PREVALENCE OF MULTI-DRUG RESISTANCE TUBERCULOSIS MDR-TB AND ASSOCIATED RISK FACTORS AMONG PATIENTS IN VHEMBE REGION OF LIMPOPO, SOUTH AFRICA

By

MAHAMUD HA (18000647)

A dissertation in full fulfilment for the requirement of Master of Science degree in Microbiology (MNMMMS)

to the

Department of Biochemistry and Microbiology

in the

FACULTY OF SCIENCE, ENGINEERING AND AGRICULTURE

University of Venda

Thohoyandou, Limpopo South

Africa

Supervisor: Prof AN Traore………………….

Co-supervisor: Prof N Potgieter…………….

Co-supervisor: Dr MC Rikhotso ……………….

February 2024

DECLARATION

I, Hafsa Ali Mahamud (18000647), declare that this research proposal is my original work and has not been submitted for any degree at any other university or institution. The dissertation does not contain other persons' writing unless specifically acknowledged and referenced accordingly.

Signed: ....Hafsa……… Date: 22/02/24

DEDICATION

I dedicate my dissertation to my family, a special feeling of gratitude to my loving parents Ali Mohamud and Farhio Ahmed Mohamud, my aunt Fowsiyo Ahmed and my siblings for their encouragement and support throughout my life.

ACKNOWLEDGEMENTS

Firstly, all praises to the Almighty, the most Gracious and the Most Merciful. I am immensely grateful for His endless mercy upon me. The Almighty made my Master's journey possible and due to His mercy; the completion of this Dissertation was possible.

Throughout the writing of this dissertation, I have worked with broad-minded individuals whose expertise and inputs always pushed me to do my best.

I would like to acknowledge my supervisor Prof Traore AN, co-supervisors Prof Potgieter N and Dr Rikhotso MC, their expertise guided me throughout the writing process, and they always encouraged me to explore new directions in life and seek my own destiny.

I would like to acknowledge Dr Kachienga LO, Dr Madala NE, Musoliwa R and Mashilo MS for assisting me throughout this research.

I would also like to acknowledge my parents (Ali Mohamud and Farhio Ahmed Mohamud), my aunt (Fowsiyo Ahmed) and my siblings (Maryam, Deeqo, Basma, Abdirahmaan and Fowsio) for their love and support throughout my life. Thank you for giving me the strength to reach for the stars and chase my dreams.

I would also like to acknowledge my friends Sana, Raisah and Asma for being the support system.

I would like to acknowledge NRF for the funding and for making this research possible.

Lastly, I would like to thank the TB research group.

Table of Contents

LIST OF ABBREVIATIONS vi

LIST OF FIGURES viii

LIST OF TABLES ix

ABSTRACT x

CHAPTER 1 12

GENERAL INTRODUCTION 12

1.1 BACKGROUND 12

1.2 STUDY RATIONALE 14

1.3 RESEARCH QUESTION 15

1.4 OBJECTIVES 15

CHAPTER 2 16

LITERATURE REVIEW 16

2.1 TUBERCULOSIS AND DRUG RESISTANCE 16

2.2 MYCOBACTERIUM TUBERCULOSIS COMPLEX 17

2.3 NON-TUBERCULOUS MYCOBACTERIA 18

2.4 PATHOGENESIS OF MYCOBACTERIUM TUBERCULOSIS 18

2.5 PREVALENCE OF TB AND MDR-TB 19

2.6 RISK FACTORS ASSOCIATED WITH TB 20

2.7 DIAGNOSIS 21

2.8 TREATMENT OF TB AND MDR-TB 25

2.9 DRUG RESISTANCE TUBERCULOSIS 26

2.10 TYPES OF DRUG RESISTANCE 28

2.10.1 MONO RESISTANCE TUBERCULOSIS 28

2.11 GENES INVOLVED IN MUTATION 29

CHAPTER 3 31

METHODOLOGY 31

3.1 ETHICAL CLEARANCE 31

3.2 STUDY SITE 31

3.3 SAMPLE COLLECTION 32

3.4 PRE-TREATMENT OF SPUTUM SAMPLES 32

3.5 EXTRACTION OF DNA AND DETECTION OF SPUTUM SAMPLES 33

3.8 GENOTYPING OF MDR-TB SAMPLES USING MASSARRAY® ASSAY TECHNIQUE 34

CHAPTER 4 36
RESULTS AND DISCUSSION 36
CHAPTER 5 53
5.1 CONCLUSION 53
5.2 LIMITATIONS 54
5.3 RECOMMENDATIONS 54
REFERENCES 55

LIST OF ABBREVIATIONS

%	Percentage
AG	Arabinogalactan
ART	Antiretroviral therapy
BCG	Bacillus Calmette Guerin
DPO	Dual Priming Oligonucleotide
DR-TB	Drug resistant Tuberculosis
DST	Phenotypic drug susceptibility
EMB	Ethambutol
HIV	Human immunodeficiency virus
INH	Isoniazid
KZN	KwaZulu Natal
LAMP	Loop-mediated Isothermal Amplification
L-J	Lowenstein-Jensen
LO	Life Orientation
LPA	Line Probe Assay
MAC	*M. avium complex*
MDR-TB	Multi-drug resistant tuberculosis
MM	Mycomembrane
MTB	*Mycobacterium Tuberculosis*
MTBC	*Mycobacterium tuberculosis* complex
NGS	Next Generation Sequencing
NTM	Non-Tuberculous Mycobacteria
PCR	Polymerase chain reaction

Pre-XDR-TB	Pre-extensively drug-resistant tuberculosis
PZA	Pyrazinamide
RIF	Rifampicin.
RR	Rifampicin-resistant
RRDR	Rifampicin resistant-determining region
RRDR	RIF-resistance determining region.
RR-TB	Rifampicin-resistant tuberculosis
SAMRC	South African Medical Council
TB	Tuberculosis
TDR-TB	Totally drug resistant TB
TOCE™	Tagging Oligonucleotide Cleavage and Extension
WHO	World Health Organization
XDR	Extensive drug resistance
XDR-TB	Extensive drug resistance tuberculosis

LIST OF FIGURES

Figure 2.1: Figure showing pathogenesis of MTB invading the human body through the respiratory tract.

Figure 2.2: Figure showing the diagram which illustrates the pathways of drug resistance in MTB.

Figure 3.1: Figure showing Vhembe district municipality which is in Limpopo, South Africa.

Figure 4.1: Figure Displaying different infections from TB patients. MTB (*Mycobacterium tuberculosis*), MTB+NTM (*Mycobacterium tuberculosis* + nontuberculous Mycobacterium), HIV (human immunodeficiency virus).

LIST OF TABLES

Table 2.1: Table showing the first-line anti-TB medications, the genes implicated in their activation, and the mechanisms involved.

Table 2.2: Table showing the medication classification for multidrug-resistant TB in 2019

Table 4.1: Table providing a detailed breakdown of the demographic composition of the TB patient population under study, offering insights into key factors such as gender, age, education, employment, family size, and religious preferences.

Table 4.2: Table providing a social behaviours, cultural factors, and stigma among the TB patients, categorizing the questions based on different aspects of the individual's experience related to TB.

Table 4.3: Table which presents the prevalence of co-morbidities among patients diagnosed with tuberculosis (TB). The percentages indicate the proportion of participants with specific co-morbid conditions, including HIV, hypertension, ulcer, diabetes, and asthma.

Table 4.5: Table showing SNPs targeted for MassArray.

Table 4.6: Table showing Socio-Demographics status, Social Behaviours, Cultural Factors, and Stigma among the TB Patients in Vhembe, South Africa.

ABSTRACT

BACKGROUND: The increasing prevalence of drug-resistant tuberculosis (DR-TB) poses a critical and significant obstacle in the management of tuberculosis (TB) worldwide. This driven by the emergence of multidrug-resistant tuberculosis (MDR-TB) strains and co-infection of TB with human immunodeficiency virus (HIV) have become major challenge in eradicating TB especially in low- and middle-income countries. Approximately half a million cases of rifampicin-resistant tuberculosis were reported in 2020, with 78% of these cases developing into MDR-TB.

AIM: This study aimed to investigate the prevalence of MDR-TB and its associated risk factors in Vhembe region (Limpopo, South Africa).

METHODOLOGY: A structured questionnaire was used to collect data on plausible risk factors. A total of 50 participants from the overall study were enrolled and sputum samples were obtained from patients receiving treatment from 20 healthcare facilities. Each patient blood sample was first tested for HIV using rapid test. For this study DNA was extracted from the sputum samples using reagents from multiplex real-time PCR kit (Allplex). Multiplex realtime PCR (Anyplex/ Allplex) was used to confirm the presence of TB and to detect MDR-TB respectively. MassArray was used to detect any mutation on the SNPs for *rpoB* and *katG* and *inhA* promoter region of the MTB.

RESULTS: Out of the 50 TB patients, 18% (9/50) were found to be MTB, 18% (9/50) to be MTB+NTM and (27/50) were NTM and 10% (5/50) could not be amplified. In this study, it was found that 2 samples were DR-TB from the patients that were MTB and MTB+NTM positive. Overall, the percentage of DR-TB was 4% (2/50). HIV prevalence in the population was 64% (32/50). The risk factors that showed high significance level of association are educational level (52%), occupation (60%), religion (80%), dusty area (72%), wearing protective mask (72%) and family support (90%). Sequence analysis showed no mutation in one patient and for the other patient the DNA not successfully genotyped.

Conclusion: There are several risk factors that are associated with TB, this includes education level, occupation, and religion. High prevalence of TB (32%) was found. However, there was low prevalence (4%) of DR-TB found (Rifampicin resistant, isoniazid resistant). Furthermore, after genotyping the resistant SNPs using MassArray, the results obtained showed that there was no mutation identified. It was observed that adolescents and young adults are more susceptible to acquiring TB and DR-TB. Timely detection of medication resistance is imperative for the efficient management of the disease and dissemination of information related to TB and MDR-TB is vital especially in low- and middle-income countries.

Keywords: *Anyplex/allplex, HIV, MDR-TB, risk factors, SNPs, TB.*

CHAPTER 1

GENERAL INTRODUCTION

1.1 BACKGROUND

Tuberculosis (TB) remains a significant global public health concern, affecting over 10 million individuals annually and resulting in nearly 1.5 million deaths. This infectious disease, caused by *Mycobacterium tuberculosis* (MTB), primarily manifests in the lungs (pulmonary TB) but can extend to other organs, leading to extrapulmonary TB. Inhalation of even minute quantities of these bacteria can result in infection (Mirzayev et al., 2021). Notably, TB stands as the second most prominent infectious cause of death worldwide, second only to COVID-19 (Chakaya et al., 2021). Despite the effectiveness of anti-TB medications, drug-resistant strains of MTB are emerging.

The evolution of drug resistance in MTB is facilitated by various mechanisms, including compensatory evolution, clonal interference, epistasis, activation of efflux pumps, impermeability of the cell envelope, target mimicry, drug degradation and modification, and the development of phenotypic drug tolerance (Rojas et al. 2019; Al-Saeedi and Al-Hajoj, 2017). In 2020 it was reported that over 500,000 patients demonstrated resistance to rifampicin (RIF), a key anti-TB drug. Alarmingly, 78% of these cases progressed to multidrug-resistant tuberculosis (MDR-TB) (Maphalle et al., 2022). This escalation of drug-resistant tuberculosis (DR-TB) strains poses a difficult challenge to global health efforts, necessitating a deeper understanding of the factors contributing to resistance and the development of strategies to mitigate its impact (Sheikh et al., 2021).

The development of TB and the emergence of drug-resistant strains are complicated processes influenced by various interconnected factors. Individual determinants such as socio-demographic characteristics, lifestyle choices, and genetic predispositions play fundamental roles in shaping susceptibility to TB (Abel et al., 2018, Irfan et al., 2017). Living conditions, including overcrowded environments and inadequate access to healthcare, contribute to the transmission dynamics of MTB (Srivastava et al., 2015). Furthermore, the rise of DR-TB strains is often linked to suboptimal adherence to treatment regimens, incomplete therapeutic courses, and challenges in healthcare infrastructure (Tiberi et al., 2022). Co-morbidities, such as human immunodeficiency virus (HIV), also elevate the risk of TB, creating a complex interactions of health conditions (Wong et al., 2020). Understanding these multifactorial influences is crucial for devising effective prevention and intervention strategies, as it necessitates a comprehensive approach addressing the diverse determinants impacting the development and spread of TB and drug-resistant strains.

There are two distinct types of resistance observed in MTB-resistant strains: genetic resistance, involving mutations in genomic regions conferring the ability to evade drug effects, and phenotypic resistance, involving epigenomic modifications that induce drug resistance without DNA mutations (Singh et al., 2020). Rifampicin-resistant tuberculosis (RR-TB) specifically denotes resistance to RIF without affecting other first- or second-line drugs (Prasad et al., 2018). MDR-TB is characterized by resistance to at least two potent anti-TB drugs, isoniazid (INH), and RIF (Singh et al., 2020).

Treatment for RR-TB and MDR-TB is typically prolonged, lasting 18 months or more, and involves a combination of selected first-line drugs along with various second-line drugs, which tend to be more expensive and toxic (Falzon et al., 2016). Despite rapid advancements in understanding the molecular genetic basis of antituberculosis drug resistance, there remain unknown mechanisms through which bacilli can resist these drugs (Zhang and Yew, 2015). The identification of clinical isolates exhibiting resistance to antituberculosis drugs is crucial for facilitating timely and accurate diagnosis, enabling the initiation of appropriate treatment strategies (Singh and Chibale, 2021).

Drug resistance in MTB displays global variability, with rural areas being particularly impacted (Chisompola et al., 2020). Rural areas confront numerous challenges, including limited healthcare facilities, a scarcity of proficient healthcare professionals, and insufficient laboratory services. These constraints impede the prompt and accurate diagnosis of TB as well as the identification of drug resistance. Rural populations, often involved in agricultural occupations, face heightened exposure to environmental factors that elevate the susceptibility to TB (Kirkhorn and Garry, 2000).

Inadequate occupational safety practices contribute to the emergence and dissemination of DR-TB (Sharma et al., 2018). The lack of awareness regarding TB, encompassing its origins, symptoms, and available treatments, fosters stigma and discrimination (Mason et al., 2016; Manzoor and Ganie, 2015). This, in turn, deters individuals from seeking timely healthcare, exacerbating the transmission of TB and drug-resistant strains.

Effectively addressing TB and DR-TB in rural areas necessitates a thorough and tailored approach. Strategies should concentrate on fortifying healthcare infrastructure, enhancing access to diagnostic and treatment services, tackling socioeconomic determinants, and promoting community engagement (WHO, 2018a). These concerted efforts aim to alleviate the burden of TB and mitigate the emergence of drug resistance in rural settings., its emergence poses significant challenges to TB control efforts. DR-TB strains, observed in both healthcare facilities and communities, exhibit varying degrees of resistance, including rifampicin resistance (RR), MDR, and extensive drug resistance (XDR) (Shibabaw et al., 2020).

1.2 STUDY RATIONALE

Drug resistance is threatening global TB control and in 2018, it was reported that, over 500,000 cases were resistant to first-line treatments. In Africa, there were 26,845 cases of multidrug- resistant and rifampicin-resistant TB (MDR/RRTB) cases reported in 2017 (WHO, 2018b). Previous study conducted by Ismail and colleagues (2018) from 2012-2014 reported that, MDR tuberculosis was found in 2.1% of new TB cases and 4.6% of retreatment cases across the country (South Africa) and an estimated 8249 cases of MDR TB were reported.

The continuing emergence and spread of DR-TB is one of the most urgent and difficult challenges faced by global TB control. TB continues to be a public health challenge in South Africa, moreover, drug-resistant tuberculosis which is also on the rise (Shah et al., 2017). TB is the driver of DR-TB, According to Cox et al. (2017) South Africa ranks the third-highest number of DR-TB patients globally, after India and Russia. It is important to investigate the risk factors associated with TB and MDR-TB in the rural area, DR-TB makes it difficult to treat.

Few studies have been conducted in Limpopo focused on the prevalence and risk factors of TB (Mlangeni et al., 2023; Ramaliba et al., 2017) where they used questionnaire to collect data, risk factors include overcrowding, inadequate ventilation, TB treatment interruption, rural settlement, working in a mine, and low income.

Nevertheless, there is scarcity of data based on drug resistant TB and the genes associated with DR-TB, that is *rpoB*, *katG* and *inhA* promoter region in the Vhembe district.

Rural areas often face specific challenges, including limited access to healthcare, poverty, and inadequate sanitation (Habib et al., 2021) Studying the prevalence of TB helps to understand the magnitude of the disease's influence in these situations (Adane et al., 2020; Dye et al., 1999).

Therefore, this study aims to investigate the multi-drug resistance TB and associated risk factors among patients in Vhembe region of Limpopo, South Africa. This study will also add to MDR profile and enhance the management of TB.

1.3 RESEARCH QUESTION

- What is the prevalence of MDR-TB in the Vhembe region?
- What are the risk factors associated with the development of TB and MDR-TB in the Vhembe region?
- What are the common TB genes leading to MDR-TB in Vhembe?

1.4 OBJECTIVES

1.4.1 PRIMARY OBJECTIVE

- To investigate the prevalence of MDR-TB drug and associated risk factors among patients in Vhembe region (Limpopo, South Africa).

1.4.2 SECONDARY OBJECTIVES

- Determine common risk factors associated with TB and MDR-TB through a questionnaire.
- Determine the prevalence of MDR-TB among TB patients in Vhembe district using multiplex real-time PCR (Anyplex/ Allplex).
- Determine selected gene mutation (*rpoB*, *katG* and *inhA* promoter region) among RR-TB patients using massArray.

CHAPTER 2

LITERATURE REVIEW

2.1 TUBERCULOSIS AND DRUG RESISTANCE

Tuberculosis (TB) is an infectious disease that is caused by a type of bacteria called *Mycobacterium tuberculosis* (MTB). It typically affects the lungs but can also spread to the other parts of the body (Batista et al., 2020). *Mycobacteria* is the only genus in the family *Mycobacteriaceae* and a part of the order *Actinomycetales*. There are currently more than 100 recognized or suggested species in the *Mycobacterium* genus, many of which are pathogens and saprophytic organisms of warm-blooded animals. This genus can be distinguished by its acid-fastness and the presence of mycolic acids. *Mycobacterium tuberculosis*, *Mycobacterium Africanum*, *Mycobacterium bovis*, *Mycobacterium canettii* and *Mycobacterium microti* make up the *Mycobacterium tuberculosis* complex (MTBC) (Hayward et al., 2018; Ogbuoji et al., 2018; Romha et al., 2018).

MTB was discovered by Robert Koch in 1882, his discovery was the most important step toward tuberculosis eradication and control (Cambau and Drancourt, 2014). According to the WHO report in 2017, there has been a consistent annual decline of 1.5% in the estimated worldwide incidence rate of tuberculosis from the year 2000. Despite these significant improvements and the extensive worldwide efforts to eliminate tuberculosis, the disease continues to contribute significantly to both morbidity and mortality on a global scale. To inform the public about the impact of TB on the world, the 24th of March was designated as World TB Day (WHO, 2005).

Individuals that are infected with HIV are more likely to contract TB disease (Twumasi et al., 2019), HIV and TB are a deadly combination because they both stimulate the progression of the other. Without sufficient treatment, the average mortality rate for HIV-negative TB patients is 45%, and HIV-positive TB patients are almost certain to die. About 187 000 persons died of HIV-associated TB in 2021 (WHO, 2021). In 2021 a significant number (76%) of TB patients were co-infected with HIV, compared to 73% individuals who were infected in 2020, thus TB/HIV co-infection is of great concern. Additionally, the WHO African Region has most HIV-associated TB cases. Additional risk factors include elderly people, malnourished, males who are alcoholics, homeless, immigrants, and prisoners (Lönnroth et al., 2010)

When one is diagnosed with active TB, the healthcare practitioner prescribes anti-TB drugs, the standard anti-TB drugs include RIF, INH, Ethambutol (EMB), Pyrazinamide (PZA) (these four drugs are known as RIFAFOUR), these drugs are administered for 6-9 months (Abdelwahab, 2021). However, improper administration of anti-TB drugs, late diagnosis, non-adherence to anti-TB drugs, evolution MTB strain (mutated), therefore drug resistant MTB arises (Borah et al., 2021).

