PREVALENCE OF DRUG-RESISTANT TUBERCULOSIS AND ASSOCIATED RISK FACTORS AMONG PATIENTS IN THE NORTHERN REGION OF SOUTH AFRICA

by

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Dissertation submitted in partial fulfilment for the requirements of Master of Science (MSc) degree in microbiology (MNMMMS)

TO

THE DEPARTMENT OF BIOCHEMISTRY AND MICROBIOLOGY

FACULTY OF SCIENCE, ENGINEERING AND AGRICULTURE

UNIVERSITY OF VENDA

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FEBRUARY 2024

DECLARATION

I, Mphaphuli Avheani Marry (student no: 15018175), hereby declare that this research dissertation titled "Prevalence of drug-resistant tuberculosis and associated risk factors among patients in the Northern region of South Africa" has been conducted and authored entirely by myself and submitted under the supervision of Dr MC Rikhotso and Professors (A.N Traore and N Potgieter). I further declare that the entire dissertation has been written entirely by myself and has never been submitted before, in part or in whole, to any college or university for diploma or degree and has been acknowledged and complemented with references.

Signature ____________________ **Date:** 04 June 2024

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DEDICATION

I would like to dedicate this research to my lovely parents.

For their encouragement, support and endless love.

ABSTRACT OF THE STUDY

Background: Drug-resistant TB is a major global health threat, driving the ongoing TB epidemic and increasing morbidity and mortality worldwide. Currently, national tuberculosis (TB) control programmes are challenged by the emergence of drug resistance to more anti-TB drugs resulting in multi-drug resistant TB (MDR-TB), pre-extensively drug-resistant TB (pre-XDR-TB) and XDR-TB. Globally, about 465,000 people have rifampicin-resistant TB (RR/TB; 78% MDR-TB), where new cases contribute about 3.5%, and 18% among previously treated cases.

Variety of factors, including demographic, behavioural, clinical, and environmental factors can favour the spread of DR-TB in the community. However, the major mechanism of acquiring DR-TB is through the spread of drug resistance (DR) mycobacterial strains in the community. Individuals who have had contact with someone who has drug-resistant TB are more likely to contract the disease. As a result, assessing the major associated risk factors contributing to the emergence of DR-TB using data from the local setting is critical in order to take appropriate action.

Objective: The study aimed to investigate the prevalence of drug-resistant tuberculosis and associated risk factors among TB patients from health care facilities in the Northern Region of South Africa.

Methodology: In order to determine the prevalence of drug-resistant TB and associated risk factors, this investigation adopted a cross-sectional study design. The study enrolled 50 tuberculosis patients of age 18 years and older from healthcare facilities in the Vhembe region between August 2022 and August 2023. Patients undergoing active TB treatment were recruited as participants in this study. Data on demographic characteristics, comorbidities, healthcare-seeking behaviour, TB-related stigma, and adherence to infection control measures were collected using a detailed questionnaire. Allplex™MTB/MDR/XDR assay (Multiplex PCR) was used for rapid detection of *M. tuberculosis* and drug-resistant TB. To study the association between risk factors and development of drug-resistant TB, Rstudio was employed (a statistical analysis software package). The chi-squared method was used to determine whether the association between two qualitative variables were statistically significant among the study population.

Results: Among the 50 sputum samples that were collected for the study, 17 (34%) remained TB active. Of these, 13 (26%) did not develop any resistance and the remaining 4(8%) presented drug-resistant TB strains which were resistant to at least one or more anti-TB drugs.

There was an equal distribution in terms of gender. Four percent (4%) of the female cases had mono-resistant TB, of which 1(2%) was isoniazid resistance (INH) and the other one being 1(2%) rifampicin resistance (RIF). Furthermore, 4% of the male cases had Extensively drug-resistant TB (XDR-TB), showing resistance to rifampicin (RIF) + isoniazid (INH) + fluoroquinolone (FQ). Having HIV infection (P=0.042), unemployment (P=0.023), non-adherence to TB treatment (P=0.042), smoking habit (P=0.023), history of contact with DR-TB patient (P=0.023) and history of previous anti TB treatment (P=0.023) were found to be significant factors for the development of drug resistance. Other factors such as age (P=0.127), gender (P=0.127) and education (p=3.246) were not significantly associated with drug resistance TB.

Conclusion: This study showed a low prevalence of drug-resistant TB in the Vhembe district with factors such as history of TB treatment, close contact with drug-resistant TB patients, non-adherence to TB medication, HIV infection and tobacco smoking, showing a significant association with drug-resistant TB. Hence, the study recommends that these factors must be taken into account in the planning and development of drug-resistant TB policies in order to work successfully towards the achievement of sustainable development goal of reducing TB by 80% before 2030. This study also highlighted that drug-resistant TB was more prevalent among the young and economically active age groups (20-40 years) than older individuals. Even though the prevalence of drug-resistant TB in this study was considered low, these findings highlight the need for health education efforts to strengthen accurate information to improve TB knowledge and correct misconceptions about TB among patients within the community especially the young age group.

Keywords: Allplex™ MTB/MDR/XDRe, drug-resistant TB, Extensively-drug resistant TB, Multi-drug resistant TB, *Mycobacterium* TB, risk factors, Tuberculosis

ACKNOWLEDGEMENTS

Firstly, I would like to appreciate and honour the Almighty God as he has blessed me with power, knowledge and strength to complete this dissertation. I am grateful for his mercy and grace, good health, wisdom and health mind.

My heartfelt thanks go:

To my lovely supervisors Dr MC Rikhotso, Prof AN Traore and Prof N Potgieter. I am forever grateful for their inspiration, gifted minds, and wisdom. Through their commitment, shared experience, tireless academic support, and passion for research they have taught and helped me to complete this great work.

The supportive assistance offered by the TB and One Health Research group, their constructive critics during meetings truly made an impact to this dissertation.

The University of Venda ethical committee and UHDC for allowing me to carry out this research.

The University of Venda Research section and NRF for granting me financial support during my study, their contribution made this study a success.

Limpopo Provincial Department of Health and Vhembe district for granting me permission to carry out this study. Not forgetting all CHC managers who permitted me to use their facilities to conduct this research and all TB Healthcare workers and TB patients who took their time and participate in this study.

Ms Munzhedzi M for working with me tirelessly reviewing my work.

Ms Marole T and Ms Mphego M for their support throughout the journey of conducting this study.

Lastly, I would also like to appreciate my family, for their prayers and support.

LIST OF ABBREVIATIONS

AFB	Acid Fast Bacillus
AIDS	Acquired Immunodeficiency Syndrome
ART	Antiretroviral therapy
°C	Degree Celsius
CDC	Centers for Disease Control
CHC	Community Health Centers
CT	Cycle threshold
DNA	Deoxyribonucleic Acid
DPO™	Dual priming oligonucleotide
DOTS	Directly Observed Therapy Short Course
DST	Drug Susceptibility Testing
DR-TB	Drug-resistant tuberculosis
EMA	European Medicines Agency
EMB	Ethambutol
FDA	Food and Drug Administration
FQ	Fluoroquinolone
HIV	Human Immunodeficiency Virus
IC	Internal Control
INH	Isoniazid
IPT	Isoniazid preventive therapy
LTBI	Latent tuberculosis infection
ml	Millilitre
MDR-TB	Multidrug-resistant tuberculosis
µl	Microliter
M-TB	*Mycobacterium Tuberculosis*
MTBC	Mycobacterium tuberculosis complex
NaOH	Sodium Hydroxide
NDOH	National department of Health

NSP	National Strategic Plan
NTM	Nontuberculous mycobacteria
NTP	National Tuberculosis Program
%	Percentage
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
Pre-XDR-TB	Pre-extensively drug-resistant tuberculosis
PZA	Pyrazinamide
READ	Real Amplicon Detection
RIF	Rifampicin
rRNA	Ribosomal ribonucleic acid
RSA	Republic of South Africa
RPM	Revolutions per minute
RT-PCR	Reverse transcriptase-polymerase chain reaction
SA	South Africa
SDGs	Sustainable development goals
STI	Sexually transmitted infections
STR	Streptomycin
TB	Tuberculosis
TDR-TB	Totally drug-resistant tuberculosis
TOCE™	Transcatheter Oily Chemoembolization Therapy
UN	United nations
USA	United state of America
WHO	World Health Organization
XDR TB	Extensively drug-resistant tuberculosis
ZN	Ziehl-Neelsen

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Chapter 1
GENERAL INTRODUCTION

1.1 Background

Tuberculosis (TB) is a life-threatening disease caused by *Mycobacterium tuberculosis* (Silva et al., 2018; Seung et al., 2015). The bacterium primarily infects the lungs; however, it can also infect other parts of the body such as the kidney, spine, and brain resulting in extrapulmonary tuberculosis (Martinez et al., 2019; Gengiah et al., 2014). The disease can spread easily when an infected person releases the bacterium into the atmosphere, this can be through coughing or sneezing (Datta et al., 2017; Cousins et al., 2016).

Mycobacterium tuberculosis can survive in the host without causing any disease, this can be referred to as latent TB infection (Silva et al., 2018). The World Health Organisation (WHO) defines latent tuberculosis infection as a state of persistent immune response to stimulation by *M. tuberculosis* antigens without evidence of clinically manifested active TB (WHO, 2017). Individuals with latent tuberculosis infection (LTBI) do not show any symptoms but typically respond positively to the tuberculin skin test or TB blood test (Migliori et al., 2020; Lohrasbi et al., 2018; Gilpin et al., 2016). Tuberculosis can progress as a newly acquired infection, or reactivation of latent infection (Martinez et al., 2019; Gengiah et al., 2014). However, the progression of the disease can be influenced by the availability of other chronic diseases within the individual and residing in areas with high TB prevalence (Lohrasbi et al., 2018; Ramaliba et al., 2017; Ejeta et al., 2016).

In 2020, 9.9 million people were reported to be infected with tuberculosis (TB), with 1.1 million children, 3.3 million women, and 5.5 million men (WHO, 2020b). According to the World Health Organization's 2022 Global TB report, 10.6 million cases of tuberculosis infections were reported in the year 2021, and 1.6 million people died from the disease (including 187 000 HIV-positive individuals) (WHO, 2022).

Tuberculosis strategies and associated targets have been identified in the United Nations' (UN) Sustainable Development Goals (SDGs) (for the period 2016-2030) and the World Health Organisation's (WHO) End TB Strategy (for the period 2016-2035).

These goals and strategies share a common objective: To end the global TB epidemic. Despite this major global drive to combat the disease, global TB indicators have not improved since 2015. Therefore, it is very important to study and understand the underlying factors that might be affecting efforts to achieve any improvements to end TB. In order to understand these underlying factors, this study was aimed at investigating the prevalence of drug-resistant TB and associated risk factors.

1.2 Study rationale

Drug-resistant tuberculosis (DR-TB) is a major global health threat, driving the ongoing TB epidemic and increasing morbidity and mortality worldwide (Chakaya et al., 2022; Dagne et al., 2021; WHO, 2019a). National tuberculosis (TB) control programmes are currently challenged by the emergence of drug resistance to more anti-TB drugs, resulting in multi-drug resistant TB (MDR-TB), pre-extensively drug-resistant TB (pre-XDR-TB), and XDR-TB (WHO, 2022; Baya et al., 2019). Despite several attempts made by the World Health Organization to increase TB treatment access, MDR-TB remains a major public health concern (Iradukunda et al., 2021; WHO 2020b).

Rifampicin-resistant tuberculosis (RR/TB; 78% MDR-TB) affects approximately 465,000 people worldwide, with new cases accounting for around 3.5% and previously treated cases accounting for 18% (WHO, 2020a). Furthermore, around 9% of RR/MDR-TB patients developed XDR-TB (WHO, 2020a; Baya et al., 2019). Furthermore, despite recent developments in treatment, the global average MDR/RR-TB treatment success rate is around 59% (WHO, 2019b).

The problem is much worse in high-burden countries such as South Africa (Zenbaba et al., 2021; Mabunda et al., 2016). An estimated 360 000 South Africans developed TB in 2019, of whom 13 000 were diagnosed with MDR-TB (Dookie et al., 2022; WHO, 2019b).

