/07/2023

pp **Head of Department** **Date**

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