Because MTB exhibit spontaneous, predictable rates of chromosomal alterations that confer drug resistance, thus DR-TB develops (Hameed et al., 2018). A susceptible strain of MTB may quickly develop drug resistance if there is monotherapy, unpredictable drug compliance, omission of one or more drugs, inappropriate dosage, poor drug absorption, or not enough active drugs in a regimen (Jang and Chung, 2020; WHO, 2018b).

There are types of resistance which include: Mono-resistance which refers to the condition where an individual is resistant to only one first-line anti-TB treatment (Lesnik and Nigulyanu, 2021). MDR-TB is caused by MTB bacteria that are resistant to at least INH and RIF (Almatar et al., 2020).

Pre-extensively drug-resistant tuberculosis (pre-XDR-TB) is resistant to INH, RIF, plus resistant to any one of the fluoroquinolones drugs and one of the second-line injectable anti-tuberculosis drugs. While XDR resistant to INH and RIF, plus resistant to any one of the fluoroquinolones drugs and one of the 3 second line injectables anti-tuberculosis drugs (amikacin, kanamycin, or capreomycin). Totally drug-resistant tuberculosis (TDR-TB), a condition characterized by resistance to all anti-tuberculosis medications now under testing, has recently brought about a more concerning situation (Stephanie et al., 2021).

Resistance to INH is due to mutations at one of two main sites, in either the *katG* or *inhA* genes. Resistance to RIF is nearly always due to point mutations in the *rpoB* gene in the beta subunit of DNA-dependent RNA polymerase (Ismail et al., 2016).

2.2 MYCOBACTERIUM TUBERCULOSIS COMPLEX

Mycobacterium tuberculosis complex (MTBC), comprise of various strains such as *M. tuberculosis M. canetti*, *M. microti*, *M. africanum*, and *M. bovis* cause TB (de Jong et al., 2010). However, MTB is the most common strain. Furthermore, within the genus of this organisms are other strains called Non-Tuberculous Mycobacteria (NTM) which share certain similarities in their biology, including the unique characteristics (Pereira et al., 2020). MTB is distinguished from other NTM and those within the MTBC involve specific genetic markers and genomic characteristics (Chin et al., 2018). These differences contribute to the unique pathogenicity, virulence, and adaptation of MTB to the human host.

2.3 NON-TUBERCULOUS MYCOBACTERIA

Non-tuberculous mycobacteria (NTM) are widely distributed in the environment (Gopalaswamy et al., 2020). These organisms have also been isolated from milk, food products, wild animals, plants, dirt, dust, and drinking water sources (Honda et al., 2018; Falkinham, 2015). There are over 170 species of mycobacteria in the NTM family. However, *M. avium complex (MAC), M. kansasii,* and *M. abscessus* species are mostly responsible for human lung disease (Johnson and Odell, 2014). NTM are distinguished by a thick, lipid-rich outer coating that encircles a thin peptidoglycan layer. This coating allows NTM to adhere to rough surfaces and provides resistance to antibiotics and disinfectants, facilitating NTM survival under low oxygen and carbon concentrations and other unfavourable environments (Falkinham, 2018).

2.4 PATHOGENESIS OF MYCOBACTERIUM TUBERCULOSIS

The pathogenicity of MTB primarily relies on three factors: the ability of the bacteria to reprogram host macrophages following initial infection, thereby evading elimination; the formation of granulomas, which allow the pathogen to coexist with the host immune response; and the regulation of bacterial central metabolism and replication, leading to a dormant state in which MTB becomes resistant to both host defences and therapy (Miggiano et al., 2020; Zhai et al., 2019).

The MTB life cycle is considered to begin upon its arrival in the airway and lung as shown in Figure 2.1. The initial interaction between the airway and bacillus is referred to as primary infection. MTB initially enters the body through the nose or mouth. It then interacts with cells in the upper airway and often proceeds to the distal lung, ultimately reaching the alveolar space (Rahlwes et al., 2023). To survive and initiate infection, MTB subsequently invades beyond the mucosal or alveolar epithelium. MTB can infect epithelial cells as it passes through the upper and lower airways (Rodrigues et al., 2020).

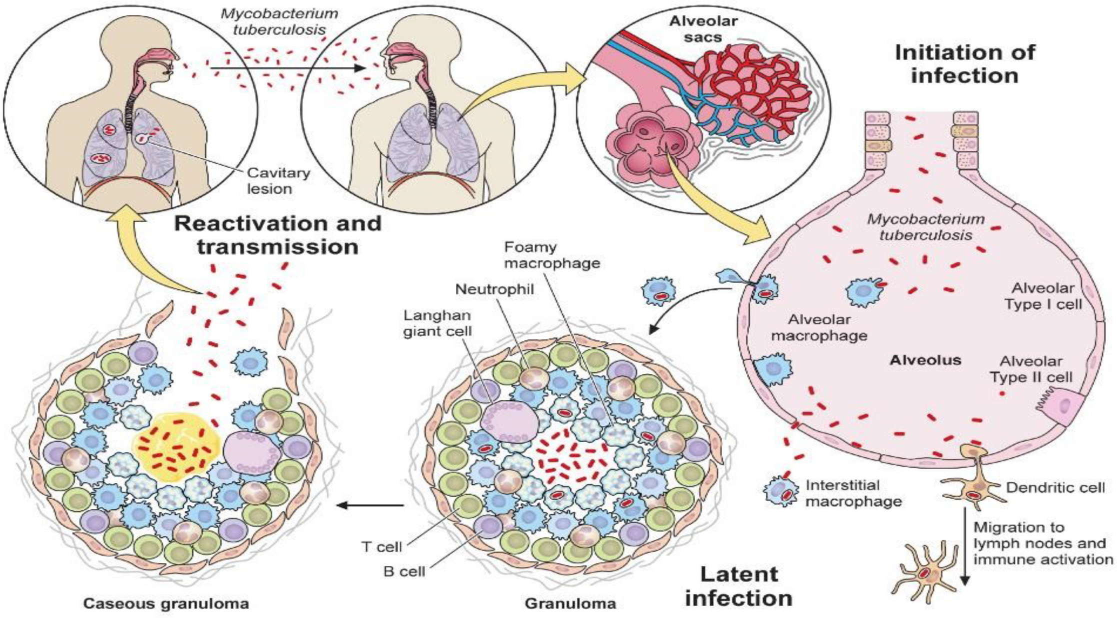


Figure 2.1: MTB invades the human body through the respiratory tract, specifically targeting the innate immune system located in the alveolar region. Macrophages and dendritic cells phagocytose the bacteria, thereby recruiting new cells and initiating adaptive immunity. The innate and adaptive immune systems work together to eliminate the bacteria or limit its replication within a granuloma. Active tuberculosis can arise from either initial infection or reactivation caused by immunodeficiency. This results in the development of symptomatic disease and the potential for transmission to a new host, initiating a new cycle of infection. (Adopted from Rahlwes et al., 2023).

2.5 PREVALENCE OF TB AND MDR-TB

Globally, TB stands as the second leading infectious cause of mortality, surpassing HIV and AIDS. In 2019, an estimated 10 million individuals were afflicted with TB, resulting in 1.41 million fatalities (Garrido-Cardenas et al., 2020). In 2022, approximately 10.6 million cases were reported globally, with 5.8 million affecting males and 3.5 million affecting females. Notably, 40% of individuals with drug-resistant tuberculosis sought medical treatment in 2022 (WHO, 2023).

Since 1994, the WHO has consistently collected and analysed data on resistance to anti-TB medications worldwide. In 2021, there were around 450,000 reported cases of MDR or RR TB globally (WHO, 2022). The rise of MDR-TB strains and TB/HIV coinfection poses significant challenges in combating tuberculosis, particularly in low- and middle-income countries (Fukunaga et al., 2021). In 2019, over 500,000 new cases of rifampicin-resistant TB were reported globally, with MDR-TB constituting 78% of these infections (Maphalle et al., 2022).

The prevalence of resistance to INH or RIF in MTB isolates varies based on the economic status and geographical location of a country. In South Africa, the frequency of anti-TB drug resistance varies between regions. Studies in KwaZulu-Natal and the Free State reported a prevalence of less than 3%, while in Gauteng, Mpumalanga, Northwest, and Limpopo, frequencies exceeding 80% were observed. Moreover, the prevalence of RIF and INH-resistant MTB was less than 15% in 91% of studies in the Western Cape, KwaZulu Natal and Free State, but exceeded 60% in 9% of studies in Gauteng, Mpumalanga, Northwest, and Limpopo (Traoré et al., 2023).

South Africa grapples with one of the most severe MDR-TB epidemics globally. Since 2002, the prevalence of MDR-TB has surged fivefold, reaching 26,000 reported cases in 2013 (Niehaus et al., 2015). Economic status and geographical location within the country contribute to the variability in anti-TB resistance (Iqbal et al., 2020).

2.6 RISK FACTORS ASSOCIATED WITH TB

Multiple factors contribute to the global persistence of tuberculosis. Poverty crowded living conditions, and limited healthcare access all play a crucial role in the disease's spread (Srivastava et al., 2015). Furthermore, the complex interaction of biological components, such as malnutrition and co-infections, increases susceptibility to TB (Whittaker et al., 2019). As patients with weaker immune systems are especially vulnerable to TB infection and development, the HIV pandemic has become a major factor in aiding the spread of TB (Olivier and Luies, 2023). MDR-TB has emerged as a key problem in the fight against TB. Misuse of antimicrobial medications, inadequate treatment regimens, and a lack of healthcare infrastructure all contribute to the establishment and spread of drug-resistant bacteria (Salam et al., 2023). Furthermore, variables such as patients' failure to comply with treatment, insufficient diagnostic capacities, and problems in implementing effective public health initiatives impact the intricate dynamics of MDR-TB (Law, 2019).

2.6.1 TB/HIV CO-INFECTION

TB and HIV/AIDS are the primary sources of infectious disease in nations with minimal resources. Within the individual host, the two infections, MTB, and HIV, mutually enhance each other, hastening the decline of immune functions and leading to early mortality if not treated (Bruchfeld et al., 2015). HIV coinfection is the predominant risk factor for developing active TB in areas with a high burden of the disease. This coinfection significantly enhances the vulnerability to both initial infection and reinfection with TB, as well as the chance of reactivating latent MTB infection in patients (Kwan and Ernst, 2011). An essential characteristic of immunosuppression in individuals with AIDS is the evident depletion of CD4+ T lymphocytes in the bloodstream, lymphoid organs, and mucosa. This depletion significantly contributes to the heightened susceptibility to developing active tuberculosis (Moir et al., 2011).

2.6.2 TB RELATED STIGMA

TB stigma is primarily driven by the perceived likelihood of TB-infected individuals transmitting the disease to susceptible members of the community (Huq et al., 2022). However, Tuberculosis is similarly susceptible to stigmatisation. in certain geographic regions because to its connections with HIV, poverty, poor social status, malnutrition, or disreputable conduct (Courtwright and Turner, 2010; Baral et al., 2007). The stigma associated with TB disproportionately affects women and individuals from financially deprived or less educated backgrounds. This is particularly worrisome because these groups are often more vulnerable to health inequalities. Hence, the stigma associated with TB has the potential to exacerbate existing health inequalities based on gender and social status (Courtwright and Turner, 2010).

2.7 DIAGNOSIS

There was widespread recognition of the need for a precise TB test to achieve substantial reductions in the global pandemic (Heemskerk et al., 2015). In general, despite some advancements in decreasing the global burden of TB, these efforts have not been enough to achieve the initial goal of the End TB Strategy (Huang et al., 2022).

2.7.1 MICROSCOPY

Sputum smear microscopy continues to be a fundamental technique for detecting MTB in underdeveloped nations (Huang et al., 2022). The most common method used is acid-fast staining utilizing a carbol fuchsin solution. The cell wall of MTB, which contains a high amount of lipids, is resistant to decolorization when exposed to reagents that contain acid. This resistance allows acid-fast organisms to be observed under a microscope when examining smears made from sputum, alveolar lavage fluid, or other samples. Smear microscopy is primarily limited by its lack of sensitivity, which exhibits significant variation (ranging from 20% to 80%) among different research investigations (Steingart et al., 2007; Steingart et al., 2006).

2.7.2 CULTURE

Specimens can be cultured to facilitate the growth of MTB (Gordhan et al., 2021). The traditional MTB culture can be conducted using either a solid media, such as Lowenstein-Jensen, or a liquid medium, such as Middlebrook 7H9 (Huang et al., 2022). Lowenstein-Jensen (L-J) media consists of egg proteins, salts, potato flour, and glycerol (Caulfield and Wengenack, 2016). Solid culture is more cost-effective and less susceptible to contamination by other bacteria compared to liquid culture. However, liquid culture offers the advantages of faster growth, increased sensitivity, and easier automatic detection of growth (Rageade et al., 2014; Chien et al., 2000).

2.7.3 PHENOTYPIC DRUG SUSCEPTIBILITY TESTING

Drug susceptibility testing (DST) for MTB is often performed following the isolation of a culture from a clinical specimen. The process is time-consuming, involving the initial isolation of a culture followed by the subsequent performance of DST (Siddiqi et al., 2012).

2.7.4 MOLECULAR DIAGNOSES

A. GENXPERT/MTB/RIF

Xpert MTB/RIF (Cepheid) is an automated molecular test that is widely used globally for diagnosing TB by detecting the presence of MTB and resistance to RIF directly from clinical specimens. The method relies on a hemi-nested real-time polymerase chain reaction (PCR) assay to amplify a particular sequence of the *rpoB* gene that is unique to MTB, it is then hybridized with five molecular beacon probes (Venter et al., 2017; Ochang et al., 2016). Every probe targets a distinct sequence and is marked with a fluorescent dye (Nguyen et al., 2019). The Xpert MTB/RIF test has a sensitivity of 99.8% for cases that are positive on both smear and culture tests, and a sensitivity of 90.2% for cases that are negative on smear but positive on culture. The predicted specificity of a single direct MTB/RIF test is 99.2% (Boehme et al., 2010). The assay has a short turnaround time of 2-3 hours.

B. LINE PROBE ASSAY (LPA)

Line probe assay (LPA) can identify TB DNA and genetic mutations linked to anti-TB drug resistance, following the process of DNA extraction and PCR amplification (Krishnakumariamma et al., 2020). LPAs are extremely precise and yield outcomes within a five-hour timeframe (Nathavitharana et al., 2017). The LPA technique utilizes reverse hybridization of DNA on a clearly readable test strip to detect specific DNA sequences linked to resistance against first-line TB treatments (RIF and INH) as well as some second-line drugs (second-line injectable therapies and fluoroquinolones) (Mäkinen et al., 2006). The current WHO guidelines suggest using GenoType MTBDRplus and GenoType MTBDRsl, both developed by Hain LifeScience GmbH in Germany, for initial drug-resistance testing on sputum smear-positive samples (WHO, 2020).

C. LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

Loop-Mediated Isothermal Amplification (LAMP) is a PCR amplification technology that operates at a constant temperature and utilizes an auto-cycling strand displacement reaction. This reaction specifically targets six areas of the 16S rRNA and *gyrB* genes. TB-LAMP is a nucleic acid amplification approach that is simple, quick, specific, and cost-effective. It does not require advanced laboratory equipment and may be performed in local settings (Iwamoto et al., 2003). Presently, the WHO suggests using the TB-LAMP assay as a possible substitute for smear microscopy, pointing to its enhanced diagnostic capabilities (WHO, 2016a).

D. NEXT-GENERATION SEQUENCING

Next-generation sequencing (NGS) is regarded as a highly promising technique for conducting DST of TB. It offers significantly faster findings compared to traditional phenotypic culture-based testing (Lee and Pai, 2017; Walker et al., 2015). WHO has released guidelines for the utilization of NGS technologies in identifying genetic changes linked to drug resistance in the MTB complex (WHO, 2018a). NGS can offer comprehensive and precise sequence data for entire genomes through the utilization of whole-genome sequencing or sequencing of multiple gene regions.

E. ALLPLEX™/ANYPLEX™

Seegene possesses two exceptional technologies in the field of PCR: dual priming oligonucleotide (DPO™) technology and Tagging Oligonucleotide Cleavage and Extension (TOCE™) technology. DPO™ is an essential technique for preventing the elongation of templates that are not appropriately primed. It enables the creation and advancement of assays with extremely high specificity. The robustness and practicality of DPO™ technology can be effectively integrated into several molecular diagnostic systems, including multiplexed molecular assays and assays designed for the identification of point mutations. TOCE™ is an innovative technology that provides real-time reading by analysing the melting temperature. Previously, the analysis of melting temperature has been limited by various inherent constraints, such as restricted probe design, limited ability to perform broad and efficient multiplexing, and high sensitivity of melting temperatures caused by sequence variation at the probe site. The TOCE™ technology addresses these constraints by offering improved multiplexing capabilities, enhancing the adaptability of probe design, delivering a readout that is unaffected by variations in target sequence, and ensuring compatibility across different platforms. The simultaneous real-time detection of numerous point mutations with excellent specificity is achieved by combining DPO™ and TOCE™ technologies (Allplex™, Seegene, Seoul, South Korea).

F. MASSARRAY

The MassArray system utilizes MALDI-TOF mass spectrometry as its foundation. The PCR-amplified product was combined with SNP sequence-specific extension primers to elongate by 1 base at the SNP location. The produced sample analytes are subsequently co-crystallized with the chip's matrix. The crystal is inserted into the vacuum tube of the mass spectrometer and subsequently stimulated by a brief nanosecond (10-9 s) laser pulse. The matrix molecules absorb energy from radiation, leading to the accumulation of energy and the rapid generation of heat, which causes the sublimation of the matrix crystals. Nucleic acid molecules undergo desorption and convert into metastable ions, with most of the produced ions being single-charged ions. The individual ions acquire equal kinetic energy in the acceleration field and are subsequently separated based on their mass-to-charge ratio in the non-electric field drift zone. The ion's velocity increases as its mass decreases, resulting in a quicker arrival at the detector (Ellis and Ong, 2017; Busó. and Iborra, 2016).

2.8 TREATMENT OF TB AND MDR-TB

The WHO recommends a set of standardized treatment regimens for TB that consist of 4 important medicines (Table 2.1) referred to as "first line" drugs.

Table 2.1: First-line anti-TB medications and the mechanisms involved (Adapted from: Palomino and Martin, 2014).

Drug	Mechanism Involved	Reference
Isoniazid	Catalase/peroxidase, enoyl reductase	Hazbón et al., 2006
Rifampicin	RNA polymerase	Caws et al., 2006; Somoskovi, et al., 2001; Ramaswamy and Musser, 1998.
Pyrazinamide	Pyrazinamidase; ribosomal protein	
Ethambutol	Arabinosyl transferase	Ando et al., 2011.

The goal of MDR-TB treatment is to recover the patient while preventing MDR-TB transmission to others (Jang and Chung, 2020). WHO established recommendations for DR-TB care in 2006. These recommendations were updated in 2011. The updated guidelines include early RR detection and a combination of four powerful drugs, including PZA, an injectable antibiotic, and a more advanced fluoroquinolone, for patients with MDR-TB (WHO, 2011). The revised 2016 WHO guidelines advised

MDRTB treatment regimens with at least 4 powerful TB medications, including pyrazinamide and four other second-line TB drugs. The treatment approach should comprise fluoroquinolone, an injectable medication, EMB or prothionamide, PZA, and cycloserine or para-aminosalicylic acid (WHO, 2016b). In 2019, WHO revised and updated the consolidated guidelines. The guidelines encompass a novel pharmacological categorization and instructions for constructing treatment plans (Chakaya et al., 2021) (Table 2.2).

Table 2.2: Medication classification for multidrug-resistant TB in 2019 (Adapted from: Chakaya et al., 2021; Jang and Chung, 2020).