The major mechanism of acquiring DR-TB is through the spread of drug resistance mycobacterial strains in the community (Alemu et al., 2022). Individuals who have had contact with someone who has DR-TB are more likely to contract the disease (Alemu et al., 2022; Li et al., 2015). In a country like South Africa with increased chances of overcrowding, transmission is more likely to be high (Dookie et al., 2022). A variety of factors, including demographic, behavioural, clinical, and environmental factors can favour the spread of DR-TB in the community (Ruru et al., 2018). These factors are likely to differ among communities as well as from one setting to the next (Varshney et al., 2021; Sigh et al., 2020). As a result, understanding the major associated risk factors contributing to the development of DR-TB using data from local setting is important in order to take appropriate action.

Mycobacterium tuberculosis infection is associated with factors such as smoking, occupation, alcoholism and TB-HIV co-infection (Amin et al., 2021). Previous TB treatment, history of contact with DR-TB patients and interruption of treatment are all risk factors which impact a larger section of the population and documented as the strongest risk factors to the development of drug resistance (Desissa et al., 2018; Stosic et al., 2018). However, to the best of our knowledge, this is the first study to report on this kind of investigation in the Vhembe district. Therefore, Identifying and understanding the risk factors that contribute to DR-TB is important in designing effective prevention and control strategies against its transmission and development of resistance to more anti-TB drugs. Thus, this study determined the prevalence of drug-resistant TB and associated risk factors among tuberculosis patients from health care facilities in the Vhembe district, Limpopo province of South Africa.

1.3 Aim of the study

- To determine the prevalence of drug-resistant TB and associated risk factors among patients from health care facilities in the Northern Region of South Africa.

1.4 Objectives of the study

1.4.1. To determine the prevalence of drug-resistant TB (MDR/XDR) in patients from health care facilities using Allplex™MTB/MDR/XDR.

1.4.2. To assess the risk factors associated with drug-resistant TB (MDR/XDR) through a detailed questionnaire.

1.4.3. To study the association between risk factors and drug-resistant TB using statistical analysis.

1.5 Research questions and hypothesis

1.5.1 Research questions

The following questions has been answered:

1.5.1.1 Is there a high prevalence of drug-resistant TB in the Vhembe district of Limpopo province?

1.5.1.2 Which risk factors contribute/ influence development of drug-resistant TB?

1.5.2 Hypothesis

- The prevalence of drug-resistant TB is high in the Vhembe district, Limpopo province due to poor living conditions and the associated risk factors in the rural communities.

1.6 Significance of the study

Vhembe district in Limpopo province is a rural setting and has received very little attention regarding TB studies from researchers. The lack of scientific based information on the contributing risk factors in drug-resistance TB patients creates a challenge in the treatment and management of TB in the region as well as the whole country. Therefore, this study aimed to determine the prevalence of DR-TB, as well as associated risk factors. These results can contribute to the management of TB in the study area.

Chapter 2
LITERATURE REVIEW

2.1. Introduction

Hunter (2018) defines a literature review as a report about an assortment of documents (published or unpublished) on a topic that has certain facts, ideas and evidence in relation to a particular research topic and the evaluation of these documents. This literature review covers the TB definition, the state of TB in South Africa, TB risk factors, TB control in South Africa, TB control through Directly Observed Therapy Short Course (DOTS) and Legislation in TB control and management.

Tuberculosis is the most dangerous bacterial infection responsible for severe increase in death cases (Varshney et al., 2021; Sweetland et al., 2017). It is caused by the bacillus *Mycobacterium tuberculosis* which belongs to the family *Mycobacteriaceae* (Silva et al., 2018; Skinner and Claassens, 2016). The *tubercle bacillus* was discovered by Robert Koch in 1882 and it was named the *tubercle bacillus* because of the nodular lesions observed in lungs, which are termed the tubercles (Wohlleben et al., 2017; Winters et al., 2015; Tortoli et al., 2012). There are several reports indicating that TB is an age-old dreadful disease even from ancient times (Martinez et al., 2019; Marahatta et al., 2018; Masjedi et al., 2016; Zhao et al., 2012). The disease was called "consumption" in the past because of the way it would consume from within anyone who became infected (Kiazyk and Ball, 2017; Klopper et al., 2013; Kolyva et al., 2012).

Infection occurs through aerosol inhalation of droplets containing *M. tuberculosis* bacilli (Makonnen and Azagew, 2018; Golub et al., 2017; Hargreaves et al., 2015). When infected people cough, sneeze, talk, or spit, droplets carrying *Mycobacterium tuberculosis* are released into the air, and those that are close-by may inhale the bacteria and become infected (Coscolla et al., 2018; Makonnen et al., 2018; Golub et al., 2017). *Mycobacterium tuberculosis* can survive for weeks as an airborne droplet suspended in the air or in house dust (Boru et al., 2017; Chaidir et al., 2015; Churchyard et al., 2014). *Mycobacterium tuberculosis* pathogenesis has two stages following infection (Fana et al., 2019; Faustine et al., 2015). The first stage is an asymptomatic state known as latent tuberculosis, which can last for many years in the host (Espinal et al., 2020; Falzon et al., 2015). When the immune system is compromised, the bacteria begin to multiply, causing symptoms such as coughing, chest pain, exhaustion, and unexplained weight loss (Duarte et al., 2018; Ejeta et al., 2016). If left untreated, the disease eventually leads to death (Datta et al., 2017). In 80% of cases, it affects the lungs, with symptoms including coughing, haemoptysis, chest pain, shortness of breath, fever, weight loss, and drenching night sweats. (Daniel and Osman., 2019; Datta et al., 2017).

2.2 Transmission and pathogenesis

More than 95% of TB infection cases results from the inhalation of droplet-nuclei containing *tubercle bacilli* into the affected site and in pulmonary TB in the alveoli of the lungs (WHO, 2020b; WHO, 2019a). The pathogen or tubercle bacilli get to be ingested by macrophages in the alveolar aiming to destroy them or inhibit multiplication (Martinez et al., 2019; Gengiah et al., 2014). Some bacilli will multiply intracellularly and would be released once the macrophage dies (Silva et al., 2018; Mabunda et al., 2016). The bacilli can be spread throughout the body (including areas of the body in which TB disease is most likely to develop such as the regional lymph nodes, apex of the lung, kidneys, brain, and bone) (Akolo et al., 2017; Kibret et al., 2013). The below figures (Figures:2.1-2.5) are demonstrating the pathogenesis of the TB disease.

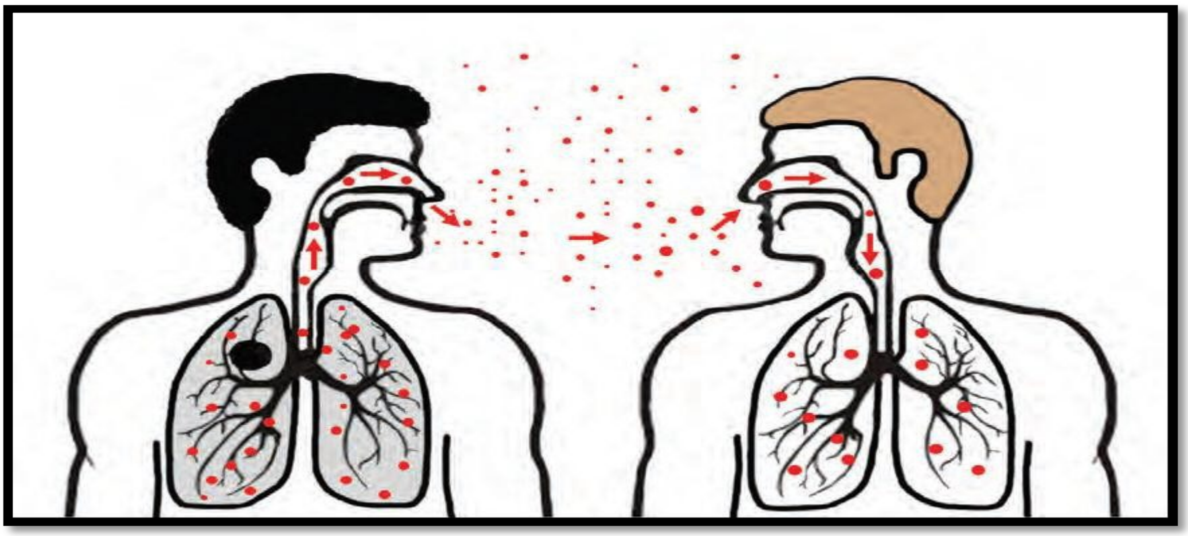

Figure 2.1: Tuberculosis Transmission. The aetiological agent of TB is carried in airborne particles known as the droplet nuclei. Infected individual can release nuclei

droplets when engaged in a conversation, coughing and other activities. The dots represent droplet nuclei containing tubercle bacilli. Droplet nuclei are inhaled, through lungs, and lodge in the alveoli (CDC, 2013).

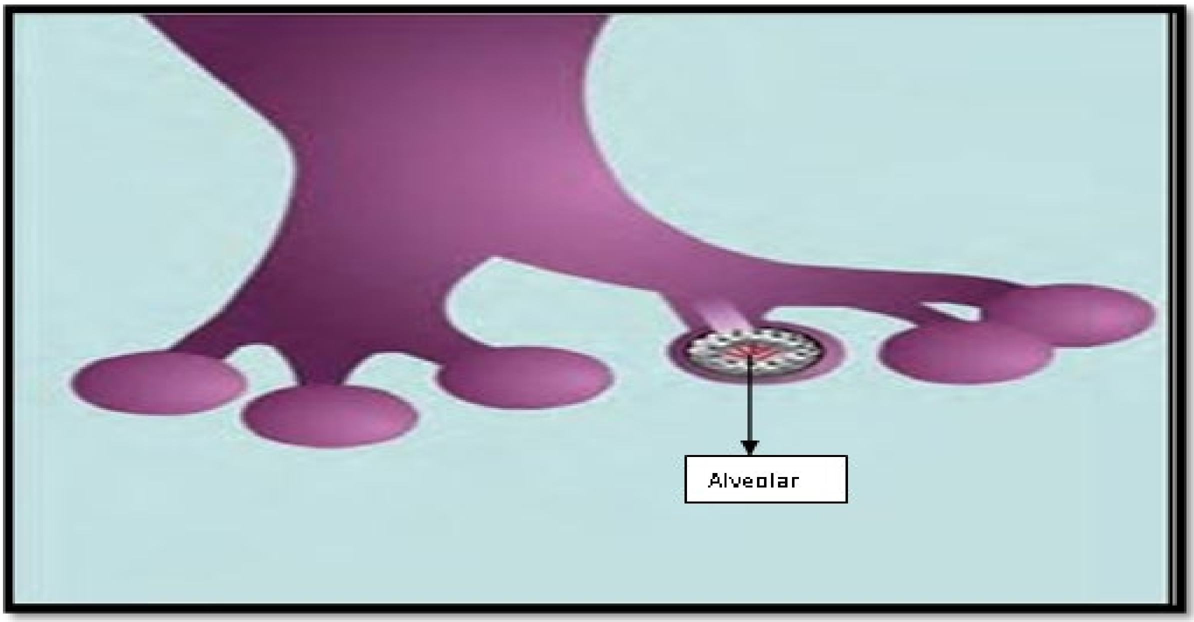

Figure 2.2: The tubercle bacilli multiply within the alveoli (CDC, 2013).

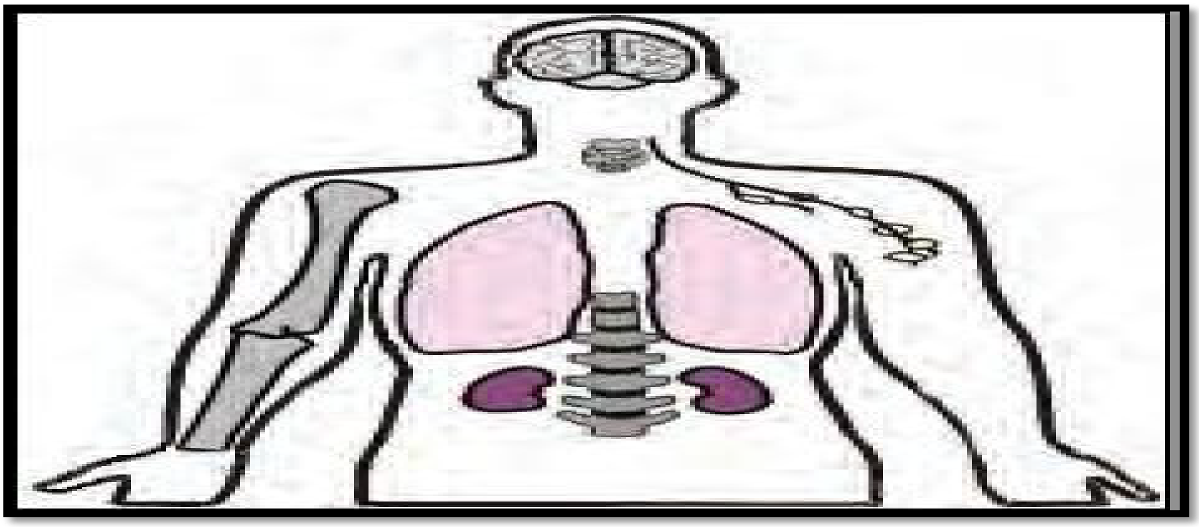

Figure 2.3: Tubercle bacilli enter the bloodstream and spread throughout the body. The development of TB disease can affect organs of the body commonly the brain, larynx, lymph node, lung, spine, bone, or kidney depending on reach of the tubercle bacilli (CDC, 2013).