Group	**Medicine**	**Step**
A	Levofloxacin or moxifloxacin Bedaquiline Linezolid	Include all three medicines (unless they cannot be used)
B	Clofazimine Cycloserine or terizidone	Add one or both medicines (unless they cannot be used)
C	Ethambutol Delamanid Pyrazinamide Imipenem-cilastatin or meropenem Amikacin or streptomycin Ethionamide or prothionamide Para-aminosalicylic acid	Add to complete a four to five regimen when medicines from group A and B cannot be used

2.9 DRUG RESISTANCE TUBERCULOSIS

Since the first anti-TB medicine was discovered, drug resistance in MTB has been identified as a biological phenomenon (Seung et al., 2015). TB treatment for and control have been complicated by DR-TB (Maggi et al., 1966). MTB acquires drug resistance by the occurrence of spontaneous genetic changes that reduce the bacterium's susceptibility to commonly used anti-TB medications. These genes can produce drug targets or drug metabolism pathways, which can impact the effectiveness of anti-TB therapy (Palomino and Martin, 2014).

2.9.1 MECHANISM OF RESISTANCE

Drug resistance is a significant barrier to the treatment of tuberculosis and creates a concern for worldwide public health and pharmaceuticals (Drobniewski et al., 2013). Drug resistance may develop in one of two ways: primary resistance or secondary resistance (WHO, 2014). Primary resistance is formed due to the exposure and infection of patients with a drug-resistant strain (Allué-Guardia et al., 2021). Secondary resistance or acquired resistance in TB patients is brought on by drug malabsorption, an inadequate regimen, and poor treatment adherence (Lange et al., 2019). The development of drug resistance in MTB is facilitated by several mechanisms, including target mimicry, efflux pumps, drug degradation and modification, and phenotypic drug tolerance (Singh et al., 2020) as shown in Figure 2.2.

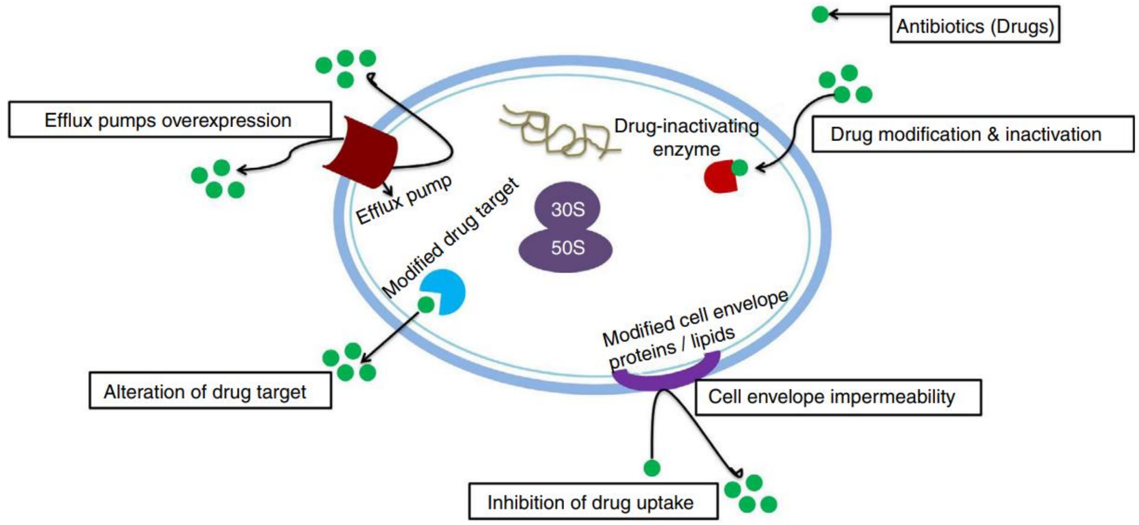


Figure 2.2: Illustrates the pathways of drug resistance in MTB (Adopted from Singh et al., 2020)

A. IMPERMEABILITY OF CELL ENVELOPE

The distinctive lipid structure and composition of mycobacterial cell envelopes play a role in their ability to cause disease and withstand the effects of drugs (Nguyen, 2016). The mycobacterial cell envelope consists of three distinct components: the capsule, the cell wall, and the cell membrane (Nasiruddin et al., 2017). The outer capsule consists mainly of proteins, glucan, and a small amount of lipids. The cellular barrier is composed of an external mycomembrane (MM), an arabinogalactan (AG) layer, and an internal peptidoglycan structure. The structure of MM is composed of two leaflets: an outer leaflet consisting of lipids such as phospholipids, trehalose mycolates, glycopeptidolipids, and lipoglycans, and an inner leaflet composed of long-chain mycolic acids (Nguyen, 2016). The mycolic acid-arabinogalactan-PG polymer creates a hydrophobic layer when combined with other lipids and the cytoplasmic membrane. The periplasmic space serves as a protective barrier for cells, shielding them from external stress and functioning as a barrier against the penetration of antibiotics (Nguyen, 2016).

2.9.2 ACQUIRED DRUG RESISTANCE

Bacteria typically develop antibiotic resistance by genetic alterations. Drug resistance in MTB generally arises from mutations occurring in the chromosome, particularly in genes that encode drug targets or drug-activating enzymes. These mutations are a direct response to the selective pressure exerted by antibiotics (Von Wintersdorff et al., 2016; Culyba et al., 2015).

2.10 TYPES OF DRUG RESISTANCE

2.10.1 MONO RESISTANCE TUBERCULOSIS

Mono-resistance refers to the condition where an individual is resistant to only one first-line anti-TB treatment.

A. RIFAMPICIN RESISTANT TUBERCULOSIS

RR-TB is characterized by resistance to the initial anti-tuberculosis medicine rifampicin. This type of TB can be detected using genotypic or phenotypic methods. (Prasad et al., 2018). Research sectors are giving greater attention to mechanisms of RIF resistance, which is still the most often used anti-TB drug in the treatment of tuberculosis (Murray et al., 2015). As a result of long-term RIF therapy, RR has grown to be a significant issue for the management of tuberculosis. The occurrence of a mutation in *rpoB,* which is the beta subunit of DNA-directed RNA polymerase in MTB, is well recognised as a primary factor contributing to RIF resistance (Kumar and Jena, 2014).

B. ISONIAZID RESISTANCE

INH is an antimicrobial agent employed in the management of tuberculosis. INH is a crucial primary anti-TB drug due to its ability to kill bacteria in the early stages of infection. Nevertheless, resistance to INH, either on its own or in conjunction with other medications, currently represents the most prevalent form of resistance to antituberculosis treatments (Jhun and Koh 2020).

2.10.2 MULTI-DRUG RESISTANT TUBERCULOSIS

MDR-TB refers to MTB strains that are resistant to both INH and RIF (Malenfant and Brewer, 2021). Even though resistance to RIF is mostly caused by one gene, resistance to INH is typically caused by mutations in either the *katG* or the *inhA* promoter genes, although high-level INH resistance is often the result of *katG* mutations and does not cause cross-resistance with any other known TB medications (Vilchèze and Jacobs, 2007). Low level of resistance to INH is caused by *inhA* promoter and gives high-level cross-resistance to ethionamide, a second-line drug utilized in most MDR- and XDR-TB regimens (Sandgren et al., 2009; Guo et al., 2006; Morlock et al.,2003; Banerjee et al.,1994).

2.11 GENES INVOLVED IN MUTATION.

2.11.1 *RPOB*

RIF specifically targets the protein *rpoB.* The enzyme *rpoB* facilitates the conversion of DNA into mRNA by utilising the four ribonucleoside triphosphates as its substrate. RIF inhibits the elongation of messenger RNA in M. tuberculosis by binding with the *rpoB*-encoded -subunit of the RNA polymerase. This prevents transcription (Piccaro et al., 2014; Carlos and Martin, 2013). *rpoB* has a gene length of 3519 base pairs, and mutations in *rpoB* can result in conformational alterations resulting in RIF resistance (Telenti et al., 1993). The "hot-spot region" (of 81 bp), often referred to as the "RIF-resistance determining region" (RRDR), which covers codons 507-533 of the *rpoB* gene, is where 96% of RIF-resistance occurs (Ramaswamy et al., 2003). Multiple studies have reported that RIF-resistant isolates most frequently had mutations in codons 516, 526, and 531 (Thirumurugan et al., 2015; Ocheretina et al., 2014).

2.11.2 *KATG* AND *INHA*

The primary cause of INH resistance in MTB is mutations in the genes *katG* and *inhA*. KatG molecular weight is 80,572.8 Da and a gene length of 2,223 bp, whereas InhA has a molecular weight of 28,527.8 Da and a gene length of 810 bp. The gene *katG* produces multipurpose enzymes with catalase-peroxidase and peroxynitritase activity, the former of which is crucial for prodrug activation and the latter of which is connected to pathways which includes oxygen intermediates and reactive nitrogen (Zhang et al., 1992).

CHAPTER 3

METHODOLOGY

3.1 ETHICAL CLEARANCE

This study is part of a South African Medical Council (SAMRC) funded project, and it has been ethically reviewed and approved by the ethical research committee of the University of Venda (SMNS/20/MBY/13: Appendix 1). Permission has been granted from the Limpopo Provincial Department of Health (LP_2021-11-001: Appendix 2) to access different health care facilities. Permission was also granted from the Vhembe District Municipality (Appendix 3) and the health care facilities selected in the study. Before enrolling in the study, all participants were given a consent form to sign (Appendix 4). Participants' rights were considered, and they had the right to withdraw without prejudice.

3.2 STUDY SITE

This study was conducted in the health care facilities located in Vhembe region of Limpopo province, South Africa. Vhembe has a total of 5 hospitals and 100 clinics. The clinics and hospitals visited include: Mhinga clinic (1), Phiphidi (9), Siloam clinic (2), Tshilidzini clinic (2), Mpheni clinic (1), Watervaal clinic (3), Donald frazer (2), Tshilidzini hospital (1), Mbokoto clinic (2), Tswinga clinic (3), Malamulele hospital (1), Thohoyandou clinic (1), Lwamondo clinic (3), Tshakuma clinic (1), Magwedzhe clinic (7), Malamulele clinic (3), William Edie clinic (7), Mphambu clinic (1) and Tshilidzini Tshedza (1).

Figure 3.1: Vhembe district municipality located in Limpopo, South Africa

3.3 SAMPLE COLLECTION

A total of 50 sputum samples were obtained from a pool of sputum samples collected from individuals over the age of 18 who were both hospitalized and outpatients. A consent form was signed, and questionnaire was answered (Appendix 5). When collecting sputum specimens, patients were provided explicit instructions. Patients took samples outside in the open air or in a private spot away from other people and samples were not collected in confined spaces such as toilets. The patients were advised to rinse their mouths with water before collecting sputum, but not brush their teeth and coughed hard to expectorate sputum into the container containing 5ml of 4% NaOH (Sigma, St Louis, MI, USA). A sputum sample of 3-5mL was collected from each patient and stored at 2-8°C. Further analyses were performed within 3 days of collection. HIV Test (Humor Diagnostica, Johannesburg, Gauteng, RSA) was done for all participants.

3.4 PRE-TREATMENT OF SPUTUM SAMPLES

The samples were Incubated for 15min at room temperature, then 1.5ml was transferred to a new sterile tube and centrifuged (Advanced Multi-Purpose Clinical

Centrifuge E-C6-4.100CP, World of Science, Pretoria, RSA) at 15,000 x g for 5min. The supernatant was discarded then 1ml of 1X PBS solution (Sigma) was added and mixed well before centrifuging at 15,000 x g for 5min and the supernatant was discarded using a pipette. A 1ml of 1X PBS solution was added and mixed well before centrifugation at 15,000 x g for 5min and then discarding the supernatant (Allplex^{TM}, Seegene, Seoul, South Korea).

3.5 EXTRACTION OF DNA AND DETECTION OF SPUTUM SAMPLES

A volume of 1 mL sterile water were added to the prepared sediment and centrifuged for 5min at 15,000 x g before discarding the supernatant. The sediment was vortexed (Vortex V1 plus; Boeco, Hamburg, Germany) for 30s after adding 100µL of DNA Extraction

Solution and 10µL of internal control. It is further placed on heat block for 20min, The sample was centrifuged at 15,000 x g for 5min then 5µL of the supernatant was used as PCR template (Allplex^{TM}, Seegene, Seoul, South Korea).

3.6 ANYPLEX™ MTB/ NTM DETECTION

The Anyplex^{TM} MTB/NTM Real-time Detection (V2.0) method relies on two key steps: the extraction of nucleic acid and the amplification of target nucleic acid utilizing Dual Priming Oligonucleotide (DPO) primers with a real-time PCR machinery.

The sputum was obtained using the Automated Nucleic Acid Extraction System (NIMBUS and STARlet). To the specimen, an equal amount of NALC-NaOH solution (consisting of 2% NaOH, 1.47% trisodium citrate, and 0.5% NALC) was added. The mixture was then vortexed for 1 minute. It was then incubated at room temperature for 15min. A volume of 1ml was transferred to a sterile tube and centrifuged for 5 minutes at a speed of 15,000 x g. The supernatant was discarded, followed by addition of 450µL Lysis Buffer to the pellet and ensuring that it was mixed well. the tube was then cap-locked and boiled on a heat block for 5min.

3.7 ALLPLEX™ MTB/MDR-TB DETECTION

Allplex was used to detect MDR-TB according to the manufacturer's instructions (Allplex^{TM}, Seegene, South Korea). Allplex™ MTB/MDR/XDRe Detection is a multiplex real-time PCR assay that permits simultaneous amplification and detection of target sequences of *Mycobacterium tuberculosis* (MTB), 7 Isoniazid (INH) resistancecausing mutations [katG S315I(ATC), S315N(AAC), S315T(ACC), S315T(ACA), inhA promoter -15(T), -8(A), -8(C)], 18 Rifampicin (RIF) resistance-causing mutations [rpoB L511P(CCG), Q513K(AAA), Q513L(CTA), Q513P(CCA), 3 amino acid-deletion in 513~516, D516V(GTC), D516Y (TAC), S522L(TTG), S522Q(CAG), H526C(TGC), H526D(GAC), H526L(CTC), H526N(AAC), H526R(CGC), H526Y(TAC), S531L(TTG), S531W(TGG), L533P(CCG)], 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)], and Internal Control (IC) (Allplex^{TM}, Seegene, South Korea).

3.8 GENOTYPING OF MDR-TB SAMPLES USING MASSARRAY® ASSAY TECHNIQUE

The MassArray technology was used to locate and detect MTB resistant gene mutation locations. anti-tuberculosis drugs and associated gene resistance loci. Two simultaneous PCR reaction systems were used. The HPLC system was used to inject 0.8μL of H2O, 0.5μL of 10XPCR buffer containing 20mM MgCl2, 0.4μL of 25mM MgCl2, and an unspecified volume of 25mM dNTP. Combine 0.1 microliters of a 0.5 micromolar primer mix. The system comprised 1 microliter (μL) of PCR enzyme, 0.2 μL of template DNA, and 2 μL of template DNA, resulting in a total volume of 5 μL. The reaction system was heated to 95°C and maintained at this temperature for 2 minutes. It was then subjected to a sequence of temperature changes: 95°C for 30 seconds, followed by a decrease to 60°C for 30 seconds, and then an increase to 72°C for 60 seconds. This cycle was repeated 45 times. Finally, the system was held at 72°C for 5 minutes and then cooled to 4°C. 2 microliters of shrimp alkaline phosphatase (SAP) were added to each well. The mixture was incubated at 37°C for 40 minutes, then heated to 85°C for 5 minutes, and finally kept at 4°C. To each well, 2μL of iPLEX extension mix (nanopure water 0.62µL, buffer 0.2µL, termination mix 0.2µL, primer mix 0.94µL, and enzyme 0.04µL, Agena Bioscience, San Diego, CA) was added. The reaction time was 95°C for 30s, 95°C for 5s, (52°C for 5s, 80°C for 5s, for 5 cycles), this was done for 40 cycles, 72°C for 3min, hold at 4°C.

The analysis was then completed at the Inqaba Biotechnical Industries (Pty) Ltd utilizing a high-throughput MassArray equipment. Typer 4.0 and plate manager 1.0 software were used to evaluate the data. Test findings quality was categorized as NoAlleles, Low Probability, Aggressive, Moderate, and Conservative.

3.7 STATISTICAL ANALYSIS

All data obtained were captured on excel spreadsheet and simple descriptive analyses were conducted. Chi square (R studio) was used which compares the measured values to the anticipated values. This test statistic is utilized to ascertain the statistical significance of the disparity between the observed and expected values.

CHAPTER 4

RESULTS AND DISCUSSION

The primary objective of this study was to investigate the prevalence of MDR-TB drug and associated risk factors among patients in the Vhembe region (Limpopo, South Africa). This study comprised of 50 patients who were diagnosed with TB from various healthcare facilities in the Vhembe district, this included both inpatients and outpatients.

4.1 SOCIO-DEMOGRAPHICS DATA OF THE STUDY POPULATION

The patient demographics, as outlined in the study (Table 4.1), reveal a gender distribution of 60% (30/50) males and 40% (20/50) females. The overall age of the participants was between 18 to 80 years of age. Notably, age groups of (18-35) and (46-60) each accounted for more than 25%, while (36-45) and (61-80) constituted less than 25% of the total. In terms of educational background, 52% (26/50) of participants had completed secondary education, while primary and tertiary education were reported by 40% (20/50), and 8% (4/50) had no formal education.

Regarding employment status, 60% (30/50) of the participants were not employed, while 32% (16 /50) were either employed or self-employed. Additionally, 8% (4/50) classified themselves as students. Family size distribution indicated that over 62% of participants had between 5 to 9 family members, while 38% (19/50) had 0 to 4 family members. In terms of religion, 80% (40/50) of patients identified with Christianity, while 20% (10/50) followed other religions. These demographic details provide a comprehensive overview of the participant characteristics in the study. High significance level of association was shown on education level (p=0.01), occupation (p=0.003), religion (p=0.0001), and family size (p=0.002).

<u>Table 4.1:</u> This table provides a detailed breakdown of the demographic composition of the TB patient population under study, offering insights into key factors such as gender, age, education, employment, family size, and religious preferences.

Category	Variables	n=50 (100%)	p-value
Gender	Male	60%	-
	Female	40%	-
Age (years)	18-35	26%	0.32
	36-45	24%	0.40
	46-60	30%	0.20
	61-80	20%	0.06
Education level	Primary	20%	0.06
	High school level	52%	0.01
	University level	20%	0.06
	No education	8%	1.0
Occupation	Unemployed	60%	0.003
	Employed	16%	0.92
	Self-employed	16%	0.92
	Student	8%	1.0
Family size	0-4	38%	0.07
	5-9	62%	0.002
Religion	Christianity	80%	0.0001
	African	14%	1.0
	Other	4%	1.0
	Atheist	2%	1.0

4.2 SOCIAL BEHAVIORS, CULTURAL FACTORS, AND STIGMA AMONG THE TB PATIENTS

In this study factors surrounding the culture, behaviours and stigma surrounding TB are outlined in Table 4.2. About 12% (6/50) with TB consulted a traditional healer for treatment, 10% (5/50) of them got remedy from the traditional healer, the other 2% (1/50) did not receive any remedy. In this study 44% (22/50) of the participants consulted health care facilities within 1-3 weeks of illness, 56% (28/50) accounts for consulting after 3 weeks of illness.

Out of the TB patients who took part, 80% (44/50) were administered the BGC vaccination. It was discovered that 88% (49/50) of the individuals did not undergo tuberculin skin testing. Majority of the participants, 94% (47/50) shared their TB status to their family, and 92% (46/50) of them informed their families within 1-7 days. where 90% (45/50) got family support during the treatment process. It was also found that 46% (23/50) of the participants also shared their TB status to their colleagues, and 42% (21/50) of them got support from colleagues. About 24% (12/50) of the participants were previously diagnosed with latent or active TB.

In the current study 50% (25/50) of the participants share bedrooms with family members. Participants that use public transport were about 62% (31/50). Majority of the participants, 74% (37/50) wear protective masks. The participants that had family members which were previously diagnosed with TB were found to be 24% (12/50). The participants that mostly spend their time on dusty areas were 72% (36/50). High significance level of association was shown on time taken to consult healthcare centre (p=0.03), family support (p=0.0001), using public transport (p=0.002), sharing room with family member (p=0.01), wearing protective mask (p=0.0004), and spending time in a dusty area (p=0.0006).

Table 4.2: Provides a social behaviours, cultural factors, and stigma among the TB patients, categorizing the questions based on different aspects of the individual's experience related to TB.

Category	Variable	n=50 (100%)	p-value
Have you ever consulted a traditional healer?	Yes No	12% 88%	1.0
Did the traditional healer give you medicine?	Yes No N/A	10% 2% 88%	1.0
After how long did you take to go to the health care centre for consultation?	≤Three weeks ≥Three weeks	44% 56%	0.03
Have you ever had a vaccine to prevent TB	Yes No	88% 12%	4.66
Have you ever had a positive/reactive TB skin Test?	Yes, No	2% 98%	1.0
When you tested positive for TB, did you tell your family?	Yes No	94% 6%	1.69
How long did you take to tell them?	≤ A week ≥Two weeks and greater N/A	92% 2% 6%	2.37
Does your family seem to be supportive?	Yes No N/A	90% 6% 4%	0.0001
Did you inform your colleagues at work, community members or friends after testing positive for TB?	Yes No	46% 54%	0.02
Do they seem to be supportive in terms of encouraging you to go to the health care centres?	Yes No N/A	42% 4% 54%	0.04
Have you ever been treated for either latent or active TB?	Yes No	24% 76%	0.40
Sharing a room with a family member	Yes No	50% 50%	0.01

What kind of transport do you use the most?	Public transport Own car Walk	62% 10% 28%	0.002
Do you wear protective mask?	Yes No	74% 26%	0.0004
Family member with TB?	Yes No	24% 76%	0.4
Spend time in dusty area?	Yes No	72% 28%	0.0006

4.3 CO-MORBIDITIES AMONG THE TB PATIENTS

Patients who were diagnosed with TB also had co-morbidities. These includes 64% (32/50) of the participants who were positive for HIV, hypertension 8% (4/50), asthma 4% (2/50), followed by 2% (1/50) with ulcer and 2% (1/50) with diabetes.

Table 4.3: Presents the prevalence of co-morbidities among patients diagnosed with tuberculosis (TB). The percentages indicate the proportion of participants with specific co-morbid conditions, including HIV, hypertension, ulcer, diabetes, and asthma.

Co-morbidity	***n=50 (100%)***	***p-value***
HIV	Yes, 64% No, 36%	0.10
Hypertension	Yes, 8% No, 92%	1.0
Ulcer	Yes, 2% No, 98%	1.0
Diabetes	Yes, 2% No, 98%	1.0
Asthma	Yes, 4% No, 96%	1.0

4.4 PREVALENCE OF MTB INFECTION, OTHER INFECTIONS AND MDR

The graph below (Figure 4.1) shows the results of Anyplex/Allplex and co-infections. Out of the 50 participants recruited the prevalence of MTB (*Mycobacterium tuberculosis*) and MTB+NTM (*Mycobacterium tuberculosis* + non-tuberculous mycobacterium) showed 36% (18/50) prevalence. Furthermore, 36% (18/50) MTB and MTB+NTM, 11.11% (2/18) were found to be drug resistant (1 Rifampicin resistant and 1 Isoniazid resistant) and both were females. Moreover, 10% (5/50) of the samples were undetectable and the Allplex showed that the remaining participants had NTM 54% (27/50). Overall, 4% (2/50) had drug resistance. High prevalence of HIV, 64% (32/50) was also found of which 40% (20/50) were male while 24% (12/50) were female.

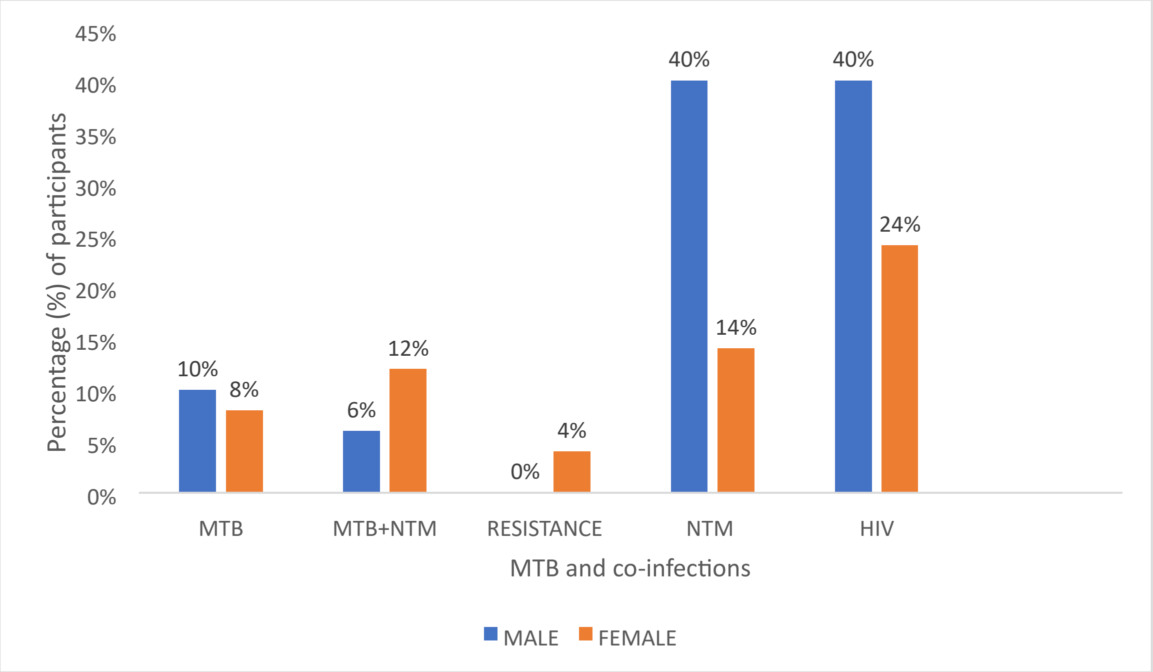


Figure 4.1: Displaying different infections from TB patients. MTB (*Mycobacterium tuberculosis*), MTB+NTM (*Mycobacterium tuberculosis* + non-tuberculous Mycobacterium), HIV (human immunodeficiency virus).

4.5 DETECTION OF MTB DRUG RESISTANT GENES USING MASSARRAY DNA MASS SPECTROMETRY

Moreover , 11.11% (2/18) drug resistant samples (RIF, INH) were further sequenced using massArray mass spectrometry to detect the mutations in the *katG*, *inhA*, and *rpoB* genes. No mutations were identified in any of these genes, independent of the presence of drug resistance as determined by the Allplex multiplex PCR test.

Table 4.5: It shows SNPs that was targeted from MassArray to detect the mutation

SNPS ANALYSED	REFERENCE ALLELE	ALTERNATIVE ALLELE	PATIENT 1 (INH-R)	PATIENT 2 (RIF-R)
INHA_1674481	T	G	-	A
RPO_B_761110	A	G	-	T
RPOB_761155	C	T	-	
KAT_G_2155168	C	G	-	

4.6 ASSOCIATION OF TB, DRUG RESISTANCE TB AND RISK FACTORS TO RESISTANCE

The table below (Table 4.6) shows the risk factors associated with resistance TB versus non-resistance TB and the significance level. High significance level of association was shown on region, consulting a traditional healer, wearing a protective mask, occupation and sharing a room with family members.

Table 4.6: Socio-Demographics status, Social Behaviours, Cultural Factors, and Stigma among the TB Patients in Vhembe, South Africa

Risk factors		**Resistant TB**	**Non-resistant TB**	**p-value**
Gender	Male		30	
	Female	2	18	0.41
Age	18-35	2	11	1
	36-45		12	
	46-60		15	
	61-80		10	
Education level	Primary		10	
	High school level	1	25	0.04
	University level	1	9	1
	No education		4	
Occupation	Unemployed	1	29	0.01
	Employed		8	
	Self-employed		8	
	Student	1	3	1
Family size (members)	0-4		19	
	5-9	2	29	0.02
Religion	Christianity	2	38	0.002
	African		7	
	other		2	
	Atheist		1	
Consulted traditional healer?	Yes		6	
	No	2	42	0.00057
Remedy from traditional healer?	Yes		5	
	No		1	

	N/A	N/A	42	
Time taken to consult health-care facilities?	≤Three weeks	1	21	0.11
	≥Three weeks	1	27	0.02
Vaccine to prevent TB?	Yes	2	42	0.00057
	No		6	
Sharing TB status with family members?	Yes	2	45	0.0002
	No		3	
Time taken to share TB status with family?	≤ A week	2	44	0.00029
	≥Two weeks and greater		1	
	N/A		3	
Support from family?	Yes	2	43	0.0004
	No		3	
	N/A		2	
Sharing TB status with community members?	Yes		23	
	No	2	25	0.08
	N/A			
Support to consult health care centers?	Yes		21	
	No		2	
	N/A	N/A	25	
Previously treated for active or latent TB?	Yes		12	
	No	2	36	0.003
Sharing a room with family member?	Yes	2	23	0.13
	No		25	
Mode of transport	Public transport	2	29	0.02
	Own car		5	
	Walk		14	
Do you wear protective face mask?	Yes	1	36	0.003
	No	1	12	0.7

Family member with TB?	Yes		12	
	No	2	36	0.003
Spend time in dusty area?	Yes	1	35	0.002
	No	1	13	0.66

4.7 DISCUSSION

The global control of TB has made significant progress in recent years. However, the widespread occurrence of multi-drug resistant tuberculosis (MDR-TB) remains a significant obstacle to effectively managing TB worldwide (Mancuso et al., 2023). A set of 50 sputum specimens were collected from confirmed TB patients receiving care in different healthcare facilities situated in the Vhembe district of Limpopo, South Africa. The demographic distribution is noteworthy, where 60% (30/50) of the study participants were male, aligning with trends observed in a study by Hertz and Schneider (2019). This gender distribution may be explained by occupational factors, highlighting the increased engagement of men in outdoor pursuits, such as mining, in contrast to women who predominantly engage in indoor activities.

The evident patterns of behaviour, notably higher prevalence rates of smoking and alcohol consumption among men, have been identified as contributing factors to an elevated risk of TB (Morojele et al., 2021). Societal norms and expectations may further influence men, potentially leading to a postponement in seeking medical attention, consequently resulting in delayed TB diagnosis and initiation of treatment (Krishnan et al., 2014).

However, the present study observed a higher propensity for females to develop resistance compared to males, although statistical evidence supporting this association was not deemed significant. This finding contrasts with the research conducted by Surkova et al. (2012) and Salari et al. (2023), wherein a substantially higher prevalence of MDR-TB was identified in males as opposed to females.

In the current study, participants within the age cohorts of 18–35, 36-45 and 46–60, each category had higher prevalence exceeding 25%. Remarkably, our study identified a higher prevalence in the demographic surrounding those below the age of 45, in contrast to the observations made by Olmo-Fontánez and Turner (2022), who identified an increased susceptibility to TB among individuals aged 65 and above. This variance may be attributed to the prevalence of risk behaviours, notably substance abuse, alcohol consumption, and smoking, prevalent among the younger age groups. The immunosuppressive effects of substances such as cocaine and methamphetamines, as well as the detrimental impact of smoking on the immune system, can compromise the body's ability to combat infections, including

Mycobacterium *tuberculosis* (MTB), the causative agent of tuberculosis (TB). Smoking-induced damage to the mucociliary clearance system and the respiratory epithelium further facilitates the evasion of TB bacteria from the body's defence mechanisms, enabling infection in the respiratory tract.

The current study revealed that individuals with drug-resistant tuberculosis (DR-TB) fell within the age range of 18-35, although there was no statistically significant evidence supporting this association. Particularly, young individuals, who are often engaged in high-risk activities, have been shown to exhibit poor medication adherence (Du et al., 2020). This lack of adherence may be influenced by the above-mentioned behaviours, contributing to the complex association of factors influencing TB prevalence within this demographic. Addressing TB risk in young populations requires a better understanding of the interconnections between high-risk behaviours, immune system function, and medication adherence to inform targeted public health interventions.

The present study found that 88% (44/50) of the participants had received Bacillus Calmette-Guérin (BCG) vaccination during childhood. Furthermore, individuals with strains exhibiting resistance had a history of BCG vaccination. BCG has been administered sporadically in South Africa since the early 1950s, primarily to schoolage children (Hesseling et al., 2009). Since 1973, the BCG vaccine has been administered to all newborn children universally, with a significantly high rate of coverage. Thus, it is highly possible that South African individuals below the age of 45 have been administered the BCG vaccine, which includes a significant portion of our healthcare workforce. However, Martinez et al. (2022) reported that the efficacy of BCG immunization is most pronounced in children under the age of five, with diminishing effectiveness as individuals age. Notably, our findings align with this observation, suggesting that BCG may confer reduced protection in adulthood. To address this, Martinez et al. (2022) propose booster shots for individuals aged 10 and above, emphasizing the need for supplementary immunization strategies to enhance the vaccine's effectiveness in older age groups.

The study cohort revealed that 52% of participants had achieved a secondary education level, deviating from Przybylski et al. (2014) findings, which indicated a higher incidence of TB among those with lower educational attainment (64.9%). Moreover, the present study identified participants with drug-resistant TB as having attained a secondary education level, with one individual being a university student. While possessing a particular educational level does not guarantee immunity against TB, as the disease is evident across diverse educational backgrounds. Nevertheless, it is anticipated that individuals with higher education levels often exhibit better awareness and health literacy (Davis et al., 2020). They are more likely to be informed about TB, its symptoms, and the significance of prompt medical attention and prevention. This increased awareness can contribute to swifter diagnosis and treatment initiation, thereby reducing overall TB prevalence.

Education can influence health-related behaviours. Individuals with higher educational levels are more inclined to adopt healthy lifestyles, including steering clear of behaviours such as smoking and excessive alcohol consumption, which are linked to an elevated risk of TB (Cutler and Lleras-Muney, 2010). Despite South Africa introducing subjects such as life orientation (LO) learning area as the main platform for imparting health education and including information about TB in schools, in accordance with the Department of Basic Education's National Strategic Plan; the full benefits are perhaps not being realized. A study conducted by George et al. (2018) uncovers obstacles in the implementation of LO. Their results indicate that the implementation and delivery of LO are not being carried out to their fullest potential, due to limitations at multiple levels, including the individual, interpersonal, school, district, and community levels. Furthermore, the scarcity of resources worsens the inconsistencies in educational standards between schools that have sufficient resources and those that lack resources.

The study found that almost 60% (30/50) of participants were without employment. These findings are consistent with the results made by Przybylski et al. (2014), who discovered that a substantial number (54%) of TB patients were without employment, frequently accompanied by a greater occurrence of smoking and alcohol misuse, which in turn led to interruptions in treatment. Unemployment might result in heightened involvement in high-risk activities, such as smoking and alcohol misuse (Henkel, 2011). Research has indicated that unemployed individuals may be more susceptible to these behaviours, which might compromise the immune system and increase the likelihood of contracting TB (Przybylski et al., 2014; Henkel, 2011).

Unemployed individuals frequently encounter financial difficulties, resulting in substandard housing, overcrowding, and poor diet (Chamie et al., 2023). These variables facilitate the spread of TB and can contribute to the development of latent TB infection into active disease. Unemployment frequently correlates with heightened levels of stress and mental health concerns (Paul and Moser, 2009). Persistent stress can weaken the immune system, rendering patients more vulnerable to illnesses, such as TB. Moreover, mental health issues can be a factor in patients not following their TB treatment plans (Acevedo et al., 2020). Unemployment is associated with other social factors that influence health, including poverty and social inequality. These factors contribute to the development of circumstances that promote the transmission of TB and impede the implementation of efficient TB control strategies (Adu et al., 2021; Bhargava et al., 2021). Moreover, in this study, among the participants diagnosed with DR-TB, one individual was unemployed, the above reasons might have contributed to the development of drug resistance.

Ninety percent (45/50) of the participants received family support during their TB treatment, indicating a positive aspect of their care. Notably, Hertz and Schneider (2019) conducted a study on family support training, revealing a significant improvement after a 5-week training period. Given the time-intensive nature of TB therapy and the necessity for patient commitment to completing the treatment course, family support plays a crucial role in this process, as emphasized in previous research (Chen et al., 2020; Ajzen, 1991). In contrast, 54% (27/50) of the patients chose not to disclose their TB diagnosis to colleagues, citing concerns related to stigma. Misconceptions about TB, often rooted in a lack of knowledge and the misconception that TB is incurable or solely associated with HIV, contribute to such stigma (Cremers et al., 2015; Chang and Cataldo, 2014). Both individuals with DR-TB revealed their health status to family members, and during the treatment period, they received support from their families.

Additionally, half of the participants (25/50) share bedrooms with family members, presenting a potential risk for the transmission of TB within households. Family members spending more time near an active TB patient are at increased risk (Eom et al., 2018). Karbito et al. (2022) conducted a study on the tracing of family members of active TB patients, reporting that 63.8% of these family members had latent TB infection. Sharing a room with an active TB patient poses an elevated risk of latent TB progressing to active TB, particularly due to prolonged exposure to the same air within the confined space (Eom et al., 2018). Furthermore, participants diagnosed with DRTB avoid shared habitation with family members, thus diminishing the likelihood of both transmission and acquisition of DR-TB.

The present study reveals that approximately 62% of participants utilize public transportation. A study conducted by Zamudio et al. (2015) reported a slightly lower figure of 53% in their participant cohort. Public transportation networks have been identified as potential sources of TB transmission, with the risk escalating proportionally with exposure time, as noted by Edelson and Phypers (2011). Notably, individuals infected with TB may transmit the infection to fellow passengers, particularly if proper protective measures, such as wearing masks, are not observed. Interestingly, most of the participants in the current study (76%) reported wearing protective masks. Nevertheless, one participant with DR-TB did not adhere to the practice of wearing a mask. The utilization of protective masks has been shown to decrease the risk of DR-TB infection transmission, as evidenced by a study conducted by Dharmadhikari et al. (2012), which reported a 56% reduction in transmission when individuals wore protective masks.

The current study showed that 74% of participants spend time in dusty areas. One of the participants with DR-TB also frequently engages in activities within dusty environments. In contrast, research by Alavi et al. (2014) suggests that soil dust may not significantly influence the prevalence of TB but could impact treatment processes, potentially leading to treatment failure and the development of MDR-TB.

Patients with TB also had co-morbidities such as HIV, diabetes, asthma, ulcer, and hypertension. There was a high prevalence (64%) of HIV positive compared to other co-morbidities . HIV is a major risk factor that leads to progression of active TB among the patients with TB (Gray and Cohn, 2013). Study conducted in Ethiopia by Belay et al. (2015) found 40.4% of TB patients were co-infected with HIV. TB/HIV coinfection enhances a patient's susceptibility to both primary infection reinfection and reactivation latent TB (Kwan and Ernst, 2011).

Co-infection of TB and HIV has been recognized as a substantial risk factor for both illnesses. People with HIV are more vulnerable to TB due to the compromised immune system induced by the HIV virus (Pawlowski et al, 2012). On the other hand, TB infection can also expedite the advancement of HIV to acquired immunodeficiency syndrome (Bhatt et al., 2023). The interaction between TB and HIV results in a combined impact, causing more severe health outcomes compared to when either infection occurs alone (Kwan and Ernst, 2011). TB is a significant contributor to illness and death in individuals with HIV, HIV infection plays a significant role in the worldwide TB crisis (Konstantinidis et al., 2023). The management of TB/HIV co-infection normally involves a complete strategy that incorporates antiretroviral therapy (ART) to regulate HIV, in addition to suitable anti-TB medication (Pooranagangadevi and Padmapriyadarsini, 2022). Rapid identification and concurrent treatment of both illnesses are crucial for enhancing results and easing the overall impact of TB and HIV on affected persons and communities (Bruchfeld et al., 2015). Public health plans frequently prioritize integrated measures to tackle TB and HIV as interrelated health issues.

In 2019, South Africa had one of the highest rates of TB among countries, with an incidence of 615 cases per 100,000 inhabitants (Conan et al., 2022). In 2017, a nationwide assessment on the prevalence of TB revealed an overall TB prevalence rate of 737 cases per 100,000 people, thus confirming the significant burden of TB. The current study 18% (9 /50) of the participants were positive for MTB.