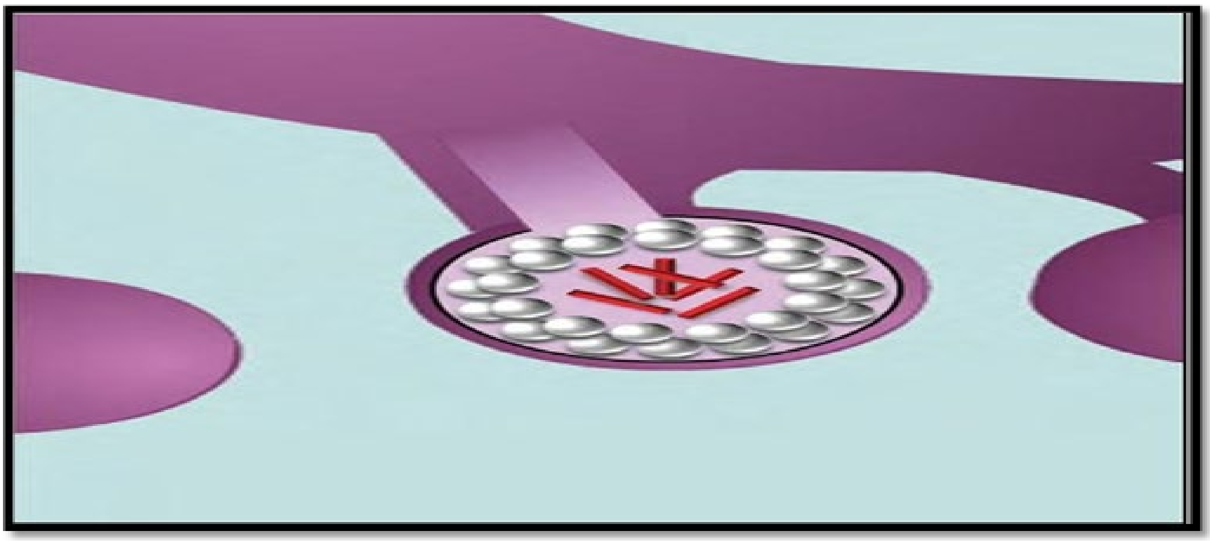

Figure 2.4: Macrophages ingest and surround the tubercle bacilli within 2-8 weeks. The macrophages form a granuloma, the barrier shell that keeps the bacilli contained and under control (LTBI) (CDC, 2013).

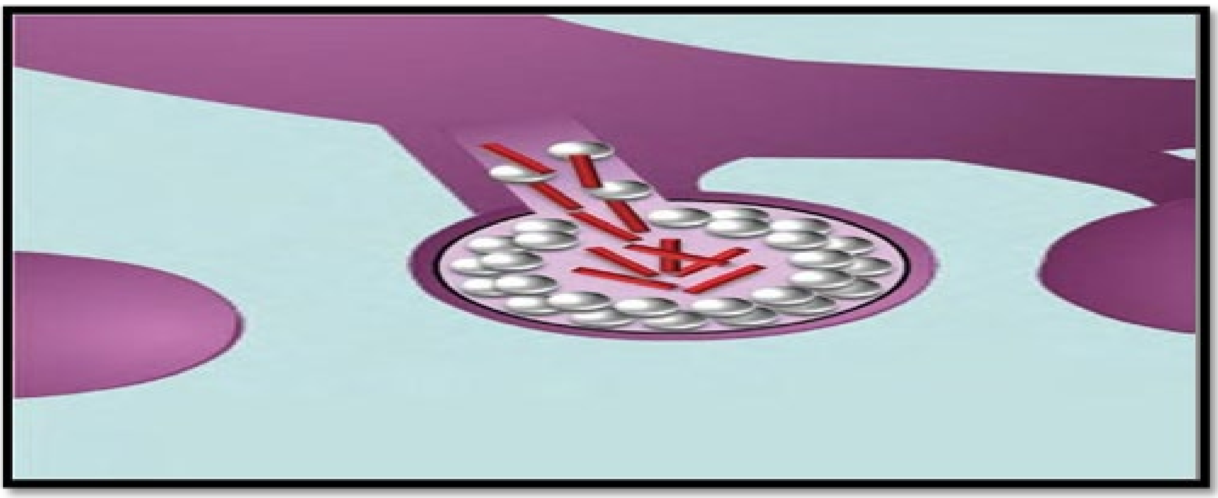

Figure 2.5: The failure to control the tubercle bacilli leads to TB disease development, at any of the body organ the bacilli can reach and multiply rapidly (TB disease occurs if the immune system cannot keep the tubercle bacilli under control) (CDC, 2013).

2.3 Symptoms

The symptoms and signs of TB are not clearly specific and are not that different from that of DR-TB as well (Guinn and Rubin, 2020; Ejeta et al., 2016; Mabunda et al., 2016). Cough is the most common symptom and at times it can be non-productive at the beginning of the illness, however as the inflammation and tissue death occur, sputum can be produced (CDC, 2013). Tuberculosis cannot be diagnosed due to cough since most acute respiratory infections involves coughing. However, if coughing persists for weeks TB diagnosis ca be done (Bark et al., 2015).

Active pulmonary TB patients normally present with a chest pain and it could be due to the infiltrations of the lung parenchyma and or even the occurrence of pneumothorax (Miranda et al., 2016; NDOH, 2016). Tuberculosis can cause severe respiratory failure and in advanced pulmonary TB dyspnoea may arise due to the involvement of lung parenchyma, severe lung fibrosis or destruction and even by tuberculous bronchopneumonia (Claassens et al., 2019; Coscolla et al., 2018; NDOH, 2016; Bark et al., 2015). There are usually systematic symptoms occurrence in active respiratory TB that include fever, tiredness, anorexia/ loss of weight (due to loss of appetite), night sweats and chills (Ruru et al., 2018; NDOH, 2017; Churchyard et al., 2014). The weight loss starts early in the disease and progresses as the disease advances (Datta et al., 2017). The symptoms of extra pulmonary TB depend on the site affected (McNabb et al., 2021; Martinez et al., 2019; Datta et al., 2017).

2.4 Manifestation

The development of TB varies in everyone depending on that individual's immune system (Duarte et al., 2018). Tuberculosis has several stages prior to its manifestation and each stage has different features, explained in detail below (Duarte et al., 2018; Datta et al., 2017; Cousins et al., 2016).

2.4.1 Latency

The majority of the people that are infected with *M. tuberculosis* do not show any clinical signs of TB, termed to be latently infected (Migliori et al., 2020; Siddique et al., 2011). Latently infected people remain as potential reservoirs of the tubercle bacilli with high possibilities of latent TB developing to active TB within few years after prime infection with *M. tuberculosis* (Duarte et al., 2018; Datta et al., 2017). Studies have been conducted on elaboration about the role of bacillary virulence determinants, host genetic and immune background as well as environmental effects in latently infected patients (Boru et al., 2017; Datta et al., 2017; Wohlleben et al., 2017; Cousins et al., 2016). Although studies were conducted, TB continues to develop to more difficult forms (Boru et al., 2017; Wohlleben et al., 2017).

2.4.2 Early primary disease

The disease at this stage is very difficult to diagnose due to non- specific signs or symptoms, the infected individual may have loss of weight, fever, frequent fatigue, non-productive cough (Migliori et al., 2020).

2.4.3 Primary progressive disease

At this stage, the sputum produced will be very much indicative of TB with more signs becoming clear (CDC, 2013). The chest radiography will be normal, and diagnosis is achievable by culture (Migliori et al., 2020; Datta et al., 2017).

2.4.4 Extra-pulmonary disease

Extra pulmonary TB affects other parts of the body excluding the respiratory site (CDC, 2013). This form of TB is usually progressive in immune-compromised patients (NDOH, 2017; NDOH, 2016). The central nervous system is mostly affected than any other extra-pulmonary sites and can lead to meningitis (Parbhoo et al., 2020; Tortoli, 2017). In some cases, the TB disseminates into the blood stream and is known to be military TB and can spread throughout the body during blood circulation (Parbhoo et al., 2020; Tortoli, 2017; CDC, 2013). Other body parts that can be affected are the pleura, bones and many more (Migliori et al., 2020; NDOH, 2016).

2.5 Prevention and control

In order to respond to the dual epidemics of HIV and TB rationally, South Africa developed an integrated National Strategic Plan (NSP) for HIV, STIs and TB (2012 - 2016) (Sigh et al., 2020; Ruru et al., 2018). The targets set in the NSP for TB was to reduce incidence and mortality by 2016 and to have no new TB infections, deaths, or stigma by 2032 (Matakanye et al., 2019; Kiazyk and Ball, 2017; Winters et al., 2015). To avoid TB deaths and reduce transmission, the central pillars of TB control are diagnosis, treatment, and prevention (Kiazyk and Ball, 2017; Churchyard et al., 2014). The National Tuberculosis Program has substantially strengthened the TB control program since 1994 (Alexander et al., 2021; Akolo et al., 2017; Abebe et al., 2016).

Encouragingly, the efforts of the NTP have contributed to a slow decline in TB case notification rates since 2009 (Matakanye et al., 2019; Kiazyk and Ball, 2017; Churchyard et al., 2014).

2.5.1 Successes and challenges

Beginning with success in fighting against TB, treatment success rate among new smear-positive and smear-negative/extrapulmonary TB patients has improved to 79% and 76%, respectively, achieved largely as a result of an increase in cure rates and a decline in the treatment default rate following the introduction of community-based tracing teams (NDOH, 2017; Churchyard et al., 2014). South Africa adopted the ‘3Is’ policy of isoniazid preventive therapy (IPT), intensified case finding and infection control in 2002 (Alexander et al., 2021; Guinn and Rubin, 2020; Mabunda et al., 2016). In 2012, 949 800 HIV-positive South Africans were screened for TB, which although impressive was still substantially below the total number of people living with HIV in care (Guinn and Rubin, 2020; Claassens et al., 2019; NDOH, 2016). During 2011 >150 000 household contacts were screened for TB and >3 000 new cases, which would not have been detected through routine means, were identified (Daniel and Osman, 2019; Bark et al., 2015; Churchyard et al., 2014).

Despite the success that has been made in the fight against TB infection, up to 25% of positive TB cases are lost to follow-up before treatment initiation, which may contribute to on-going transmission and an increased risk of death (Claassens et al., 2019; Freiman et al., 2018; Fogel, 2015). Moreover, the mortality rate remains high even after completion of TB treatment, probably due to HIV disease (Nwendamutswu, 2020; Omrani et al., 2014). Among persons on long-term antiretroviral therapy (ART) the prevalence of undiagnosed TB remains high and underscores the need for ongoing TB screening (Golub et al., 2017; Hargreaves et al., 2015). South Africa introduced a TB infection control program in 2007, the implementation of infection control guidelines remains sub- optimal (Lohrasbi et al., 2018; Helb et al., 2017; Gandhi et al., 2014).

2.5.2 Directly Observed Therapy Short Course (DOTS)

Directly Observed Therapy Short Course (DOTS) is the internationally recommended strategy to help eradicate tuberculosis (Wells et al., 2019). It is based on five basic concepts common to disease control strategies, with a focus on early detection and treatment of infectious cases to prevent the spread of tuberculosis (Winters et al., 2015; WHO, 2018b; Wells et al., 2019). The proposed TB control plan includes the following principles (Wells et al., 2019; WHO, 2018b).