Non-tuberculous mycobacteria (NTM) are found in the environment and have the ability to infect individuals with weakened immune systems as well as those with normal immune systems. However, they are less likely to cause disease compared to MTB (Sharma and Upadhyay, 2020). In the current study a total of 54% of the participants were infected with NTM. NTM species have been implicated in the development of lung diseases in humans, according to recent epidemiological research (Gopalaswamy et al., 2020). Distinguishing between MTB and NTM species in clinical samples poses a considerable difficulty and is frequently deceptive since both MTB and NTMs exhibit positive results in the traditional smear acid-fast staining technique. Consequently, the prevalence of NTM has been inaccurately assessed in numerous areas where TB is widespread (Gopalaswamy et al., 2020). The implementation of Xpert MTB/RIF has significantly transformed TB diagnosis in low-income and middle-income nations, facilitating prompt commencement of treatment and strategies for infection control (Cummings and Schluger, 2018).

The rising prevalence of NTM infections poses a significant global public health risk (Donohue and Wymer, 2016; Thomson and Laboratory, 2010). NTM often exhibit resistance to first line anti-tuberculosis medications. Cases involving co-infections of

MTB and NTM may be misdiagnosed as MDR-TB (Huang et al., 2022). In the current study 18% (9/50) MTB+NTM co-infection.

In the current study 2% (1/50) of the samples was detected with rifampicin resistance (RR), this resistance may occur independently or in combination with resistance to other medications used to treat TB. In 2019, around 465,000 people worldwide contracted RR-TB (Lecai et al., 2021). The development of resistance to RIF in MTB is mostly due to mutations occurring in the rifampicin resistance-determining region (RRDR) of the RNA polymerase β subunit (*rpoB*) gene (Somoskovi et al., 2001). These alterations disrupt the interaction between RIF and the enzyme, reducing the drug's efficacy in suppressing bacterial activity (Traoré et al., 2023). The WHO endorsed the Xpert MTB/RIF assay as the primary diagnostic test for TB in December 2010 (WHO, 2020). Global guidelines typically advocate for utilization of MDR-TB treatment protocols for individuals who are diagnosed with RR-TB (Chakaya et al., 2021; Migliori et al., 2018; Turnbull et al., 2017)

Furthermore 2% (1/50) of the sample were detected to have isoniazid (INH) resistance, INH is an antimicrobial agent employed in the management of TB (Bansal et al., 2018). The resistance to INH seems to be more complex and has been associated with several genes, particularly *katG* and the *inhA* promoter area. *KatG* is a multifunctional enzyme that acts as both a catalase and a peroxidase. This gene mutation is responsible for the DR-TB particularly to INH (Krewall et al., 2020). The presence of S315T mutations in the *katG* gene results in decreased activation of INH, hence diminishing the efficacy of the medication in eradicating MTB (Unissa et al., 2016).

Genotyping was performed on the DNA samples of two drug-resistant participants in this investigation. However, the genotyping of patient 1's DNA sample in Table 4.5 was unsuccessful, while two single nucleotide polymorphisms (SNPs) were successfully genotyped from patient 2. This difference can be related to insufficient DNA amplification. Subsequent analysis of drug-resistant samples using mass-array genotyping indicated the absence of mutations on the two SNPs. This observation suggests the potential involvement of other SNPs within the gene in the development of resistance.

CHAPTER 5

CONCLUSION, LIMITATIONS AND RECOMMENDATIONS

5.1 CONCLUSION

The main objective of this study was to determine the prevalence of drug resistant Tuberculosis (TB) amongst TB patients in the Vhembe district. This was achieved by looking at a set of secondary objectives.

In the 1st secondary objective set to determine the common risk factors associated with drug resistance TB, a survey was used. The results obtained indicate the following risk factors showed high level of significance association: educational level, occupation, religion, dusty area, wearing protective mask and family support.

In the 2nd secondary objective set to determine the prevalence of drug resistance using Allplex™ Multiplex PCR protocol. The samples were subjected to Anyplex™ to determine the presence of TB and then TB positive samples were further analysed with Allplex™ to determine drug-resistant TB. Out of the TB samples analysed 18 (36%) were found to be positive for TB. Furthermore, 2 (4%) were found to have drug resistant (MDR).

In the 3rd secondary objective set to determine MTB mutation, the mutation status of genotypes conferring resistance for INH and RIF were determined using MassArray technique. For Patient 1, there was no detection observed, while in patient 2, no mutations were detected.

In conclusion, MTB is an opportunistic pathogen that relies on the immune system being weak, therefore the current study found that majority of the people who engaged in activities that are likely to cause immune system suppression were at more risk of developing TB, such as men doing outdoor activities. Young people are more at risk of developing TB and DR-TB. Most of the participants were educated. Early diagnosis of drug resistance is necessary to effectively manage patients in considering the emergence and global spread of MDR-TB.

5.2 LIMITATIONS

The current study was limited to the age group of above 18 therefore we cannot confidently report on effectiveness of BCG vaccine children below 18. Furthermore, the present study questionnaire did not include smoking and alcohol behaviour which would fore tell the higher prevalence of TB amongst men. Questionnaire did not include initial date of treatment.

5.3 RECOMMENDATIONS

Execute public awareness initiatives to enlighten the population about TB, its mode of transmission, and the significance of timely identification and treatment. Highlight the importance of adhering to the entire prescribed treatment regimen to avoid the emergence of drug resistance. Allocate resources towards enhancing diagnostic capacities, specifically by ensuring the widespread availability and easy accessibility of speedy and precise TB tests. Prompt and precise diagnosis is essential to promptly begin suitable therapy, minimizing the likelihood of developing MDR-TB. Engage in cooperation with transportation authorities to execute strategies that mitigate the potential for TB transmission on public transit. These measures may involve advocating for increased air circulation, implementing strict mask mandates, and regularly evaluating the health of public transportation employees. Enhance contact tracing initiatives to detect and evaluate persons who might have been into touch with MDR-TB. This is especially crucial in domestic environments where the likelihood of transmission is heightened.

REFERENCES

Abdelwahab, M.T., 2021. Population pharmacokinetic and pharmacodynamic modelling to improve tuberculosis treatment.

Abel, L., Fellay, J., Haas, D.W., Schurr, E., Srikrishna, G., Urbanowski, M., Chaturvedi, N., Srinivasan, S., Johnson, D.H. and Bishai, W.R., 2018. Genetics of human susceptibility to active and latent tuberculosis: present knowledge and future perspectives. *The Lancet infectious diseases*, *18*(3), p.e64-e75.

Acevedo, P., Mora-Urda, A.I. and Montero, P., 2020. Social inequalities in health: duration of unemployment unevenly effects on the health of men and women. *European Journal of Public Health*, *30*(2), p.305-310.

Adane, A., Damena, M., Weldegebreal, F. and Mohammed, H., 2020. Prevalence and associated factors of tuberculosis among adult household contacts of smear positive pulmonary tuberculosis patients treated in public health facilities of Haramaya district, Oromia region, eastern Ethiopia. *Tuberculosis Research and Treatment*, *2020*.

Adu, P.A., Spiegel, J.M. and Yassi, A., 2021. Towards TB elimination: how are macrolevel factors perceived and addressed in policy initiatives in a high burden country?. *Globalization and Health*, *17*(1), p.1-11.

Ajzen, I., 1991. The theory of planned behavior. *Organizational behavior and human decision processes*, *50*(2), p.179-211.

Alavi, S.M., Bakhtiyariniya, P., Eghtesad, M. and Salmanzadeh, S., 2014. Prevalence of pulmonary tuberculosis before and after soil dust in Khuzestan, southwest Iran. *Caspian Journal of Internal Medicine*, *5*(4), p.190.

Allué-Guardia, A., García, J.I. and Torrelles, J.B., 2021. Evolution of drug-resistant Mycobacterium tuberculosis strains and their adaptation to the human lung environment. *Frontiers in Microbiology*, *12*, p.612675.

AlMatar, M., Var, I., Kayar, B. and Köksal, F., 2020. Differential expression of resistant and efflux pump genes in MDR-TB isolates. *Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders)*, *20*(2), p.271-287.

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

Ando, H., Kitao, T., Miyoshi-Akiyama, T., Kato, S., Mori, T. and Kirikae, T., 2011. Downregulation of katG expression is associated with isoniazid resistance in Mycobacterium tuberculosis. Molecular microbiology, 79(6), pp.1615-1628.

Banerjee, A., Dubnau, E., Quemard, A., Balasubramanian, V., Um, K.S., Wilson, T., Collins, D., De Lisle, G. and Jacobs Jr, W.R., 1994. inhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. *Science*, *263*(5144), p.227230.

Bansal, R., Sharma, D. and Singh, R., 2018. Tuberculosis and its treatment: an overview. *Mini reviews in medicinal chemistry*, *18*(1), p.58-71.

Baral, S.C., Karki, D.K. and Newell, J.N., 2007. Causes of stigma and discrimination associated with tuberculosis in Nepal: a qualitative study. *BMC public health*, *7*, p.1-10.

Batista, L.A.F., Silva, K.J.S., e Silva, L.M.D.C., de Moura, Y.F. and Zucchi, F.C.R., 2020. Tuberculosis: A granulomatous disease mediated by epigenetic factors. *Tuberculosis*, *123*, p.101943.

Belay, M., Bjune, G. and Abebe, F., 2015. Prevalence of tuberculosis, HIV, and TBHIV co-infection among pulmonary tuberculosis suspects in a predominantly pastoralist area, northeast Ethiopia. *Global health action*, *8*(1), p.27949.

Bhargava, A., Bhargava, M. and Juneja, A., 2021. Social determinants of tuberculosis: context, framework, and the way forward to ending TB in India. *Expert Review of Respiratory Medicine*, *15*(7), p.867-883.

Bhatt, A., Syed, Z.Q. and Singh, H., 2023. Converging Epidemics: A Narrative Review of Tuberculosis (TB) and Human Immunodeficiency Virus (HIV) Coinfection. *Cureus*, *15*(10).

Boehme, C.C., Nabeta, P., Hillemann, D., Nicol, M.P., Shenai, S., Krapp, F., Allen, J., Tahirli, R., Blakemore, R., Rustomjee, R. and Milovic, A., 2010. Rapid molecular detection of tuberculosis and rifampin resistance. *New England Journal of Medicine*, *363*(11), p.1005-1015.

Borah, P., Deb, P.K., Venugopala, K.N., Al-Shar'i, N.A., Singh, V., Deka, S., Srivastava, A., Tiwari, V. and Mailavaram, R.P., 2021. Tuberculosis: an update on pathophysiology, molecular mechanisms of drug resistance, newer anti-TB drugs, treatment regimens and host-directed therapies. *Current Topics in Medicinal Chemistry*, *21*(6), p.547-570.

Bruchfeld, J., Correia-Neves, M. and Källenius, G., 2015. Tuberculosis and HIV coinfection. *Cold Spring Harbor perspectives in medicine*, p.a017871.

Busó, E.J. and Iborra, M., 2016. Sequenom MassARRAY Technology for the Analysis of DNA Methylation: Clinical Applications. In *Epigenetic Biomarkers and Diagnos*137153). Academic Press.

Carlos Palomino, J. and Martin, A., 2013. Tuberculosis clinical trial update and the current anti-tuberculosis drug portfolio. *Current medicinal chemistry*, *20*(30), p.37853796.

Caulfield, A.J. and Wengenack, N.L., 2016. Diagnosis of active tuberculosis disease: From microscopy to molecular techniques. *Journal of Clinical Tuberculosis and Other Mycobacterial Diseases*, *4*, pp.33-43.

Caws, M., Duy, P.M., Tho, D.Q., Lan, N.T.N., Hoa, D.V. and Farrar, J., 2006. Mutations prevalent among rifampin-and isoniazid-resistant Mycobacterium tuberculosis isolates from a hospital in Vietnam. *Journal of clinical microbiology*, *44*(7), p.2333-2337.

Chakaya, J., Khan, M., Ntoumi, F., Aklillu, E., Fatima, R., Mwaba, P., Kapata, N., Mfinanga, S., Hasnain, S.E., Katoto, P.D. and Bulabula, A.N., 2021. Global Tuberculosis Report 2020–Reflections on the Global TB burden, treatment and prevention efforts. *International journal of infectious diseases*, *113*, p.S7-S12.

Chamie, G., Hahn, J.A., Kekibiina, A., Emenyonu, N.I., Beesiga, B., Marson, K., Fatch, R., Lodi, S., Adong, J., Thirumurthy, H. and McDonell, M.G., 2023. Financial incentives for reduced alcohol use and increased isoniazid adherence during tuberculosis preventive therapy among people with HIV in Uganda: an open-label, factorial randomised controlled trial. *The Lancet Global Health*, *11*(12), p.e1899-e1910.

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), p.168-173.

Chen, X., Du, L., Wu, R., Xu, J., Ji, H., Zhang, Y., Zhu, X. and Zhou, L., 2020. The effects of family, society and national policy support on treatment adherence among newly diagnosed tuberculosis patients: a cross-sectional study. *BMC infectious diseases*, *20*(1), p.1-11.

Chien, H.P., Yu, M.C., Wu, M.H., Lin, T.P. and Luh, K.T., 2000. Comparison of the BACTEC MGIT 960 with Löwenstein-Jensen medium for recovery of mycobacteria from clinical specimens. *The International Journal of Tuberculosis and Lung Disease*, *4*(9), p.866-870.

Chin, K.L., Sarmiento, M.E., Norazmi, M.N. and Acosta, A., 2018. DNA markers for tuberculosis diagnosis. *Tuberculosis*, *113*, p.139-152.

Chisompola, N.K., Streicher, E.M., Muchemwa, C.M.K., Warren, R.M. and Sampson, S.L., 2020. Molecular epidemiology of drug resistant Mycobacterium tuberculosis in Africa: a systematic review. *BMC Infectious Diseases*, *20*(1), p.1-16.

Conan, N., Simons, E., Ohler, L., Mbatha, M., Van Cutsem, G. and Huerga, H., 2022. Prevalence of TB and health-seeking behaviour. *The International Journal of Tuberculosis and Lung Disease*, *26*(5), p.463.

Courtwright, A. and Turner, A.N., 2010. Tuberculosis and stigmatization: pathways and interventions. *Public health reports*, *125*(4_suppl), p.34-42.

Cox, H., Dickson-Hall, L., Jassat, W., Moshabela, M., Kielmann, K., Grant, A., Nicol, M., Black, J., Mlisana, K., Vanleeuw, L. and Loveday, M., 2017. Drug-resistant tuberculosis in South Africa: history, progress and opportunities for achieving universal access to diagnosis and effective treatment. South African Health Review, 2017(1), p.157-167.

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., Mo, C.Y. and Kohli, R.M., 2015. Targets for combating the evolution of acquired antibiotic resistance. *Biochemistry*, *54*(23), p.3573-3582.

Cummings, M.J. and Schluger, N.W., 2018. The diagnosis of pulmonary tuberculosis: Established and emerging approaches for clinicians in high-income and low-income settings. *Clinical Pulmonary Medicine*, *25*(5), p.170-176.

Cutler, D.M. and Lleras-Muney, A., 2010. Understanding differences in health behaviors by education. *Journal of health economics*, *29*(1), p.1-28.

Davis, S.N., Wischhusen, J.W., Sutton, S.K., Christy, S.M., Chavarria, E.A., Sutter, M.E., Roy, S., Meade, C.D. and Gwede, C.K., 2020. Demographic and psychosocial factors associated with limited health literacy in a community-based sample of older Black Americans. *Patient education and counseling*, *103*(2), p.385-391.

De Jong, B.C., Antonio, M. and Gagneux, S., 2010. Mycobacterium africanum—review of an important cause of human tuberculosis in West Africa. PLoS neglected tropical diseases, 4(9), p.e744.

Dharmadhikari, A.S., Mphahlele, M., Stoltz, A., Venter, K., Mathebula, R., Masotla, T., Lubbe, W., Pagano, M., First, M., Jensen, P.A. and van der Walt, M., 2012. Surgical face masks worn by patients with multidrug-resistant tuberculosis: impact on infectivity of air on a hospital ward. *American journal of respiratory and critical care medicine*, *185*(10), p.1104-1109.

Donohue, M.J. and Wymer, L., 2016. Increasing prevalence rate of nontuberculous mycobacteria infections in five states, 2008–2013. *Annals of the American Thoracic Society*, *13*(12), p.2143-2150.

Drobniewski, F., Nikolayevskyy, V., Maxeiner, H., Balabanova, Y., Casali, N., Kontsevaya, I. and Ignatyeva, O., 2013. Rapid diagnostics of tuberculosis and drug resistance in the industrialized world: clinical and public health benefits and barriers to implementation. *BMC medicine*, *11*, p.1-11.

Du, L., Chen, X., Zhu, X., Zhang, Y., Wu, R., Xu, J., Ji, H., Zhou, L. and Lu, X., 2020. Determinants of medication adherence for pulmonary tuberculosis patients during continuation phase in Dalian, Northeast China. *Patient preference and adherence*, p.1119-1128.

Dye, C., Scheele, S., Pathania, V. and Raviglione, M.C., 1999. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. *Jama*, *282*(7), p.677-686.

Ellis, J.A. and Ong, B., 2017. The MassARRAY® system for targeted SNP genotyping. *Genotyping: Methods and Protocols*, p.77-94.

Eom, J.S., Kim, I., Kim, W.Y., Jo, E.J., Mok, J., Kim, M.H., Lee, K., Kim, K.U., Park, H.K. and Lee, M.K., 2018. Household tuberculosis contact investigation in a tuberculosis-prevalent country: Are the tuberculin skin test and interferon-gamma release assay enough in elderly contacts?. *Medicine*, *97*(3).

Falkinham III, J.O., 2018. Challenges of NTM drug development. *Frontiers in microbiology*, *9*, p.1613.

Falkinham, J.O., 2015. Environmental sources of nontuberculous mycobacteria. *Clinics in chest medicine*, *36*(1), p.35-41.

Falzon, D., Schünemann, H.J., Harausz, E., González-Angulo, L., Lienhardt, C., Jaramillo, E. and Weyer, K., 2017. World Health Organization treatment guidelines for drug-resistant tuberculosis, 2016 update. *European Respiratory Journal*, *49*(3).

Fukunaga, R., Glaziou, P., Harris, J.B., Date, A., Floyd, K. and Kasaeva, T., 2021. Epidemiology of tuberculosis and progress toward meeting global targets—worldwide, 2019. *Morbidity and Mortality Weekly Report*, *70*(12), p.427.

Garrido-Cardenas, J.A., de Lamo-Sevilla, C., Cabezas-Fernández, M.T., ManzanoAgugliaro, F. and Martínez-Lirola, M., 2020. Global tuberculosis research and its future prospects. *Tuberculosis*, *121*, p.101917.

George, G., Tucker, L.A., Panday, S. and Khumalo, F., 2018. Challenges facing life orientation educators in the delivery of sexuality education in South African schools. *Southern African Review of Education with Education with Production*, *24*(1), p.43-57.

Gopalaswamy, R., Shanmugam, S., Mondal, R. and Subbian, S., 2020. Of tuberculosis and non-tuberculous mycobacterial infections–a comparative analysis of epidemiology, diagnosis and treatment. *Journal of biomedical science*, *27*(1), p.1-17.

Gordhan, B.G., Peters, J.S., McIvor, A., Machowski, E.E., Ealand, C., Waja, Z., Martinson, N., and Kana, B.D., 2021. Detection of differentially culturable tubercle bacteria in sputum using mycobacterial culture filtrates. *Scientific Reports*, *11*(1), p.6493.

Gray, J.M. and Cohn, D.L., 2013, February. Tuberculosis and HIV coinfection.

In *Seminars in respiratory and critical care medicine* (Vol. 34, No. 01, p. 032-043). Thieme Medical Publishers.

Guo, H., Seet, Q., Denkin, S., Parsons, L. and Zhang, Y., 2006. Molecular characterization of isoniazid-resistant clinical isolates of Mycobacterium tuberculosis from the USA. *Journal of medical microbiology*, *55*(11), p.1527-1531.

Habib, S.S., Jamal, W.Z., Zaidi, S.M.A., Siddiqui, J.U.R., Khan, H.M., Creswell, J., Batra, S. and Versfeld, A., 2021. Barriers to access of healthcare services for rural women—applying gender lens on TB in a rural district of Sindh, Pakistan. *International Journal of Environmental Research and Public Health*, *18*(19), p.10102.

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.

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*.