1. The government's commitment to maintaining ongoing, comprehensive TB control activities.

2. Case detection using sputum smear microscopy among symptomatic patients who self-report to health providers.

3. Standardized short-course chemotherapy regimens lasting six to eight months for at least all confirmed smear positive cases. Direct observed therapy (DOT) during the intense phase is recommended for all new sputum positive cases, as is the continuation phase of rifampicin-containing regimens and the entire re-treatment schedule.

4. A consistent, ongoing supply of all necessary anti-TB medications.

5. A systematic recording and reporting system for assessing case-finding and treatment outcomes for each patient, as well as the TB control program.

2.6 Treatment

2.6.1 Regimens set by the World Health Organization

The World Health Organization has recommended standard primary drugs for tuberculosis treatment, known as first-line drugs, for the first six months: isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB) (WHO, 2020b; Finnie et al., 2019; Grobler et al., 2018; Ramaliba et al., 2017).

The first two months are known to be the most crucial stage of the treatment and at this point all the drugs are administered (Hillemann et al., 2020; Gilpin et al., 2016). Meanwhile for the remaining four months of treatment only INH and RIF are used (WHO, 2020b). Streptomycin is one of the first line drugs which was usually used in the initial stage of treatment but its administration has decreased over a period of time due to the elevated levels of resistance (McNerney et al., 2017; Ramaliba et al., 2017).

The new and improved regimens for the treatment of DR-TB were made possible with the emergence of three new drugs into the market during the last decade, namely bedaquiline (approved by the Food and Drug Administration (FDA) in 2012), delamanid (approved by the European Medicines Agency (EMA) and the Japanese Pharmaceuticals and Medical Devices Agency in 14 2014) and protionamide (approved by the FDA in 2019) (Alexander et al., 2021; Hillemann et al., 2020).

This recommendation of the TB treatment is based on the mode of administration, duration of treatment and clinical efficacy trials in terms of dosage (Ramaliba et al., 2017; Perry et al., 2014). The treatment period is divided into intensive (initial) and continuation phases (Palomino and Martin, 2014; Phillips, 2013). The intensive phase is a period where there is a need for rapid killing of the bacteria and it is based on all first line drugs (Silva et al., 2018; Sweetland et al., 2017). Patients who were infectious rapidly change into non-infectious (usually within two weeks) and symptoms start to be reduced (Silva et al., 2018; Tortoli, 2017). The majority of patients who were initially smear positive start to produce smear negative sputum within two months (Padayatchi et al., 2019; Skinner and Claassens, 2016; Zarir et al., 2012). The continuation phase is a period where only few drugs are necessary but for a prolonged period of time to allow the sterilizing effect of drugs to eliminate remaining bacilli (Sweetland et al., 2017; Tshitangano, 2013).

Tuberculosis patients without treatment usually show poor quality of life but administration of treatment has proven to have a promising effect in improving the quality of life (Lawn and Nicol, 2020; Wells et al., 2019). However, treatment of TB remains as one of the most important challenges to global health because many of these anti-TB drugs are associated with side effects that usually determine the outcomes of the treatment (Palomino and Martin, 2014). These side effects reduce the patient acquiescence to the treatment programme, which leads to increased rates of TB recurrence and increased mortality (Silva et al., 2018; Tortoli, 2017). Adverse effects of anti-TB drugs mostly experienced include: neurological disorders, gastrointestinal disorders, arthralgia, skin reactions and hepatotoxicity (which is the most serious and can be fatal if not addressed early) (Hillemann et al., 2020; Mekonnen and Azagew, 2018). Anti-TB drug induced hepatotoxicity has been observed to be between 2 – 28% of treated TB cases especially in multidrug resistance cases (McNabb et al., 2021; Grobler et al., 2018). This requires discontinuation of one or more of the drugs used in the treatment programme, interruption in the treatment course may result in resistant TB strains and even death (Hillemann et al., 2020; Finnie et al., 2019; Faustini et al., 2015). The TB treatment regimen requires the intake of multiple drugs and prolonged treatment period, making it difficult to guarantee patient treatment compliance, and therefore it leads to treatment failure (McNabb et al., 2021; Grobler et al., 2018; Perry et al., 2014).

Tuberculosis strains often render the first line regimen ineffective and that highlights that the drugs used are not effective in controlling that particular strain therefore, the second line anti-TB drugs are to be considered (Matakanye et al., 2019; Kiazyk and Ball, 2017). The second line drugs usually comprise of fluoroquinolone's and injectables (Migliori et al., 2020; Marahatta et al., 2018; Miranda et al., 2016; Masjedi et al., 2016). The cure rate of resistant strains is much less than normal TB infection, for example XDR-TB case have a cure rate which is generally much lesser than 50% with the current available chemotherapy (McNabb et al., 2021; Marsh et al., 2019; Marahatta et al., 2018). Although basic TB regimens are broadly applicable, there are modifications that should be made under special circumstances (i.e., Pregnancy, infant treatment and HIV infection) (Hillemann et al., 2020; Mekonnen and Azagew, 2018; Masjedi et al., 2016).

2.7 Drug resistance

Drug resistance is the ability of the bacteria to grow and multiply in the presence of an antimicrobial agent (Boru et al., 2017; Bark et al., 2015). Drug resistant TB emerge as a result of wrong prescription of the drug, length or dose of the treatment and incomplete course of the treatment which inadequately expose the bacteria to the drug, thus it undergoes mutation which enables it to tolerate the drug (McNabb et al., 2021; Grobler et al., 2018; WHO, 2017).

2.7.1 Primary resistance

Primary resistance is a form of resistance that occurs in the new TB patients without any history of previous TB treatment (Daniel and Osman, 2019; Boru et al., 2017; Bark et al., 2015). This describes resistance in patients who have had received TB treatment for less than a one month (Sweetland et al., 2017). The resistance in such subjects provide a measure of the degree of transmission of the *M. tuberculosis* strains (NDOH, 2017; Sweetland et al., 2017).

2.7.2. Acquired resistance

Acquired drug resistance is a term used to define a drug resistant case of TB which was previously treated (NDOH, 2017; Skinner and Claassens, 2016; Churchyard et al., 2014). The strains isolated from the cultured specimens after treatment completion that are still resistant from patients with at least one treatment episode of more than a month are referred to as retreatment cases (Sweetland et al., 2017; Skinner and Claassens, 2016; CDC, 2013). The term retreatment is usually used to describe the acquired drug resistant cases (Varshney et al., 2021; Winters et al., 2015). Patients with acquired drug resistance have always shown a high level of resistance than in the newly infected patients and they provide an indication of the extent to which a patient was approximately treated, like the quality of TB control (Espinal et al., 2020; Merza et al., 2014).

2.7.3 Multiple Drug Resistance TB (MDR-TB)

Inadequate tuberculosis treatment, such as an inappropriate combination of TB medications, insufficient dose or duration, or irregular drug use, promotes the development of anti-TB drug resistance (Kolappan and Gopi, 2019; Kale, 2016; Huyen et al., 2014). Tuberculosis is treated with a variety of drugs, and if these drugs are ineffective against the bacilli, MDR-TB develops, which is resistant to RIF and INH (Freiman et al., 2018; Golub et al., 2017; Kale, 2016). This resistance can be selectively induced by direct or indirect mono-chemotherapeutic drugs, allowing drug-resistant microorganisms to take over the host (Fana et al., 2019; Ejeta et al., 2016). The *M. tuberculosis* population that has evolved resistance to the agent will continue to multiply, while susceptible organism will be suppressed, allowing drug-resistant strains to become dominant in the host and, if mismanaged, could lead to additional drug resistance (Lohrasbi et al., 2018; Mekonen et al., 2015).

The appearance of MDR-TB was after the introduction of Rifampicin in the 1966 (Kolyva and Karakousis, 2017; Varahram et al., 2014). The global surveillance data recorded 150 000 MDR-TB deaths in 2008 with relatively high estimations of MDR-TB cases (650 000) in 2010 (WHO, 2017). MDR-TB has a highly unequal regional distribution, with 0.7% of new cases in established market economies and approximately 2% in Africa (WHO, 2018b). Thus, the worldwide assessment demonstrated that the magnitude and trends of drug-resistant tuberculosis vary at the regional and national levels (WHO, 2018b; Falzon et al., 2015). South Africa had a high MDR-TB rate, with over 7000 cases diagnosed in 2018, with Limpopo Province accounting for approximately 126 cases (Guinn and Rubin, 2020). In the 2008 report of the anti-TB drug resistance surveillance global project, the incidence of MDR-TB cases ranged from 0 to 22.3%, whereas the proportion of XDR-TB ranged from 0 to 30% globally (WHO, 2017).

2.7.4 Pre-Extensively Drug-resistant TB (pre-XDR TB)

Pre-Extensively Drug-resistant TB (pre-XDR TB) is a type of MDR TB caused by TB bacteria that are resistant to rifampin, isoniazid, and a fluroquinolone OR by TB bacteria that are resistant to rifampin, isoniazid, and a second-line injectable (kanamycin, capreomycin and amikacin) (Espinal et al., 2020; Padayatchi et al., 2019).

2.7.5 Extensively Drug Resistant TB (XDR-TB)

Treatment to MDR-TB can be challenging due to the prolonged procedure, which can lead to patient noncompliance and a great potential for the development of resistance to second-line drugs (Ruru et al., 2018; Ramaliba et al., 2017; Falzon et al., 2015). Such strains can develop into XDR-TB (Sigh et al., 2020). Extensively drug-resistant tuberculosis (XDR-TB) is defined as an MDR-TB strain resistant to any fluoroquinolone drug and one of the second-line injectable drugs (Sigh et al., 2020; Skinner and Claassen, 2016). The emergence of XDR-TB in every region of the world has prompted significant concerns regarding the future of TB control (Matakanye et al., 2019; Ruru et al., 2018).

An incident of XDR-TB outbreak was firstly reported in one of the South African Provinces with increased drug resistant TB in 2005 (Zegeye et al., 2019; Wurie et al., 2018). During the year 2006 there were 53 XDR-TB cases noted at a rural hospital in KwaZulu-Natal representing 24% of MDR-TB cases (Wurie et al., 2018; McNerney et al., 2017; Phillips, 2013). The median time period from which diagnosis was made to death of XDR-TB patients was only 16 days (Zenbaba et al., 2021; Klopper et al., 2013). The XDR-TB was notable in patients without TB history, where TB treatment was successfully completed and it was believed that there was a nosocomial transmission (Padayatchi al., 2019; Klopper et al., 2013). Drug-resistant TB strains pose difficulties in the diagnosis and treatment, with lower survival rates (Lange et al., 2018; Klopper et al., 2013).

2.7.6 Totally Drug Resistant TB (TDR-TB)

The mismanagement of drug resistance continues to result in more aggressive forms of tuberculosis (WHO, 2020; Padayatchi et al., 2019; WHO, 2018a). Strains isolated from TDR-TB have been shown to be resistant to all first and second-line anti-TB drugs (Wurie et al., 2018; Zhao et al., 2012). According to a study published in the European respiratory journal, the isolated TB strains have transformed into adapted forms that produce round or oval shaped bacilli (Zarir et al. 2012). They had patients who were treated with second-line drugs and remained smear and culture positive after 18 months of treatment (Wohlleben et al., 2017; Winters et al., 2015).

The probability of treatment to eradicate a disease is mostly decreased with the emergence of new drug resistant strains (Tortoli, 2017; Tortoli et al., 2012). The cell wall of the TDR-TB strains is extra ordinarily thick (21-26nm) and such bacilli has never been found among susceptible and or MDR TB forms (Boru et al., 2017; Abebe et al., 2016). Below in figure 2.6 is an illustration of TDR-TB.