Hazbón, M.H., Brimacombe, M., Bobadilla del Valle, M., Cavatore, M., Guerrero, M.I., Varma-Basil, M., Billman-Jacobe, H., Lavender, C., Fyfe, J., García-García, L. and León, C.I., 2006. Population genetics study of isoniazid resistance mutations and evolution of multidrug-resistant Mycobacterium tuberculosis. *Antimicrobial agents and chemotherapy*, *50*(8), p.2640-2649.

Heemskerk, D., Caws, M., Marais, B. and Farrar, J., 2015. Tuberculosis in adults and children.

Henkel, D., 2011. Unemployment and substance use: a review of the literature (19902010). *Current drug abuse reviews*, *4*(1), p.4-27.

Hertz, D. and Schneider, B., 2019, March. Sex differences in tuberculosis. In *Seminars in i* Mariani, H., Afriandi, I., Setiawati, E.P., Gondodiputro, S., Wiwaha, G., Nataprawira, H.M., Arisanti, N. and Sumantri, S., 2022. Tuberculosis Family Support

Training's (TB FaST) Influence on Encouraging TB Treatment Compliance. *The Open Public Health Journal*, *15*(1). *mmunopathology* (Vol. 41, p. 225-237). Springer Berlin Heidelberg.

Hesseling, A.C., Caldwell, J., Cotton, M.F., Eley, B.S., Jaspan, H.B., Jennings, K., Marais, B.J., Nuttall, J., Rabie, H., Roux, P. and Schaaf, H.S., 2009. BCG vaccination in South African HIV-exposed infants: risks and benefits. *SAMJ: South African Medical Journal*, *99*(2), p.88-91.

Hirsh, A.E., Tsolaki, A.G., DeRiemer, K., Feldman, M.W. and Small, P.M., 2004. Stable association between strains of Mycobacterium tuberculosis and their human host populations. *Proceedings of the National Academy of Sciences*, *101*(14), p.48714876.

Honda, J.R., Virdi, R. and Chan, E.D., 2018. Global environmental nontuberculous mycobacteria and their contemporaneous man-made and natural niches. *Frontiers in microbiology*, *9*, p.2029.

Huang, M., Tan, Y., Zhang, X., Wang, Y., Su, B., Xue, Z., Wang, J. and Pang, Y., 2022. Effect of mixed infections with Mycobacterium tuberculosis and nontuberculous Mycobacteria on diagnosis of multidrug-resistant tuberculosis: A retrospective multicentre study in China. *Infection and Drug Resistance*, p.157-166.

Huang, Y., Ai, L., Wang, X., Sun, Z. and Wang, F., 2022. Review and Updates on the Diagnosis of Tuberculosis. *Journal of Clinical Medicine*, *11*(19), p.5826.

Huq, K.E., Moriyama, M., Krause, D., Shirin, H., Awoonor-Willaims, J.K., Rahman, M. and Rahman, M.M., 2022. Perceptions, Attitudes, Experiences and Opinions of Tuberculosis Associated Stigma: A Qualitative Study of the Perspectives among the Bolgatanga Municipality People of Ghana. *International Journal of Environmental Research and Public Health*, *19*(22), p.14998.

Iqbal, F., Defer, M.K., Latif, A. and Hadi, H., 2020. Understanding how geographic, demographic and treatment history impact health outcomes of patients with multi-drugresistant tuberculosis in Pakistan, 2014–2017. *Epidemiology & Infection*, *148*, p.e253.

Irfan, S.D., Faruque, M.O., Islam, M.U., Sanjoy, S.S., Afrin, D. and Hossain, A., 2017. Socio-demographic determinants of adult tuberculosis: a matched case-control study in Bangladesh. *Am J Infect Dis*, *13*(3), p.32-37.

Ismail, N.A., Ismail, M.F., Noor, S.S.M. and Camalxaman, S.N., 2016. Genotypic detection of rpoB and katG gene mutations associated with rifampicin and isoniazid resistance in Mycobacterium tuberculosis isolates: A local scenario (Kelantan). *The Malaysian journal of medical sciences: MJMS*, *23*(1), p.22.

Ismail, N.A., Mvusi, L., Nanoo, A., Dreyer, A., Omar, S.V., Babatunde, S., Molebatsi, T., Van der Walt, M., Adelekan, A., Deyde, V. and Ihekweazu, C., 2018. Prevalence of drug-resistant tuberculosis and imputed burden in South Africa: a national and subnational cross-sectional survey. The Lancet Infectious Diseases, 18(7), p.779-787.

Iwamoto, T., Sonobe, T. and Hayashi, K., 2003. Loop-mediated isothermal amplification for direct detection of Mycobacterium tuberculosis complex, M. avium, and M. intracellulare in sputum samples. *Journal of clinical microbiology*, *41*(6), p.2616-2622.

Jang, J.G. and Chung, J.H., 2020. Diagnosis and treatment of multidrug-resistant tuberculosis. *Yeungnam University Journal of Medicine*, *37*(4), p.277-285.

Jhun, B.W. and Koh, W.J., 2020. Treatment of isoniazid-resistant pulmonary tuberculosis. *Tuberculosis and Respiratory Diseases*, *83*(1), p.20-30.

Johnson, M.M. and Odell, J.A., 2014. Nontuberculous mycobacterial pulmonary infections. *Journal of thoracic disease*, *6*(3), p.210.

Karbito, K., Susanto, H., Adi, M.S., Sulistiyani, S., Handayani, O.W.K. and Sofro, M.A.U., 2022. Latent tuberculosis infection in family members in household contact with active tuberculosis patients in Semarang City, Central Java, Indonesia. *Journal of Public Health in Africa*, *13*(2).

Kirkhorn, S.R. and Garry, V.F., 2000. Agricultural lung diseases. *Environmental health perspectives*, *108*(suppl 4), p.705-712.

Konstantinidis, I., Crothers, K., Kunisaki, K.M., Drummond, M.B., Benfield, T., Zar, H.J., Huang, L. and Morris, A., 2023. HIV-associated lung disease. *Nature Reviews Disease Primers*, *9*(1), p.39.

Krewall, J.R., Minton, L.E. and Goodwin, D.C., 2020. KatG Structure and Mechanism: Using Protein-Based Oxidation to Confront the Threats of Reactive Oxygen.

In *Mechanistic Enzymology: Bridging Structure and Function* (p. 83-120). American Chemical Society.

Krishnakumariamma, K., Ellappan, K., Muthuraj, M., Tamilarasu, K., Kumar, S.V. and Joseph, N.M., 2020. Molecular diagnosis, genetic diversity and drug sensitivity patterns of Mycobacterium tuberculosis strains isolated from tuberculous meningitis patients at a tertiary care hospital in South India. *PloS one*, *15*(10), p.e0240257.

Krishnan, L., Akande, T., Shankar, A.V., McIntire, K.N., Gounder, C.R., Gupta, A. and Yang, W.T., 2014. Gender-related barriers and delays in accessing tuberculosis diagnostic and treatment services: a systematic review of qualitative studies. *Tuberculosis research and treatment*, *2014*.

Kumar, S. and Jena, L., 2014. Understanding rifampicin resistance in tuberculosis through a computational approach. *Genomics & informatics*, *12*(4), p.276.

Kwan, C.K. and Ernst, J.D., 2011. HIV and tuberculosis: a deadly human syndemic. Clinical microbiology reviews, 24(2), p.351-376.

Lange, C., Aarnoutse, R.E., Alffenaar, J.W.C., Bothamley, G., Brinkmann, F., Costa, J., Chesov, D., Van Crevel, R., Dedicoat, M., Dominguez, J. and Duarte, R., 2019. Management of patients with multidrug-resistant tuberculosis. *The international journal of tuberculosis and lung disease*, *23*(6), p.645-662.

Law, S., 2019. *Exploring TB patient journeys and the emergence of MDR-TB in India and South Africa: A multi-methods study*. McGill University (Canada).

Lecai, J., Mijiti, P., Chuangyue, H., Mingzhen, L., Qian, G., Weiguo, T. and Jihong, C., 2021. Predictors and trends of MDR/RR-TB in Shenzhen China: a retrospective 2012–2020 period analysis. *Infection and Drug Resistance*, p.4481-4491.

Lee, R.S. and Pai, M., 2017. Real-time sequencing of Mycobacterium tuberculosis: are we there yet?. *Journal of clinical microbiology*, *55*(5), p.1249-1254.

Lesnik, E. and Nigulyanu, A., 2021. The peculiarities of the patients with monoresistant and poly-resistant tuberculosis. *Arta Medica*, *81*(4), p.4-11.

Lönnroth, K., Jaramillo, E., Williams, B., Dye, C. and Raviglione, M., 2010. Tuberculosis: the role of risk factors and social determinants. Equity, social determinants and public health programmes, 219, p.241.

Maggi, N., Pasqualucci, C.R., Ballotta, R. and Sensi, P., 1966. Rifampicin: a new orally active rifamycin. *Chemotherapy*, *11*(5), p.285-292.

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), p.350-352.

Malenfant, J.H. and Brewer, T.F., 2021, February. Rifampicin mono-resistant tuberculosis—a review of an uncommon but growing challenge for global tuberculosis control. In *Open forum infectious diseases* (Vol. 8, No. 2, p. ofab018). US: Oxford University Press.

Mancuso, G., Midiri, A., De Gaetano, S., Ponzo, E. and Biondo, C., 2023. Tackling drug-resistant tuberculosis: new challenges from the old pathogen Mycobacterium tuberculosis. *Microorganisms*, *11*(9), p.2277.

Manzoor, S. and Ganie, Z.A., 2015. Social Stigma, Discrimina-tion, Marginalization and Prejudice towards Persons with Tuberculosis: A Systematic Review Study. *Int J Clin Med Allergy*, *3*(2), p.14-18.

Maphalle, L.N., Michniak-Kohn, B.B., Ogunrombi, M.O. and Adeleke, O.A., 2022. Pediatric Tuberculosis Management: A Global Challenge or Breakthrough?. *Children*, *9*(8), p.1120.

Mason, P.H., Roy, A., Spillane, J. and Singh, P., 2016. Social, historical and cultural dimensions of tuberculosis. *Journal of biosocial science*, *48*(2), p.206-232.

Miggiano, R., Rizzi, M. and Ferraris, D.M., 2020. Mycobacterium tuberculosis pathogenesis, infection prevention and treatment. *Pathogens*, *9*(5), p.385.

Migliori, G.B., Sotgiu, G., Rosales-Klintz, S., Centis, R., D'Ambrosio, L., Abubakar, I., Bothamley, G., Caminero, J.A., Cirillo, D.M., Dara, M. and De Vries, G., 2018. ERS/ECDC Statement: European Union standards for tuberculosis care, 2017 update. *European Respiratory Journal*, *51*(5).

Mirzayev, F., Viney, K., Linh, N.N., Gonzalez-Angulo, L., Gegia, M., Jaramillo, E., Zignol, M. and Kasaeva, T., 2021. World Health Organization recommendations on the treatment of drug-resistant tuberculosis, 2020 update. *European Respiratory Journal*, *57*(6).

Moir, S., Chun, T.W. and Fauci, A.S., 2011. Pathogenic mechanisms of HIV disease. *Annual review of pathology: mechanisms of disease*, *6*, p.223-248.

Morlock, G.P., Metchock, B., Sikes, D., Crawford, J.T. and Cooksey, R.C., 2003. ethA, inhA, and katG loci of ethionamide-resistant clinical Mycobacterium tuberculosis isolates. *Antimicrobial agents and chemotherapy*, *47*(12), p.3799-3805.

Morojele, N.K., Shenoi, S.V., Shuper, P.A., Braithwaite, R.S. and Rehm, J., 2021. Alcohol use and the risk of communicable diseases. *Nutrients*, *13*(10), p.3317.

Murray, J.F., Schraufnagel, D.E. and Hopewell, P.C., 2015. Treatment of tuberculosis. A historical perspective. *Annals of the American Thoracic Society*, *12*(12), p.17491759.

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

Nathavitharana, R.R., Cudahy, P.G., Schumacher, S.G., Steingart, K.R., Pai, M. and Denkinger, C.M., 2017. Accuracy of line probe assays for the diagnosis of pulmonary and multidrug-resistant tuberculosis: a systematic review and metaanalysis. *European Respiratory Journal*, *49*(1).

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

Nguyen, T.N.A., Anton-Le Berre, V., Bañuls, A.L. and Nguyen, T.V.A., 2019. Molecular diagnosis of drug-resistant tuberculosis; a literature review. *Frontiers in microbiology*, *10*, p.794.

Niehaus, A.J., Mlisana, K., Gandhi, N.R., Mathema, B. and Brust, J.C., 2015. High Prevalence of inh A Promoter Mutations among Patients with Drug-Resistant Tuberculosis in KwaZulu-Natal, South Africa. PloS one, 10(9), p.e0135003.

Ochang, E.A., Udoh, U.A., Emanghe, U.E., Tiku, G.O., Offor, J.B., Odo, M., Nkombe, E., Owuna, O.E., Obeten, S.M. and Meremikwu, M.M., 2016. Evaluation of rifampicin resistance and 81-bp rifampicin resistant determinant region of rpoB gene mutations of Mycobacterium tuberculosis detected with XpertMTB/Rif in Cross River State, Nigeria. *International journal of mycobacteriology*, *5*, p.S145-S146.

Ocheretina, O., Escuyer, V.E., Mabou, M.M., Royal-Mardi, G., Collins, S., Vilbrun, S.C., Pape, J.W. and Fitzgerald, D.W., 2014. Correlation between genotypic and phenotypic testing for resistance to rifampin in Mycobacterium tuberculosis clinical isolates in Haiti: investigation of cases with discrepant susceptibility results. *PloS one*, *9*(3), p.e90569.

Ogbuoji, O., Qi, J., Olson, Z.D., Yamey, G., Nugent, R., Norheim, O.F., Verguet, S. and Jamison, D.T., 2018. Annual Rates of Decline in Child, Maternal, Tuberculosis, and Noncommunicable Disease Mortality across 109 Low-and Middle-Income Countries from 1990 to 2015.

Olivier, C. and Luies, L., 2023. WHO Goals and Beyond: Managing HIV/TB Coinfection in South Africa. *SN Comprehensive Clinical Medicine*, *5*(1), p.251.

Olmo-Fontánez, A.M. and Turner, J., 2022. Tuberculosis in an aging world. Pathogens, 11(10), p.1101.

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

Paul, K.I. and Moser, K., 2009. Unemployment impairs mental health: Metaanalyses. *Journal of Vocational behavior*, *74*(3), p.264-282.

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

Pereira, A.C., Ramos, B., Reis, A.C. and Cunha, M.V., 2020. Non-tuberculous mycobacteria: Molecular and physiological bases of virulence and adaptation to ecological niches. *Microorganisms*, *8*(9), p.1380.

Piccaro, G., Pietraforte, D., Giannoni, F., Mustazzolu, A. and Fattorini, L., 2014. Rifampin induces hydroxyl radical formation in Mycobacterium tuberculosis. *Antimicrobial agents and chemotherapy*, *58*(12), p.7527-7533.

Pooranagangadevi, N. and Padmapriyadarsini, C., 2022. Treatment of tuberculosis and the drug interactions associated with HIV-TB co-infection treatment. *Frontiers in Tropical Diseases*, *3*, p.834013.

Prasad, R., Gupta, N. and Banka, A., 2018. Multidrug-resistant tuberculosis/rifampicinresistant tuberculosis: Principles of management. *Lung India: official organ of Indian chest society*, *35*(1), p.78.

Rageade, F., Picot, N., Blanc-Michaud, A., Chatellier, S., Mirande, C., Fortin, E. and Van Belkum, A., 2014. Performance of solid and liquid culture media for the detection of Mycobacterium tuberculosis in clinical materials: meta-analysis of recent studies. *European Journal of Clinical Microbiology & Infectious Diseases*, *33*, p.867870.

Rahlwes, K.C., Dias, B.R., Campos, P.C., Alvarez-Arguedas, S. and Shiloh, M.U., 2023. Pathogenicity and virulence of Mycobacterium tuberculosis. *Virulence*, *14*(1), p.2150449.

Ramaliba, T.M., Tshitangano, T.G., Akinsola, H.A. and Thendele, M., 2017. Tuberculosis risk factors in Lephalale local municipality of Limpopo province, South Africa. South African Family Practice, 59(5), p.182-187.

Ramaswamy, S. and Musser, J.M., 1998. Molecular genetic basis of antimicrobial agent resistance inMycobacterium tuberculosis: 1998 update. *Tubercle and Lung disease*, *79*(1), p.3-29.

Ramaswamy, S.V., Reich, R., Dou, S.J., Jasperse, L., Pan, X., Wanger, A., Quitugua, T. and Graviss, E.A., 2003. Single nucleotide polymorphisms in genes associated with isoniazid resistance in Mycobacterium tuberculosis. *Antimicrobial agents and chemotherapy*, *47*(4), p.1241-1250.

Rojas Echenique, J.I., Kryazhimskiy, S., Nguyen Ba, A.N. and Desai, M.M., 2019. Modular epistasis and the compensatory evolution of gene deletion mutants. *PLoS Genetics*, *15*(2), p.e1007958.

Romha, G., Gebru, G., Asefa, A. and Mamo, G., 2018. Epidemiology of Mycobacterium bovis and Mycobacterium tuberculosis in animals: Transmission dynamics and control challenges of zoonotic TB in Ethiopia. *Preventive veterinary medicine*, *158*, p.1-17.

Salari, N., Kanjoori, A.H., Hosseinian-Far, A., Hasheminezhad, R., Mansouri, K. and Mohammadi, M., 2023. Global prevalence of drug-resistant tuberculosis: a systematic review and meta-analysis. *Infectious Diseases of Poverty*, *12*(1), p.1-12.

Sandgren, A., Strong, M., Muthukrishnan, P., Weiner, B.K., Church, G.M. and Murray, M.B., 2009. Tuberculosis drug resistance mutation database. *PLoS medicine*, *6*(2), p.e1000002.

Seung, K.J., Keshavjee, S. and Rich, M.L., 2015. Multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. *Cold Spring Harbor perspectives in medicine*, *5*(9).

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), p.243-253.

Sharma, D., Sharma, J., Deo, N. and Bisht, D., 2018. Prevalence and risk factors of tuberculosis in developing countries through health care workers. *Microbial pathogenesis*, *124*, p.279-283.

Sharma, S.K. and Upadhyay, V., 2020. Epidemiology, diagnosis & treatment of nontuberculous mycobacterial diseases. *The Indian journal of medical research*, *152*(3), p.185.

Sheikh, B.A., Bhat, B.A., Mehraj, U., Mir, W., Hamadani, S. and Mir, M.A., 2021. Development of new therapeutics to meet the current challenge of drug resistant tuberculosis. *Current pharmaceutical biotechnology*, *22*(4), p.480-500.

Shibabaw, A., Gelaw, B., Gebreyes, W., Robinson, R., Wang, S.H. and Tessema, B., 2020. The burden of pre-extensively and extensively drug-resistant tuberculosis among MDR-TB patients in the Amhara region, Ethiopia. *PloS one*, *15*(2), p.e0229040.

Siddiqi, S., Ahmed, A., Asif, S., Behera, D., Javaid, M., Jani, J., Jyoti, A., Mahatre, R., Mahto, D., Richter, E. and Rodrigues, C., 2012. Direct drug susceptibility testing of Mycobacterium tuberculosis for rapid detection of multidrug resistance using the Bactec MGIT 960 system: a multicenter study. *Journal of clinical microbiology*, *50*(2), p.435-440.

Singh, A., Gupta, A.K. and Singh, S., 2020. Molecular mechanisms of drug resistance in Mycobacterium tuberculosis: Role of nanoparticles against multi-drug-resistant tuberculosis (MDR-TB). *NanoBioMedicine*, p.285-314.

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), p.1547-1567.

Singh, V. and Chibale, K., 2021. Strategies to combat multi-drug resistance in tuberculosis. *Accounts of chemical research*, *54*(10), p.2361-2376.

Somoskovi, A., Parsons, L.M. and Salfinger, M., 2001. The molecular basis of resistance to isoniazid, rifampin, and pyrazinamide in Mycobacterium tuberculosis. *Respiratory research*, *2*, p.1-5.

Srivastava, K., Kant, S. and Verma, A., 2015. Role of environmental factors in transmission of tuberculosis. *Dynamics of Human Health*, *2*(4), p.12.

Steingart, K.R., Ng, V., Henry, M., Hopewell, P.C., Ramsay, A., Cunningham, J., Urbanczik, R., Perkins, M.D., Aziz, M.A. and Pai, M., 2006. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. *The Lancet infectious diseases*, *6*(10), p.664-674.

Stephanie F, Saragih M, Tambunan US. Recent progress and challenges for drugresistant tuberculosis treatment. Pharmaceutics. 2021 Apr 21;13(5):592.

Surkova, L., Horevich, H.L., Titov, L.P., Sahalchyk, E., Arjomandzadegan, M., Alinejad, S. and Sadrnia, M., 2012. A study on demographic characteristics of drug resistant Mycobacterium tuberculosis isolates in Belarus. *International journal of mycobacteriology*, *1*(2), p.75-81.

Telenti, A., Imboden, P., Marchesi, F., Matter, L., Schopfer, K., Bodmer, T., Lowrie, D., Colston, M.J. and Cole, S., 1993. Detection of rifampicin-resistance mutations in Mycobacterium tuberculosis. *The Lancet*, *341*(8846), p.647-651.

Thirumurugan, R., Kathirvel, M., Vallayyachari, K., Surendar, K., Samrot, A.V. and Muthaiah, M., 2015. Molecular analysis of rpoB gene mutations in rifampicin resistant Mycobacterium tuberculosis isolates by multiple allele specific polymerase chain reaction in Puducherry, South India. *Journal of Infection and Public Health*, *8*(6), p.619-625.

Thomson, R.M. and Laboratory, Q.M.R., 2010. Changing epidemiology of pulmonary nontuberculous mycobacteria infections. *Emerging infectious diseases*, *16*(10), p.1576.

Tiberi, S., Utjesanovic, N., Galvin, J., Centis, R., D'Ambrosio, L., van den Boom, M., Zumla, A. and Migliori, G.B., 2022. Drug resistant TB–latest developments in epidemiology, diagnostics and management. *International Journal of Infectious Diseases*, *124*, pp.S20-S25.

Traoré, A.N., Rikhotso, M.C., Mphaphuli, M.A., Patel, S.M., Mahamud, H.A., Kachienga, L.O., Kabue, J.P. and Potgieter, N., 2023. Isoniazid and Rifampicin Resistance-Conferring Mutations in Mycobacterium tuberculosis Isolates from South Africa. *Pathogens*, *12*(8), p.1015.

Turnbull, L., Bell, C. and Child, F., 2017. Tuberculosis (NICE clinical guideline 33). *Archives of Disease in Childhood-Education and Practice*, *102*(3), p.136-142.

Twumasi, C., Asiedu, L. and Nortey, E.N., 2019. Markov chain modeling of HIV, tuberculosis, and Hepatitis B Transmission in Ghana. *Interdisciplinary perspectives on infectious diseases*, *2019*.

Unissa, A.N., Subbian, S., Hanna, L.E. and Selvakumar, N., 2016. Overview on mechanisms of isoniazid action and resistance in Mycobacterium tuberculosis. *Infection, Genetics and Evolution*, *45*, p.474-492.

Venter, R., Derendinger, B., De Vos, M., Pillay, S., Dolby, T., Simpson, J., Kitchin, N., Ruiters, A., Van Helden, P.D., Warren, R.M. and Theron, G., 2017. Mycobacterial genomic DNA from used Xpert MTB/RIF cartridges can be utilised for accurate second-line genotypic drug susceptibility testing and spoligotyping. *Scientific reports*, *7*(1), p.14854.

Vilchèze, C. and Jacobs, Jr, W.R., 2007. The mechanism of isoniazid killing: clarity through the scope of genetics. *Annu. Rev. Microbiol.*, *61*, p.35-50.

Von 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.

Walker, T.M., Kohl, T.A., Omar, S.V., Hedge, J., Elias, C.D.O., Bradley, P., Iqbal, Z., Feuerriegel, S., Niehaus, K.E., Wilson, D.J. and Clifton, D.A., 2015. Whole-genome sequencing for prediction of Mycobacterium tuberculosis drug susceptibility and resistance: a retrospective cohort study. *The Lancet infectious diseases*, *15*(10), p.1193-1202.

Whittaker, E., López-Varela, E., Broderick, C. and Seddon, J.A., 2019. Examining the complex relationship between tuberculosis and other infectious diseases in children. Frontiers in pediatrics, 7, p.233.

Wong, K., Nguyen, J., Blair, L., Banjanin, M., Grewal, B., Bowman, S., Boyd, H., Gerstner, G., Cho, H.J., Panfilov, D. and Tam, C.K., 2020. Pathogenesis of human immunodeficiency virus-Mycobacterium tuberculosis co-infection. Journal of Clinical Medicine, 9(11), p.3575.

World Health Organization (WHO), 2005. Global tuberculosis control: surveillance, planning, financing: WHO report 2005. World Health Organization.

World Health Organization (WHO), 2014. *Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis*. World Health Organization.

World Health Organization (WHO), 2016a. The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis: policy guidance

World Health Organization (WHO), 2018a. *A vision for primary health care in the 21st century: towards universal health coverage and the Sustainable Development Goals* (No. WHO/HIS/SDS/2018.15). World Health Organization.

World Health Organization (WHO), 2018b. Tuberculosis: multidrug-resistant tuberculosis (MDR-TB). *Geneva: World Health Organisation*, *7*.

World Health Organization (WHO)., 2022. Global tuberculosis report 2022. Accessed from https://www.who.int/teams/global-tuberculosis-programme/tb-reports/globaltuberculosis-report-2022/tb-disease-burden/2-3-drug-resistant-tb#refs [Accessed 20 June 2022].

World Health Organization (WHO)., 2023. Tuberculosis. Accessed from https://www.who.int/news-room/fact-sheets/detail/tuberculosis [Accessed 15 November 2023].

World Health Organization, 2011. *Guidelines for the programmatic management of drug-resistant tuberculosis: emergency update 2011* (No. WHO/HTM/TB/2011.402). World Health Organization.

World Health Organization, 2016b. *WHO treatment guidelines for drug-resistant tuberculosis*. World Health Organization.

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

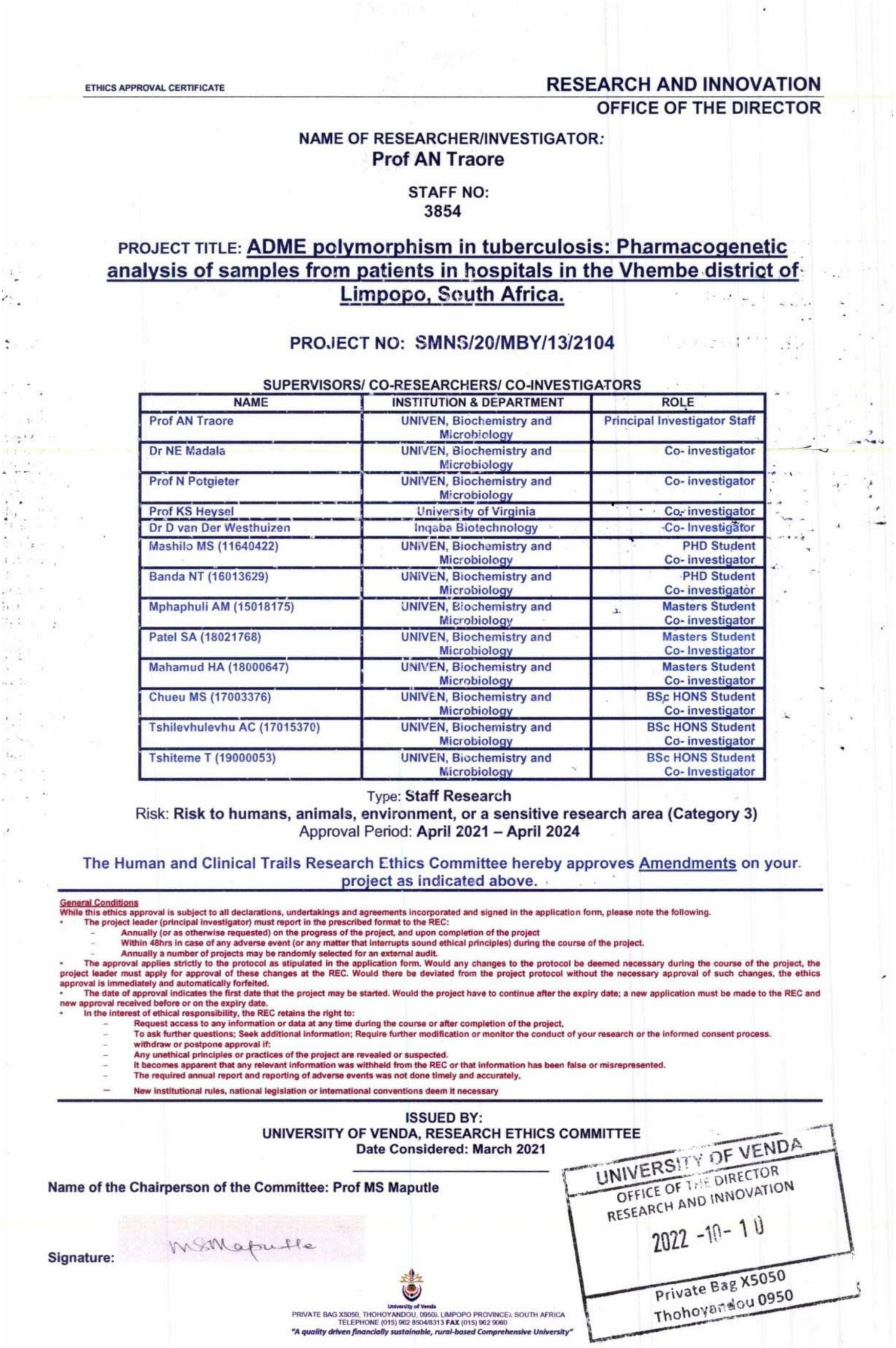
World Health Organization, 2021. Global tuberculosis report, 2020. Geneva, Switzerland: WHO, 2020. *ISBN 9789240037021. [Google Scholar]*, p.57.

Zhai, W., Wu, F., Zhang, Y., Fu, Y. and Liu, Z., 2019. The immune escape mechanisms of Mycobacterium tuberculosis. *International journal of molecular sciences*, *20*(2), p.340.

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

Appendix 1: Ethical clearance granted by University Of Venda



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 Heysel	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
Chueu 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

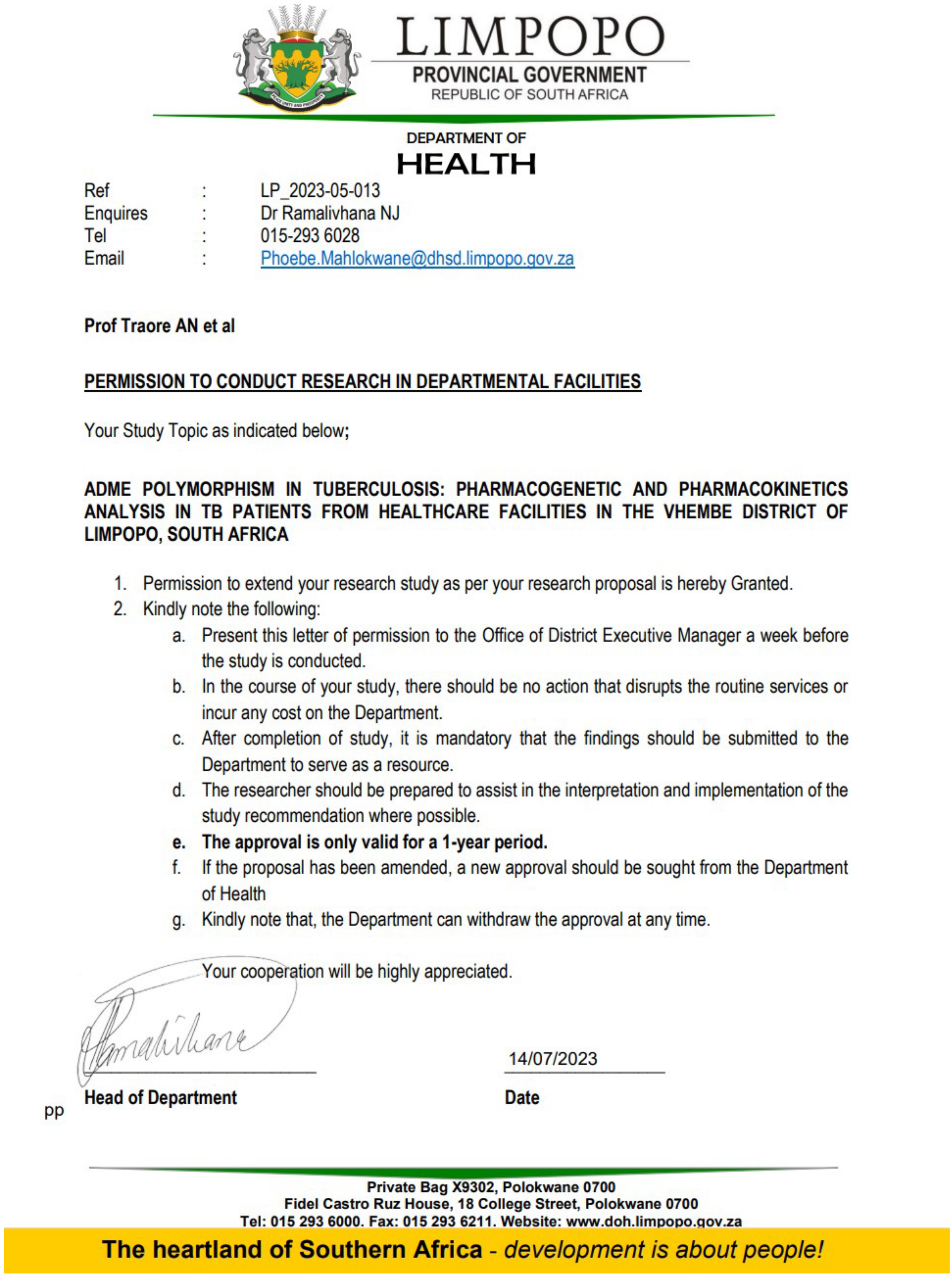
Signature: MSMaputle

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

University of Venda
PRIVATE BAG X5050, THOHOYANDOU, 0950), LIMPOPO PROVINCE), SOUTH AFRICA
TELEPHONE (015) 962 8504/8313 **FAX** (015) 962 9060
"A quality driven financially sustainable, rural-based Comprehensive University"

:

Appendix 2: Letter of permission granted by Limpopo Department of Health



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.

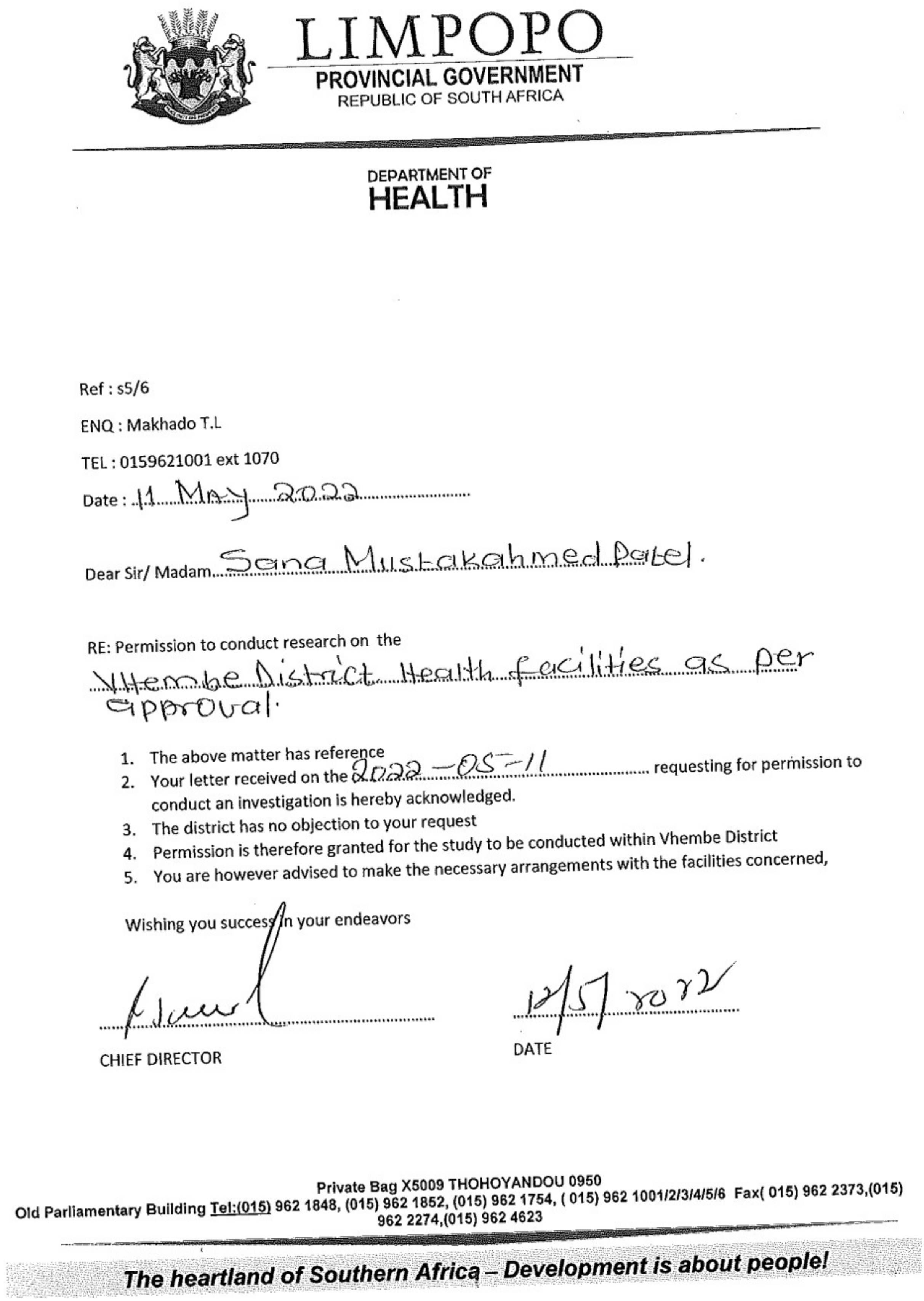
pp **Head of Department**

14/07/2023
Date

Private Bag X9302, Polokwane 0700
Fidel Castro Ruz House, 18 College Street, Polokwane 0700
Tel: 015 293 6000. Fax: 015 293 6211. Website: www.doh.limpopo.gov.za

The heartland of Southern Africa - *development is about people!*

Appendix 3: Letter of permission granted by Vhembe District



LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF
HEALTH

Ref : s5/6

ENQ : Makhado T.L

TEL : 0159621001 ext 1070

Date : 11 May 2022

Dear Sir/ Madam Sana Mustakahmed Patel.

RE: Permission to conduct research on the Vhembe District Health facilities as per approval.