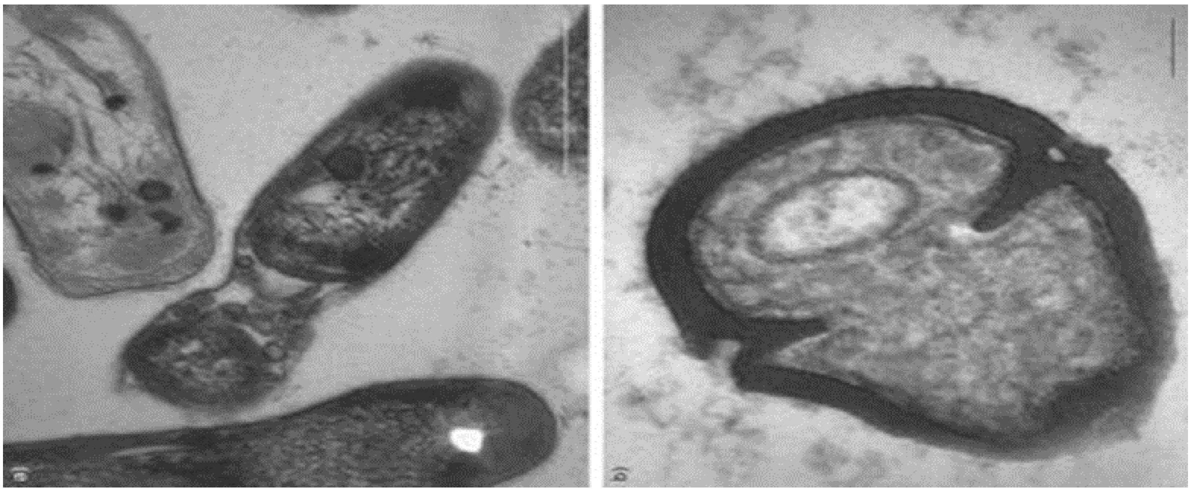

Figure 2.6: The totally drug-resistant (TDR) bacilli shown under a microscope. (a) Oval or spherical bodies among TDR TB bacilli (b) immobile or anaerobic dormant bacilli in the dividing stage (Zarir et al., 2012).

2.8 Mechanisms of drug resistance in TB

Drug resistance can be classified as either primary or secondary (Akolo et al., 2017; Bark et al., 2015). Primary resistance defined as infections resulting from the already resistant *M. tuberculosis* complex (MTBC) strains in most cases it is due to previously undocumented or unreported treatment (Masjedi et al., 2016; Chaidir et al., 2015; Churchyard et al., 2014). Acquired resistance is resistance that develops as a result of improper exposure of the *M. tuberculosis* complex strain to anti-TB drugs and followed by selection of resistant mutant bacilli (McNabb et al., 2021).The understanding of mycobacterial resistance mechanisms to the anti-TB drugs will not possibly only enable the advanced development of more rapid diagnostic tests and effective drug development, but help in the implementation of relevant measures in preventing and managing the development of such resistance in the future (Martinez et al., 2019; Zhang et al., 2013).

Mechanisms in which resistance to drugs develops has been identified and broadly categorized: The spontaneous chromosomal mutations that affect key drug targets, enzyme production that degrades or modify compounds (rendering them useless), the formation of a layer that is rich in lipids reducing permeability to drugs and the efflux of drugs through protein pumps (Zegeye et al., 2019; Maswanganyi et al., 2014). Through the understanding of these mechanisms; several molecular diagnostic tests for identification of TB and its resistance to drugs have been developed (Daniel and Osman, 2019; Seung et al., 2015).

2.9 Drug-resistant TB in South Africa

South Africa is considered a high burden MDR-TB country, with an anticipated 13,000 cases reported in 2018 (WHO, 2018a). In a 2017 nationwide survey in South Africa, 20.2% of all notified TB cases were isoniazid-resistant, with MDR-TB accounting for nearly half (9.6% of all cases) (Zegeye et al., 2019; Stats, 2017; Wohlleben et al., 2017). This represents a threefold increase from 2004, when 3.1% of all TB cases (new and retreatment) tested positive for MDR-TB (Zegeye et al., 2019; Wohlleben et al., 2017).

These findings were further supported by a report from the National Health Laboratory Service (NHLS) that indicated a consistent increase in the incidence of MDR-TB patients since 2004, as well as a study in Khayelitsha that revealed MDR-TB in 4.4% of all TB cases in 2008 (Zenbaba et al., 2021; Zhang et al., 2013). According to NHLS statistics, 6.3% of diagnosed MDR-TB cases were XDR-TB (following second-line drug susceptibility testing, DST), while World Health Organization estimates for South Africa were considerably lower, at 10.5% (Stats, 2017; Varahram et al., 2014; Siddiqui et al., 2011).

In 2006, researchers revealed that an outbreak of XDR-TB had killed over 50 people in less than a year in KwaZulu-Natal Province (Khumalo, 2021; Fana et al., 2019; Datta et al., 2017). Between June 2005 and August 2007, 539 cases of MDR-TB were recorded from a single hospital (the Church of Scotland Hospital at Tugela Ferry), in a remote area of Kwazulu-Natal (Sigh et al., 2020). The World Health Organization ascribed the high prevalence of MDR-TB to poor adherence to the six-month treatment regimen of anti-TB drugs required to treat the disease (Khumalo, 2021; Fana et al., 2019).

The ever-increasing MDR-TB epidemic in South Africa places a significant pressure on the National TB Control Programme's budget (Wells et al., 2019; Grobler et al., 2018; Statistics, 2018). Multi-drug resistant TB treatment is expected to cost almost 70% of the funds allocated for fighting the overall TB epidemic in South Africa (Akolo et al., 2017; Abebe et al., 2016). As a result, important resources are redirected away from treating the DR-TB epidemic, which has already reached alarming proportions, with an estimated 500,000 cases identified each year in South Africa (Lohrasbi et al., 2018; Bark et al., 2015).

Despite the establishment of the DOTS program in 1996, the current TB control strategy is ineffective in preventing the development and dissemination of MDR-TB (Stats, 2018). This could be due in part to the DOTS program's inadequate implementation in some South African regions (Lange et al., 2018). A recent study in Kwazulu-Natal discovered that just 18% of patients with smear-positive pulmonary disease completed treatment, potentially contributing to the province's drug resistance development (Espinal et al., 2020; Khumalo, 2021). To reduce tuberculosis morbidity and mortality, a thorough understanding of the disease's clinical pathology is required.

2.10 Risk factors associated with drug-resistant TB

According to Narasimhan et al. (2013), the risk of tuberculosis infection is mostly determined by various factors. As a result, knowledge and understanding of the risk factors associated with DR-TB is critical for establishing effective prevention strategies. According to Padayatchi et al. (2019), identifying the risk factors associated with DR-TB is a critical step towards tuberculosis control and to end-TB. Figure 2.7 demonstrates the significant characteristics which influence an individual's likelihood of developing infection and disease, with key risk factors summarized below.

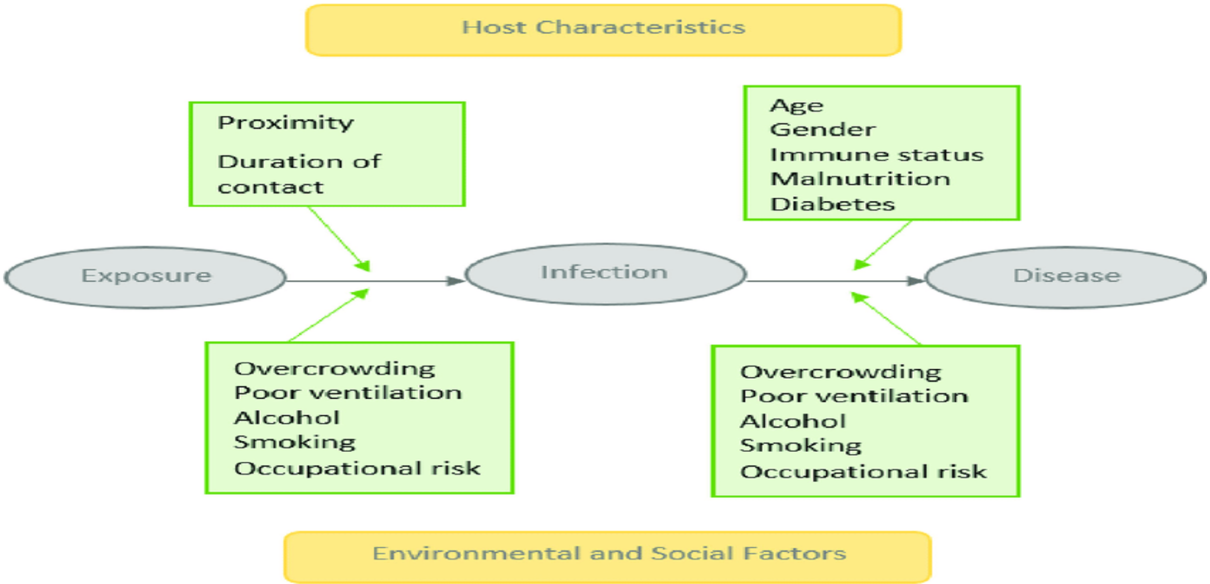

Figure 2.7: Characteristics or behaviors that increase the likelihood of an individual to develop drug-resistant Tuberculosis (Loxton, 2019; Narasimhan et al., 2013)

2.10.1 Co-infection with HIV/AIDS

Tuberculosis is a leading cause of death among HIV patients, especially in high-burden countries (Naidoo et al., 2022). TB and HIV co-infection increases the likelihood of harboring and developing MDR/XDR-TB strains (Niward, 2019). HIV coinfection with MDR/XDR-TB complicates the management of the disease and is regarded as a major threat which requires challenging management (Niward, 2019; Caminero et al., 2018). Due to an increased pill burden, adherence to therapy, overlapping or additive adverse drug reactions (ADRs), and drug-drug interactions, remains a concern (Caminero et al., 2018).

It is possible that HIV infection, compounded by advanced immunosuppression and antiretroviral therapy, might lead to lower concentrations and variable metabolism of TB drugs within the body, creating an environment conducive to the development of drug resistance (Naidoo et al., 2022; CDC, 2019).

2.10.2 Use of cultural/alternative medicines

Most people from rural areas believe that each and every sickness has a traditional remedy that they can use and get healed within few weeks. They believe in using traditional remedies other than prescribed medication from hospital or clinic (Zegeye et al., 2019). Similar findings were reported in KwaZulu-Natal that most cases of DR-TB were due to patients who abandon their drugs and switch to traditional medication before they even complete their treatment period (Skinner and Claassens, 2016). Along the same line, findings by different study done in Indonesia reported that TB patients developed drug- resistance because they were using traditional medicine instead of their hospital treatment (Duarte et al., 2018). The patients responded that they decided to go for traditional medicines as the treatment period is too long and the drugs given has some side effects (Ruru et al., 2018; Chaidir et al., 2015).

2.10.3 Unemployment

Although TB treatment in South African public health facilities is free, there are hidden costs such as that of transportation (Fana et al., 2019). Skinner and Claassens, (2016) in a study conducted in South Africa, reported that participants who were diagnosed with DR-TB were not adhering to their treatments during their initial stage of TB. It was further elaborated that the main reason for their failure to adhere to treatments was lack of transport money to go collect their drugs on the scheduled day of appointment, which made them skip days or even months without taking their medications. Similarly, TB patients in Brazil struggled to cover transport cost, food, and other medical costs during TB treatment due to unemployment (Ruru et al., 2018).

2.10.4 Close contact with drug-resistant TB individuals

Being in close contact with DR-TB patients pose a greater risk of infection to the next person (Golub et al., 2017). Siddique et al. (2011) in a study done at Karachi in Pakistan reported that patients who were mostly diagnosed with DR-TB were sharing bedroom with someone who has already been diagnosed with the disease. Consistent with these findings, a study done in India by Sigh et al., (2018) reported that, participants who have been in a close contact with house members who are drug-resistant TB, were more likely to carry the resistant bacteria as compared to those who have never been in contact with someone who have been diagnosed with DR-TB. As previously stated, tuberculosis can be spread over a short duration of contact, and such interactions are common in endemic areas with additional risk factors such as poverty, overcrowding, and high infection pressure (Golub et al., 2017).

2.10.5 Substance abuse (Smoking, alcohol consumption)

The increasing evidence of tobacco effect has been worldwide documented as a considerable increase of death among TB patients (Khan et al., 2020; Brunet et al., 2011). According to recent studies, active and passive tobacco smoking while taking TB medications increases the risk of developing DR-TB (Bay et al., 2022). Smoking has been linked to DR-TB because it impairs the functioning of TB drugs, resulting in poorer rates of treatment success and increased TB-related death (Bay et al., 2022; Dookie et al., 2018). Furthermore, smoking has been related with more frequent cavitation and bilateral radiological abnormalities, larger bacillary load, delayed smear and culture conversion, and increased risk of reactivation (Duarte et al., 2018; Tiberi et al., 2018). Other studies have found a dose-response relationship between quantity of cigarettes smoked, years of smoking, and tuberculosis (Kim et al., 2022; Thomas et al., 2019).

The link between heavy alcohol use and TB has long been established, but only recently has it been possible to assess the alcohol-related disease burden (Matzopoulos et al., 2022). According to latest estimations, avoiding high alcohol intake could prevent 17% of DR-TB cases and 15% of deaths (Starshinova et al., 2022).

A number of studies have investigated the influence of excessive drinking of alcohol on tuberculosis, and there is a consistent link between alcohol abuse and more infectious and severe disease symptoms (Maja and Maposa, 2022; Wigger et al., 2022). As per the findings by Kolappan and Gopi (2019), alcohol consumption in TB patients increase health problems and increases the chances of DR-TB development. Freiman et al. (2018) explained that substance abuse among TB patients renders the drugs ineffective thereby activating DR-TB. Wurie et al. (2018) added that when TB patients are under the influence of alcohol, they become too drunk and forget to drink their medication, bringing up high chances of resistance development.

2.11 Laboratory diagnosis of tuberculosis

Though, TB and its drug resistance is common in most countries of the world and well documented in literature, most patients are under diagnosed for DR-TB resulting from inadequate laboratory resources and rapid accurate point-of-care tests (Martinez et al., 2019; WHO, 2017). The key component of correct and effective drug administration depends on the rapid and accurate diagnosis of the disease (Hillemann et al., 2020; Omrani et al., 2014; Tortoli et al., 2012). The treatment of TB and the drug resistant profiles of TB are in most settings based on the epidemiological data from periodic drug resistant surveys (WHO, 2017).

The slow growth of the bacterium is a potential factor affecting the diagnostic procedures, especially in culture (phenotypic) methods for direct detection in clinical specimens (Claassens et al., 2019; Coscolla et al., 2018; NDOH, 2017; Bark et al., 2015).

Appropriate diagnosis of TB and drug resistant forms in patients is more challenging, particularly in resource poor settings like Limpopo Province (Hillemann et al., 2020). In these settings, diagnosis rests on the detection of acid-fast bacilli in sputum coupled with expensive drug susceptibility tests (Hillemann et al., 2020; Omrani et al., 2014; Tortoli et al., 2012). The diagnosis depends on the detection of TB bacilli in sputum or any other specimen depending on the site of infection (CDC, 2013). In most of the health care facilities of poor resourced settings, routine diagnosis is based on sputum smear microscopy and culture that are associated with low sensitivity and long turnaround time (Palomino and Martin, 2014). The inability to rapidly and accurately detect and treat TB leads to the spread of disease in communities (Kiazyk and Ball, 2017; NDOH, 2016).

A good quality sample is required for diagnosis of TB depending on the site of infection (i.e., good quality sputum from lung infection) (Klopper et al., 2013). The samples should always be collected aseptically in an air tight container and be transported to the laboratory at favourable conditions to prevent the multiplication of the contaminating bacteria (Lawn and Nicol, 2020). There are several sequential activities thus, after collection, samples are subjected to slide preparation to be viewed under the microscope (Martinez et al., 2019; Maja and Maposa, 2022).

2.11.1 Microscopy

This technique is used widely to identify TB either directly or indirectly due to its low cost (Feng et al., 2018; Perry et al., 2014). Microscopy has the overall sensitivity of 50-60% (Campbell et al., 2022; Caminero et al., 2018). Mycobacteria are composed of a rich lipid cell wall that is not easy to be stained with ordinary dyes and resist decolorization by acidified alcohol (Helb et al., 2017; Philips, 2013; Zhao et al., 2012). This characteristic of mycobacteria is then said to be acid-fast bacilli (Feng et al., 2018). Currently only two methods are used for staining namely, auramine-o fluorochrome staining for florescence microscopy and Ziehl-Neelsen (ZN) for light microscopy (Gandhi et al., 2014; Gilpin et al., 2016.) The auramine staining is easy to perform, requires less time even for reading thus it is more preferred than the ZN staining (Campbell et al., 2022; Zhao et al., 2012). In Figure 2.8 is an illustration of acid-fast bacilli under a microscope.

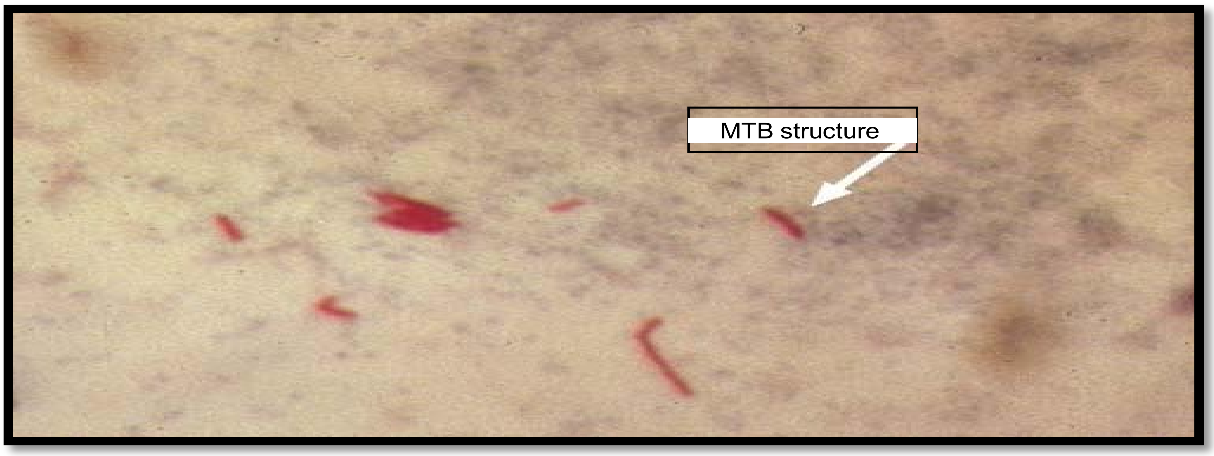

Figure 2.8: The acid-fast TB bacilli visualized under a bright field microscope (CDC, 2013).

2.11.2 Culture

Culture is referred to as the gold standard for diagnosis of TB regardless of the form of media used (solid or liquid) (Mechal et al., 2019). Cultures used have the ability to differentiate *M. tuberculosis* from the rest of mycobacteria due to particular enzyme that is not found in any but only MTB strain (Palomino and Martin, 2014). The specimen is pre-treated by decontamination to prevent other bacteria that can disrupt the identification of *M. tuberculosis* (Campbell et al., 2022; Alexander et al., 2021). Although solid media are still used due to the low cost, liquid media is advised due to its ability of producing growth of the bacilli rapidly (McNerney et al., 2017; Zhao et al., 2012). A Lowenstein-Jensen medium which is egg-based and Middle brook media (agar-based) is a commonly used medium for culturing of *M. tuberculosis* (Dookie et al., 2018; Palomino and Martin, 2014). Figure 2.9 shows the picture of *M. tuberculosis* on LJ media.

Figure 2.9: Colonies of *M. tuberculosis* on Lowenstein-Jensen medium (CDC, 2013).

2.12 Molecular diagnostic methods

The rapid and accurate diagnosis of TB is the cornerstone of global strategies for TB control (Khan et al., 2020; Mechal et al., 2019; McNerney et al., 2017). The use of molecular technology is beneficial to TB diagnostics due to its short turnaround time (Acharya et al., 2020; Gilpin et al., 2016). Tuberculosis is currently diagnosed by total assessment of symptoms, clinical signs, and results from diagnostic methods (chest radiography, microscopy, culture and molecular diagnostics) (Marsh et al., 2019; Gandhi et al., 2014). Together with culture as the gold standard, molecular diagnostic methods based on PCR are broadly used for the early diagnosis of TB (WHO, 2019b). The WHO encourages the implementation of these assays due to their reliability, accuracy and reproducibility (Nguyen et al., 2019; WHO, 2017). New assays are developed to keep track with new mutations and minimize the time of diagnosis (Helb et al., 2017; NDOH, 2017; Huyen et al., 2014). The newly developed technologies are highly specific and sensitive for detection of TB and the drug resistant strains thereby simplifying management of TB (Nguyen et al., 2019).

2.12.1 Drug Susceptibility Testing (DST)

Drug susceptibility testing (DST) is the test used to determine the strain susceptibility to drugs utilized in therapy (Dookie et al., 2018; Chaidir et al., 2015). A “sensitive” indicates high probability of treatment success the patient has with that strain, while a “resistant” result translates that there are high chances of treatment failure and highlights a need for therapy to be changed (Omrani et al., 2014; Perry et al., 2014). The amplification of drug mono-resistance to multiple drug resistant is on the rise and is indicated by exponential occurrence of the MDR and XDR-TB strains which are undermining the advancement made in the control and management of TB indicating that there is a need for accurate drug susceptibility testing (Chopra, 2020).

2.12.2 Conventional (phenotypic) DST

The phenotypic DST is based on the detection of drugs effects on the bacterial multiplication in comparison to the controls which are not exposed to any drug (Niward, 2019; Bark et al., 2015; Tortoli et al., 2012). These conventional methods are probably more accurate, less costly and are more appropriate for surveillance but they are very slow to produce results (Hillemann et al., 2020). Therefore, various laboratories use different methods that are inclusive of medium based, slide DST, absolute concentration, micro-colony, colorimetric redox indicator and automated MGIT 460/960/940 methods (Lange et al., 2018; Akolo et al., 2017). The turnaround time for these methods contributes to delay in clinical intervention (Starshinova et al., 2022; WHO, 2015). There are colorimetric methods that are rapid but not reliable and the macrophage-based method (fast plague assay) that can give results within two days, but due to lack of accuracy these assays are not widely used (Lawn and Nicol, 2020; WHO, 2015).

2.13 Genotypic methods

The molecular DST methods depend on the identification of resistance-conferring mutations of the bacillary genome (Tiberi et al., 2018; Abebe et al., 2016). These techniques do not require viable mycobacteria but always requires extraction of DNA (Wells et al., 2019). Genotypic DST methods create a capacity for early treatment of MDR-TB cases with them requiring less expensive infrastructure for their implementation (Huyen et al., 2014). Safety of personnel performing these methods is secured than in conventional techniques (Wells et al., 2019; Huyen et al., 2014). The techniques can be easily centralized and their specificity and sensitivity for RIF which is a surrogate predictor of MDR-TB is proven to be excellent (Tiberi et al., 2018; Abebe et al., 2016).

However, that does not rule out the fact that even the genotypic methods have limitations (Acharya et al., 2020; Miranda et al., 2016). The most problematic issue with the techniques (excluding sequencing) is their limited ability to detect known mutations in a defined region and are not adequately sensitive to detect unknown mutations due to their probe design (Migliori et al., 2020).

Numerous commercially available molecular tests endorsed by WHO can only detect resistance to RIF and INH; although rare contamination can easily be introduced in these methods (Mechal et al., 2019; Masjedi et al., 2016). The molecular techniques to be performed can be very demanding based on the logistic issues of reagents storage and transportation at specific conditions (Marsh et al., 2019). Most of the consumables are shipped (short expiry dates) and that could be a great challenge to low-income settings considering that the instrumentation used have to be serviced regularly (Maja and Maposa, 2022; Tortoli et al., 2012). Their operation also requires well trained personnel and modern infrastructure namely GeneXpert, MTBDR*plus* and Allplex assays (Maja and Maposa, 2022; Starshinova et al., 2022; Tortoli et al., 2012).

2.13.1 GeneXpert MTB/RIF

PCR-based tests are currently employed in clinical laboratories to provide early diagnosis, which is often part of a routine diagnostic work-up (Lawn and Nicol, 2020). The GeneXpert (Cepheid, Sunnyvale, CA, USA) is an automated real-time PCR machine that requires minimal technical knowledge and has been validated for use in clinical research in pulmonary and extra-pulmonary clinical samples (Lawn and Nicol, 2020; Veluchamy et al., 2013). This is a fast diagnostic test that can detect both tuberculosis and RIF resistance (Hillemann et al., 2020; Helb et al., 2010).