1. The above matter has reference
2. Your letter received on the 2022-05-11 requesting for permission to conduct an investigation is hereby acknowledged.
3. The district has no objection to your request
4. Permission is therefore granted for the study to be conducted within Vhembe District
5. You are however advised to make the necessary arrangements with the facilities concerned,

Wishing you success in your endeavors

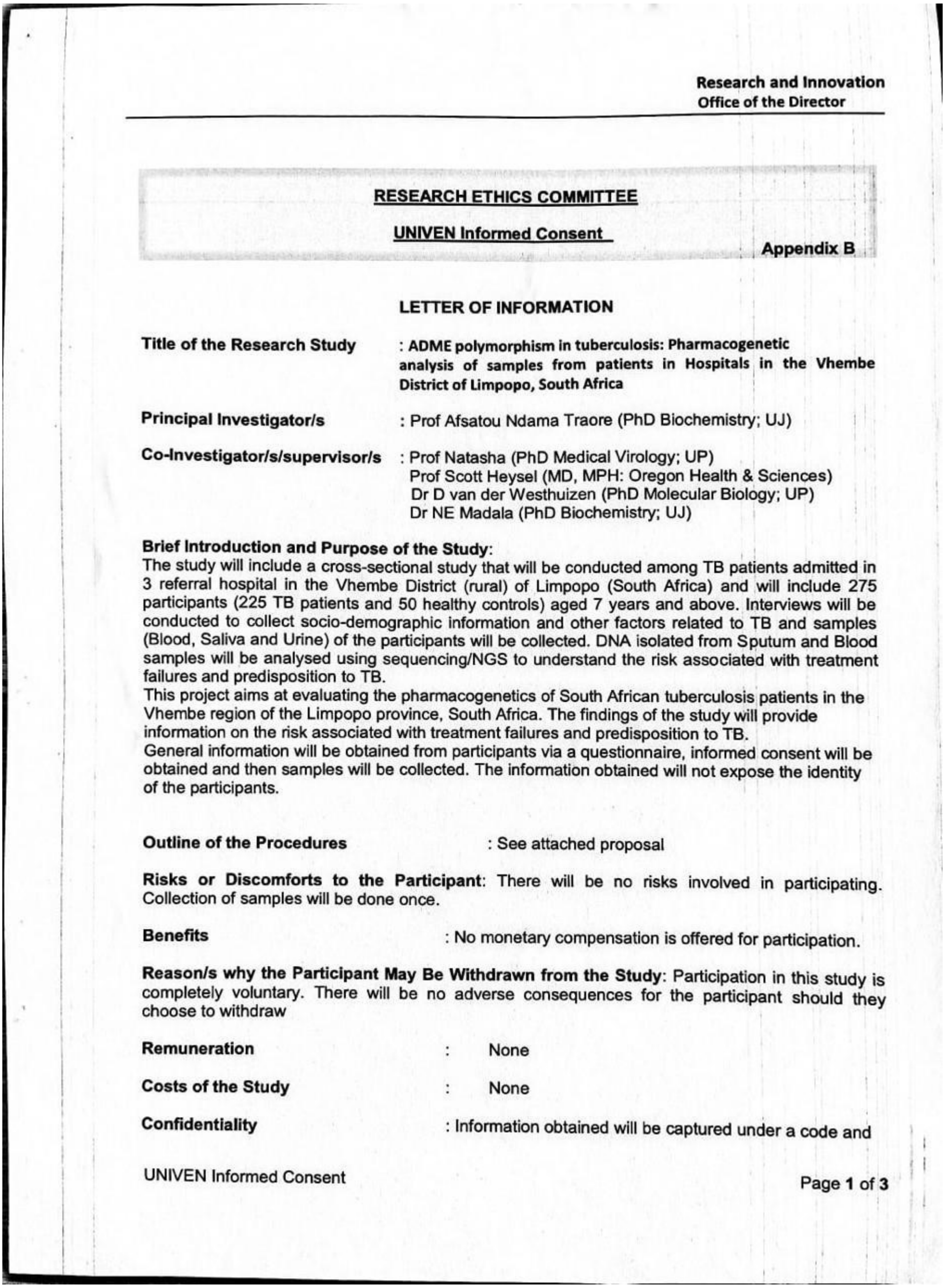
CHIEF DIRECTOR

12/5/2022
DATE

Private Bag X5009 THOHOYANDOU 0950
Old Parliamentary Building Tel:(015) 962 1848, (015) 962 1852, (015) 962 1754, (015) 962 1001/2/3/4/5/6 Fax(015) 962 2373,(015) 962 2274,(015) 962 4623

The heartland of Southern Africa – Development is about people!

Appendix 4: Consent form



Research and Innovation
Office of the Director

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.

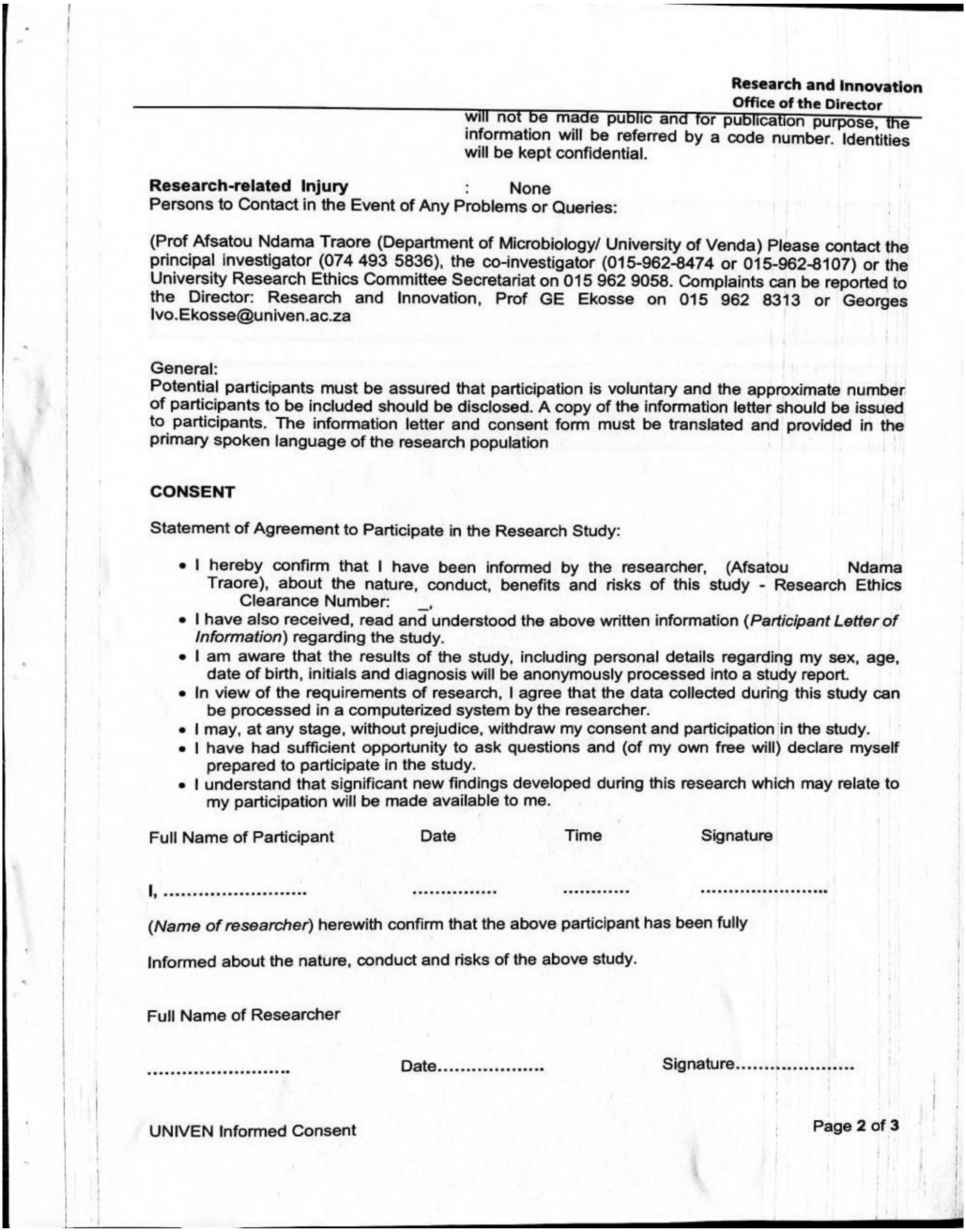
Benefits : No monetary compensation is offered for participation.

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

UNIVEN Informed Consent

Page **1** of **3**



Research and Innovation
Office of the Director

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
....	....	....	....

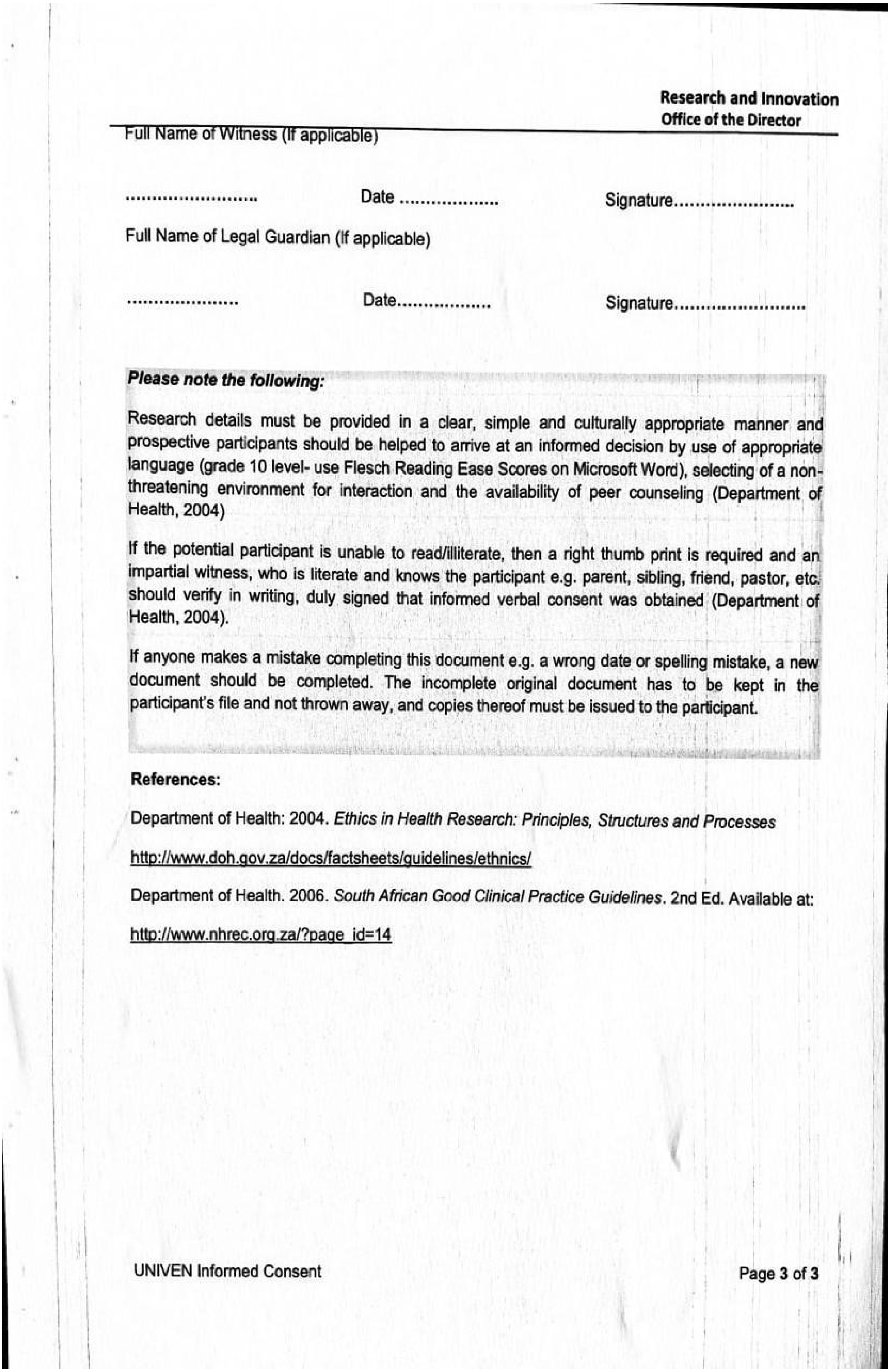
I, (*Name of researcher*) herewith confirm that the above participant has been fully

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

Full Name of Researcher

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

UNIVEN Informed Consent Page **2** of **3**



Research and Innovation
Office of the Director

Full Name of Witness (If applicable)

……………………… Date ………………… Signature……………………

Full Name of Legal Guardian (If applicable)

…………………… Date……………… Signature……………………

Please note the following:

Research details must be provided in a clear, simple and culturally appropriate manner and prospective participants should be helped to arrive at an informed decision by use of appropriate language (grade 10 level- use Flesch Reading Ease Scores on Microsoft Word), selecting of a non-threatening environment for interaction and the availability of peer counseling (Department of Health, 2004)

If the potential participant is unable to read/illiterate, then a right thumb print is required and an impartial witness, who is literate and knows the participant e.g. parent, sibling, friend, pastor, etc. should verify in writing, duly signed that informed verbal consent was obtained (Department of Health, 2004).

If anyone makes a mistake completing this document e.g. a wrong date or spelling mistake, a new document should be completed. The incomplete original document has to be kept in the participant's file and not thrown away, and copies thereof must be issued to the participant.

References:

Department of Health: 2004. *Ethics in Health Research: Principles, Structures and Processes*

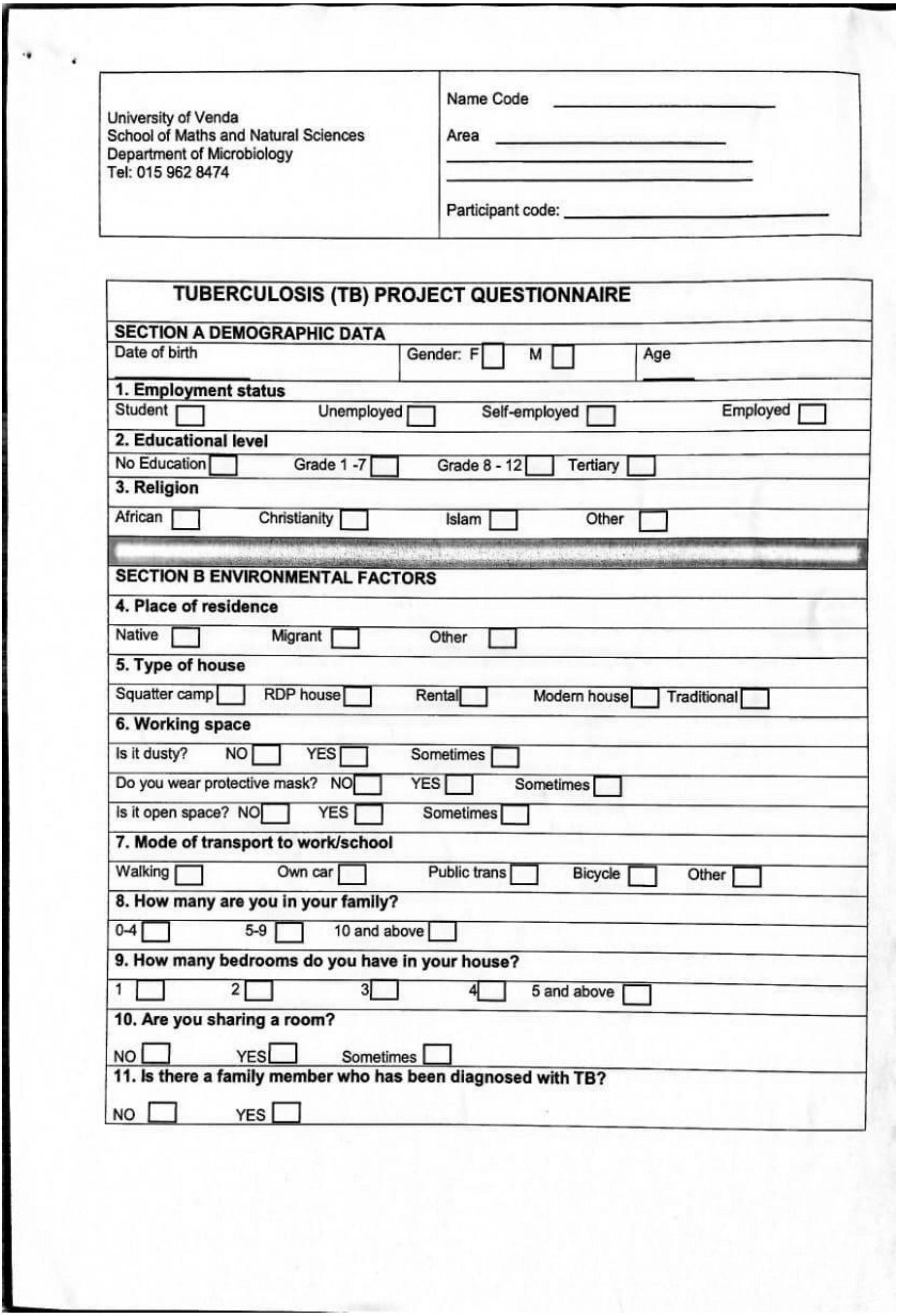
http://www.doh.gov.za/docs/factsheets/guidelines/ethnics/

Department of Health. 2006. *South African Good Clinical Practice Guidelines*. 2nd Ed. Available at:

http://www.nhrec.org.za/?page_id=14

UNIVEN Informed Consent Page 3 of 3

Appendix 5: 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?

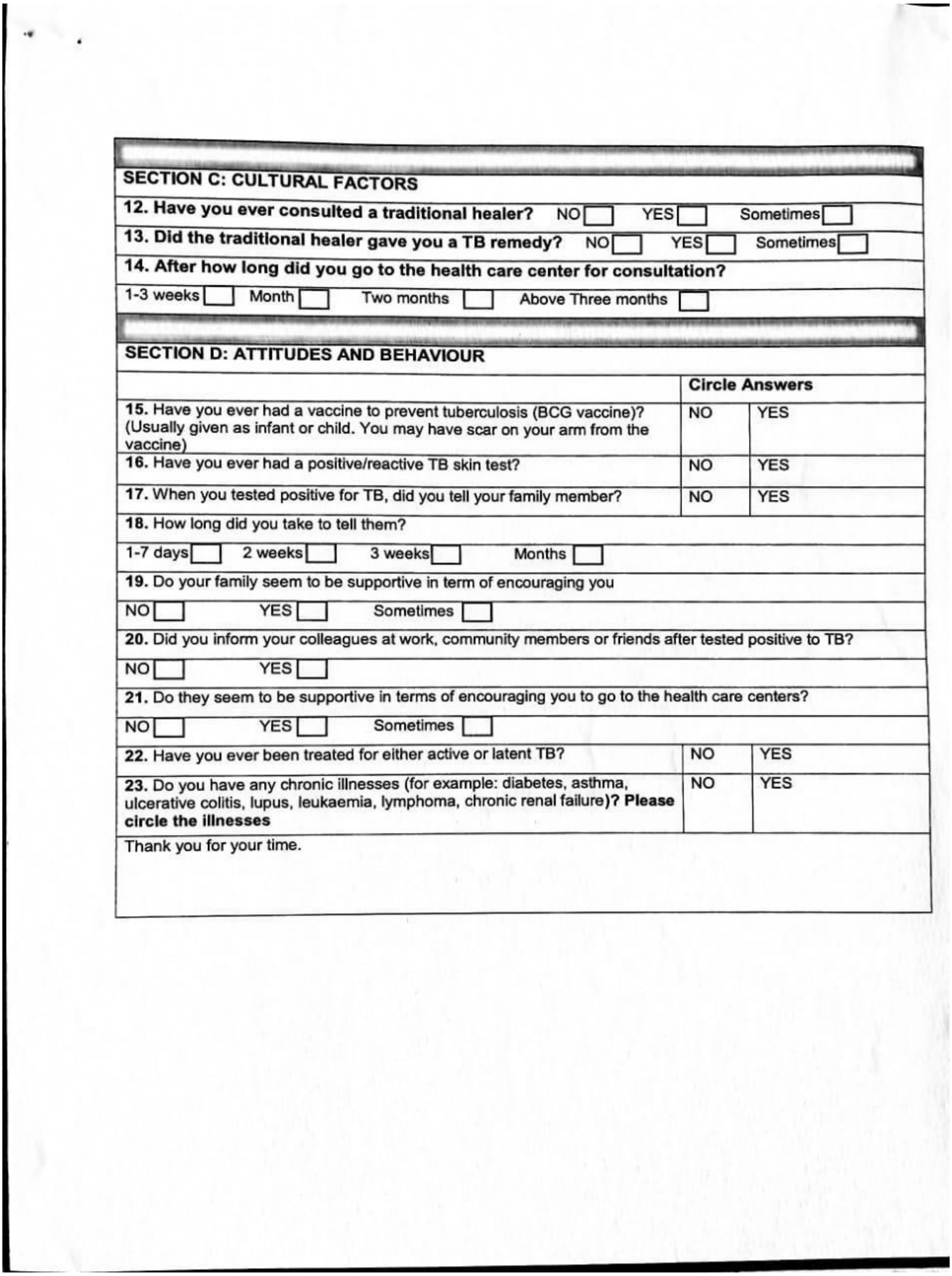
1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 and above ☐

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.