The World Health Organization has approved the assay for the diagnosis of pulmonary tuberculosis as part of its effort to improve TB diagnostics in clinical laboratories around the world (WHO, 2015). Several studies have shown that the GeneXpert MTB/RIF assay is effective for diagnosing both pulmonary and extra-pulmonary tuberculosis (Hillemann et al., 2020; Omrani et al., 2014; Tortoli et al., 2012).

The GeneXpert MTB/RIF assay uses molecular beacon technology, which includes five distinct nucleic acid hybridization probes used in the same multiplex reaction (Naidoo et al., 2022; Marahatta, 2017). The five overlapping molecular probes are responsible for detecting *M. tuberculosis* collectively in the entire 81 base pair core region. Each molecular probe is assigned or rather complementary to a specific target sequence within the rpoB gene of RIF-susceptible *M. tuberculosis*, as indicated by a different colored fluorophore (Nambi, 2015). *Mycobacterium tuberculosis* is recognized when at least two of the five molecular probes produce a positive signal with a cycle threshold of less than or equal to 38 cycles (Chopra et al., 2020; Palomino and Martin, 2014). The results are interpreted based on the presence or absence of *M. tuberculosis*, the presence or absence of RIF resistance, and semi-qualitative estimations of bacilli concentrations as indicated by the CT range (high, <16; medium, 16-22; low, 22-28; very low, >28) (Wells et al. 2019; Marahatta 2017; Nambi 2015).

2.13.2 Allplex™ assay

The Seegene™ innovative Multiplex Real-time PCR method, READ (Real Amplicon Detection), assay is rapid and does not require changes in the laboratory structures or highly qualified individuals to perform and interpret results (Seegene Inc, Korea). Allplex™ MDR-TB Screening Test an assay that is based on two technologies which are PCR related (Allplex, D-66386 St. Ingbert, Germany). The Dual priming oligonucleotide that optimizes PCR, provide freedom on primer design and further maximizing the PCR sensitivity and specificity by fundamentally blocking the non-specific priming (Allplex, D-66386 St. Ingbert, Germany). The real amplicons detection is a new concept that installs the real high-throughput multiplexing in to real time PCR platform (Perry et al., 2014). Single test is completed within three hours. There are fifteen probes designed to detect for RIF resistance and six for INH resistance (Bio-Rad, Hercules, California U.S).

2.14 Summary of literature review

This literature review discusses tuberculosis (TB) in South Africa, focusing on its definition, state, risk factors, control, Directly Observed Therapy Short Course (DOTS), and legislation. Tuberculosis is a dangerous bacterial infection caused by the *Mycobacterium tuberculosis* bacillus, which causes severe death cases (Varshney et al., 2021; Sweetland et al., 2017). Infection occurs through aerosol and inhalation of droplets containing *M. tuberculosis* bacilli (Makonnen et al., 2018; Golub et al., 2017; Hargreaves et al., 2015). If untreated, the disease can lead to death, affecting 80% of cases (Daniel and Osman., 2019; Datta et al., 2017). Over 95% of TB infections occur due to inhalation of droplet-nuclei containing tubercle bacilli into the affected site and pulmonary TB in the alveoli of the lungs (WHO, 2020b; WHO, 2019a). Macrophages in the alveolar ingest the pathogen, which can multiply intracellularly and spread throughout the body, including areas most likely to develop TB disease (Akolo et al., 2017; Kibret et al., 2013).

Tuberculosis treatment involves the use of various drugs, and if these drugs are not effective against the bacilli, MDR-TB develops, which is resistant to RIF and INH (Freiman et al., 2018; Golub et al., 2017; Kale, 2016). This resistance can be selectively exerted by direct or indirect mono-chemotherapeutic agents, allowing drug-resistant strains to become dominant in the host (Kale, 2016; Huyen et al., 2014). Pre-Extensively Drug-resistant TB (pre-XDR TB) is caused by TB bacteria resistant to isoniazid, rifampin, and a fluroquinolone, while XDR-TB is resistant to any fluoroquinolones drug and one of the second-line injectable drugs (Kolappan and Gopi, 2019). The occurrence of XDR-TB in every region raises further alarm about the future of TB control (Guinn and Rubin, 2020).

Mechanisms in which resistance to drugs develops has been identified and broadly categorized: The spontaneous chromosomal mutations that affect key drug targets, enzyme production that degrades or modify compounds (rendering them useless), the formation of a layer that is rich in lipids reducing permeability to drugs and the efflux of drugs through protein pumps (Zegeye et al., 2019; Maswanganyi et al., 2014). Through the understanding of these mechanisms; several molecular diagnostic tests for identification of TB and its resistance to drugs have been developed (Daniel and Osman, 2019; Seung et al., 2015).

Chapter 3
METHODOLOGY

3.1 Introduction

The previous chapter presented literature on TB and various risk factors that may increase the likelihood of developing DR-TB. This chapter elaborates the research methods used to answer the objectives of the study. In addition, study setting, data analysis, ethical considerations, data collection and study population are elaborated in detail.

3.2 Ethical consideration

This research work was conducted as part of a bigger project “ADME polymorphism in tuberculosis: pharmacogenetic and pharmacokinetics analysis in TB patients from health care facilities in the Vhembe district of Limpopo province, South Africa”. Ethical clearance was granted by the University of Venda Human and Clinical Trails Research Ethics Committee under the reference number (SMNS/20/MBY/13/2104: Appendix 1). Additionally, permission to access health care centres was granted by the Limpopo Provincial Department of Health (LP_2023-05-013: Appendix 2). Furthermore, permission to gain access to the specific hospitals and clinics was obtained from the respective managers prior to sample collection.

3.3 Study design

This investigation adopted a cross-sectional study design with the primary objective of determining the prevalence of drug-resistant tuberculosis (TB) and associated risk factors among patients from health care facilities, including hospitals and clinics, within the Vhembe region, Limpopo province, South Africa.

3.3 Study setting

The study was conducted in the Vhembe district, one of the four districts which are situated in the Limpopo province, South Africa. Figure 3.1 is a map of Limpopo province, with the red circled part representing Vhembe district. Limpopo is the fifth largest province and the northernmost province of South Africa (Mokhaukhau, 2022). It is named after the Limpopo River, which makes up the province's western and northern boundaries (Barratt, 2018). Limpopo province is the gateway to the rest of Africa, with shared borders that make it well-suited for economic cooperation with the rest of Southern Africa (Makhanya, 2021).

The province shares borders with Botswana to the West, Zimbabwe to the North, and Mozambique to the East. The eastern region covers the northern half of the amazing Kruger National Park (Makhanya, 2021; Barratt, 2018). Approximately 80% of the population in Limpopo is based in rural areas (Makhanya, 2021). It comprises mainly of Tshivenda, Xitsonga and Sepedi speaking people (Ndou, 2019). The province experiences high numbers of immigrants both legal and illegal from Zimbabwe, Mozambique, and Botswana which made it more necessary to conduct this study as immigrants might carry resistance strains (Ndou, 2019).

The size of Vhembe district is approximately 25 597/km^2 and consists of 821 local communities (villages) with a population size of 1 472 615 (Vhembe district municipality profile, 2010/2011). Vhembe district has eight rural-based hospitals including one (psychiatric) hospital which does not admit TB patients and 116 clinics. However, not all of these hospitals and clinics had TB patients during the time of sample collection. Thus, this study was conducted across 12 healthcare facilities strategically chosen based on the presence of TB patients actively seeking medical consultation at these facilities. The data collected were from the following Health care facilities: Mhinga (10), Malamulele Hospital (4), Tshilidzini hospital (7), Tshilidzini clinic (3), Dzingahe clinic (1), Xigalo (1), Phiphidi clinic (5), Magwedzhe clinic (8), Mbokota (2), Tshakhuma clinic (1), Tswinga (6), Tshedza clinic (2).

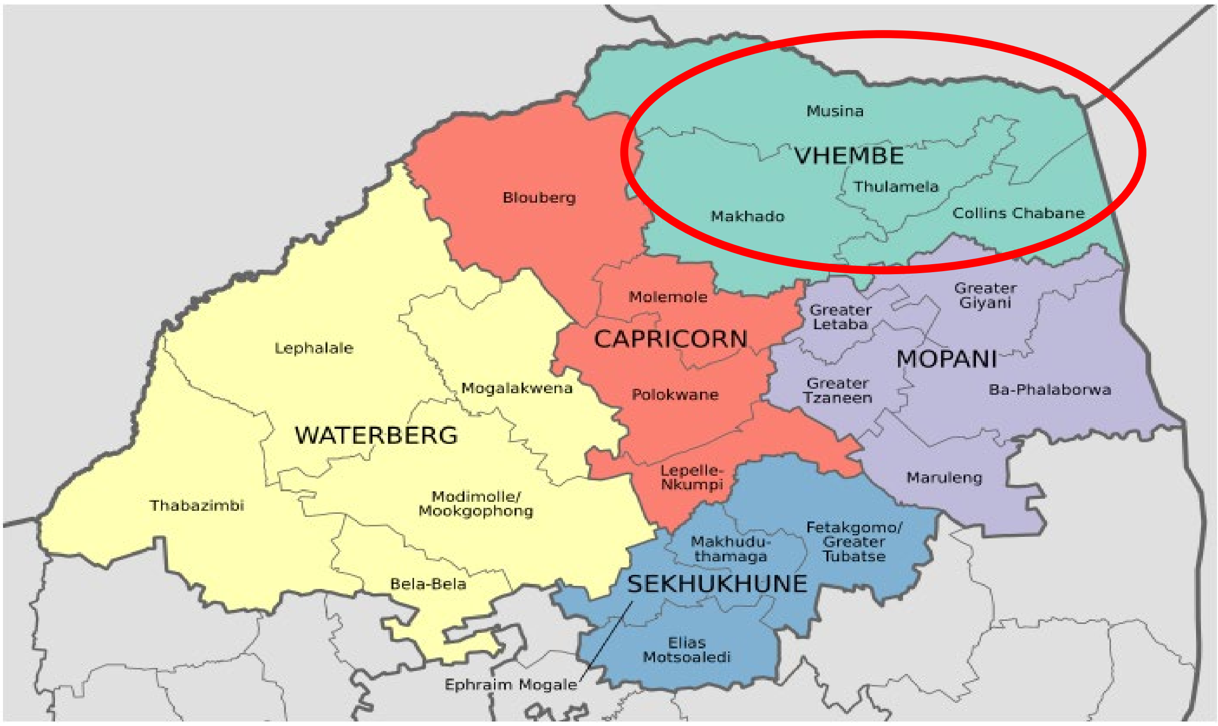

Figure 3.1: Map of the study area (Vhembe district) within Limpopo province (Source: African special tours (2015).

3.4 Sample collection

The study was conducted between August 2022 and August 2023. A total of 50 sputum samples and 50 blood samples were collected from different health care facilities (hospitals and clinics) from patients aged 18 years and older. Patients recruited for this study were those who had received a confirmed diagnosis of TB and were currently undergoing anti-TB treatment. Before commencing with sample collection, a brief explanation about the study was given to patients and they were allowed to give consent if they would like to participate in the study (Appendix 3). After obtaining informed consent from the participants, a face-to-face interview was conducted using a detailed questionnaire to collect relevant clinical information, demographic data, socioeconomic data and all possible associated risk factors (Appendix 4).

From each patient, early morning sputum sample and blood sample was collected aseptically with the assistance from a qualified nurse. The process of sputum collection is shown by Figure 3.2 below. After collection, the sputum containers and blood collection tubes were immediately kept in a cooler box with ice for transportation to the University of Venda TB laboratory, in the department of Biochemistry and Microbiology for further analysis.

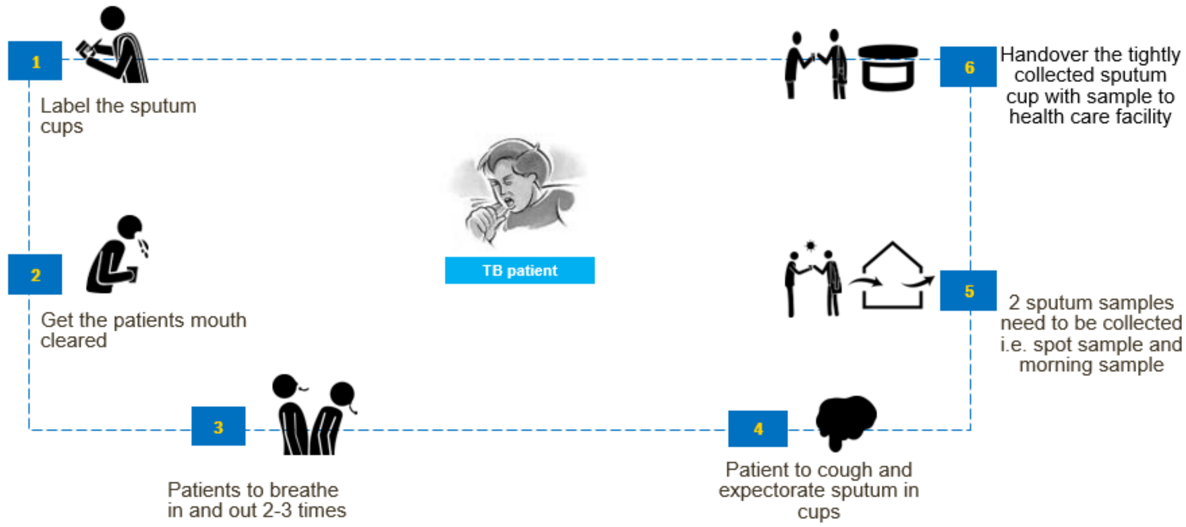

Figure 3.2: Procedure followed during sputum collection (Patil et al., 2019)

3.5 Methodology applied in the study

3.5.1 Sample processing

After collection of samples and upon arrival to the laboratory, rapid HIV testing (Humor Diagnostica, Pretoria, Gauteng, RSA) was done to confirm the status of the patients.

3.5.2 Pretreatment and decontamination of sputum samples

In each container holding the sputum sample, 5ml of 4% inactivating solution (Sodium hydroxide [NaOH], Sigma, St. Louis, Mi, USA) was added and the sample was vortexed for a minute, then incubated for 15 minutes at room temperature. About 1.5 ml of each sample was transferred into a new sterile 2ml tube (Eppendorf, Hamburg, Germany) and centrifuged (Heraeus megafuge16, Thermo Scientific, Waltham, MA, USA) at 15,000 x g (13,000 rpm) for 5 minutes. The supernatant was then discarded and 1 ml of 1X PBS (Sigma) solution was added next. The solution was mixed well and placed on the centrifuge at 15,000 x g (13,000 rpm) for 5 minutes and supernatant was discarded carefully with a pipette. 1 ml of 1X PBS was added again, mixed well and gets to centrifuge at 15,000 x g (13,000 rpm) for 5 minutes and the resulting supernatant was discarded carefully with a pipette according to the manufacturer's instruction (Allplex, D-66386, Seegene, Seul, South Korea). The resulting pellet was preserved at -20ºC for further DNA extraction analysis. Figure 3.3 below is an illustration of some of the steps followed during decontamination of sputum samples as mentioned above.

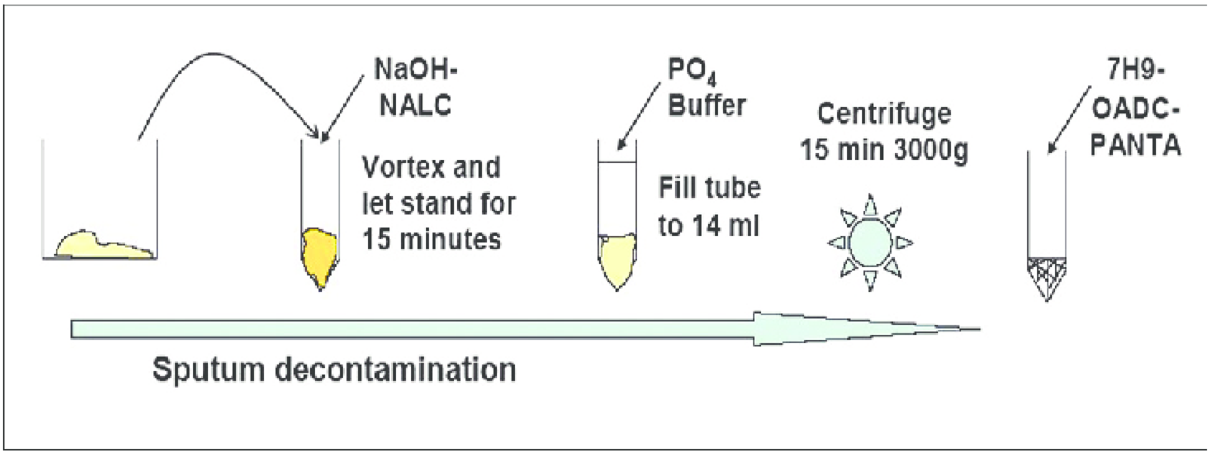

Figure 3.3: Sputum decontamination by NaOH method (Lazarus et al., 2012)

3.5.3 DNA extraction

The DNA was extracted using the Allplex™ MTB/MDR/XDRe kit (Seegene) according to the manufacturer's instructions. The prepared sediments were taken out of -20ºC for thawing before DNA extraction. A volume of 1 ml of sterile water was added to the prepared sediment and centrifuged at 15,000 x g (13,000 rpm) for 5 minutes and the supernatant was discarded with a pipette. A volume of 100 µl of DNA Extraction Solution and 10 µl of MTB/DRe internal control which was included in the kit was added to the sediment and vortexed for 30 seconds. The tube caps were closed properly and gets to boil for 20 minutes on a heat block at 100ºC. After that the tubes were placed on a centrifuge at 15,000 x g (13,000 rpm) for 5 minutes. Then 100 µl of the supernatant was preserved at -20ºC to be used as PCR template.

3.5.4 Real- time PCR to detect *Mycobacterium* TB in sputum samples using ALLPLEX™ MTB/MDR/XDRE CFX96™ Real-time PCR System (Bio-Rad).

The Allplex™ MTB/MDR/XDR procedure was performed according to the manufacturer's instruction (Seegene). According to the number of reactions the master mix reaction tubes were prepared. In each sample 3 tubes were prepared. First tube was allowed to have 5 µl of MTBe TOM, 5 µl EM1, 5 µl of RNase-free water, second tube was allowed to have 5 µl of MDRe TOM, 5 µl EM1, 5 µl of RNase-free water and third tube was allowed to have 5 µl of XDRe TOM, 5 µl EM1 and 5 µl of RNase-free water to make 15 µl of the master mix in each tube. Everything was mixed by quick vortex and briefly centrifuged. Then 15 µl of the PCR Master mix was aliquoted into the PCR tubes.

About 5 µl of each sample's nucleic acids was then added into the tubes containing aliquot of the PCR Master mix to make a final volume of 20 µl in each tube. The caps of the tubes were closed and the tubes were placed in centrifuge. After centrifuging verification was done to check if the liquid containing all PCR components was at the bottom of each PCR tube, if not centrifugation was done again at a higher speed and for a longer time. The protocol was carried out with provided controls every time it was performed. The tubes were then loaded into the CFX96 Real time PCR system (Bio-Rad, Hercules, California U.S) and results were generated. Strict laboratory procedures were being followed to prevent PCR contamination, including separate spaces for handling of samples, Master Mix ingredients, and plasmid templates.

3.6 Data analysis

Data collected was recorded in an Excel spreadsheet. Descriptive statistical analyses were conducted to characterize the TB patient population. To explore the influence of risk factors associated with drug-resistant TB, we employed Rstudio, a statistical analysis software package (Kronthaler and Zöllner, 2021). The association between educational status, employment status, HIV infection, and the presence of family members diagnosed with TB was evaluated among drug-resistant TB patients using the Pearsons chi-squared method. The chi-squared method was used to determine whether the association between two qualitative variables were statistically significant among the study population. A p-value of less than 0.05 was considered statistically significant.

Chapter 4
RESULTS AND DISCUSSION

4.1 Introduction

In this chapter, the results of the study are presented and discussed. Responses from study respondents were converted into percentages and presented in charts and tables to facilitate easy analysis and understanding of the data of the study that sought to investigate the prevalence of drug-resistant tuberculosis and associated risk factors among patients in the northern region of South Africa. The data was analysed based on the study specific objectives and results related to specific study objectives are presented in the subsequent sections.

4.2 Prevalence of TB and drug-resistant TB

A total of 50 TB patients, aged 18 years and older, who had received a confirmed diagnosis of tuberculosis and undergoing anti-TB treatment were recruited as participants for this study. All the patients were taking the same treatment drugs and all on their fourth month taking the anti-TB drugs, however during detection, only 17 (34%) remained TB positive. Of which, 13(26%) were normal TB cases with no resistance while 4 (8%) presented drug-resistant TB strains. The remaining 33 (66%) were no longer showing the presence of MTB in their sputum, however they were positive for Nontuberculous Mycobacteria (NTM) Figure 4.1.

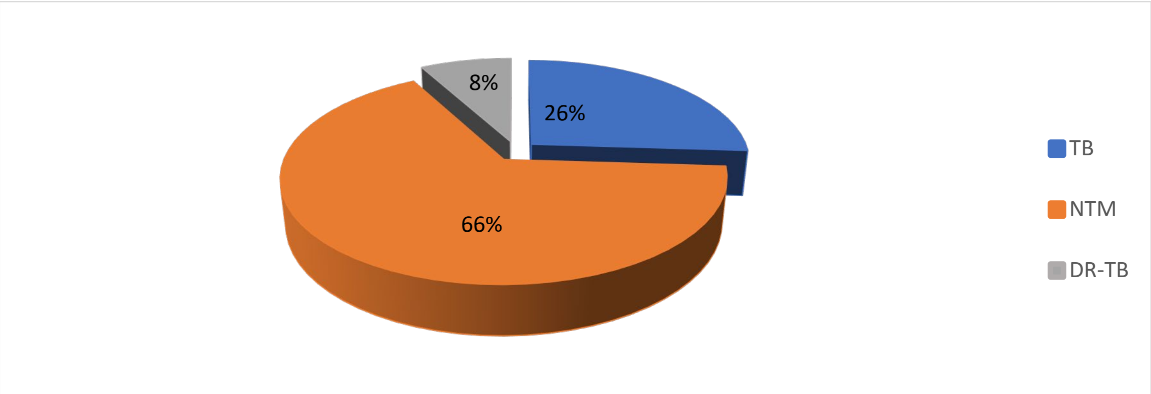

Figure 4.1 Tuberculosis prevalence among patients in the Vhembe district (n=50).

4.2.1 Different TB resistant strains observed among the patients

Below, Table 4.1 shows different TB resistant strains that were dominant among the respondents. Within the 4 (8%) cases of drug-resistant TB, 2(4%) had mono-resistant TB, of which 1(2%) was isoniazid resistance (INH) and 1(2%) was rifampicin resistance (RIF). Furthermore, about 2(4%) of these cases had extensively drug-resistant TB (XDR-TB), showing resistance to isoniazid (INH)+rifampicin (RIF)+ fluoroquinolone (FQ).

Table 4.1 Resistance profile among study respondents in the Vhembe district

Variables	Category	Male n=50 (%)	Female n=50(%)
Age (years)	20-30		2 (4%)
	31-40	2 (4%)	
Resistance profiles	Mono resistance TB		
	INH-R		1 (2%)
	RIF-R		1 (2%)
	Extensively drug resistance TB (XDR-TB)		
	INH-R +RIF-R +FQ-R	2 (4%)	

4.2.2 Socio-demographic characteristics of TB and related resistant patients

In order to gather demographic information about the study respondents, questions on issues such as age, gender, level of education, and occupation were asked in the first section of the questionnaire. Table 4.2 presents demographic variables of respondents as explained above. Majority of non-resistant (26%) respondents and more than half (52%) of NTM respondents belonged to the age group of ≥50 years, while all (8%) those who developed resistance fell within the age bracket of 20 to 40 years.

Equal gender distribution among resistant participants was observed of which males presented 4% of the cases, with females comprising the remaining 4% of the study population (Table 4.2). However more female cases (36%) were observed from NTM as compared to fewer (16%) of those who had normal tuberculosis. It is clear from these two groups that female cases were dominant over male cases. Very few (6%) of these participants reported not having education at all with majority of them having secondary level (32%) of education and tertiary level (52%) of education. Notably all participants (8%) who developed resistance TB were unemployed and they were only depending on piece jobs (self-employment) to sustain their lives. Close to half (40%) of NTM cases were respondents who are employed and can thus afford to cover all costs during their illness (Table 4.2).

Table 4.2 Socio-Demographics of TB patients who participated in the study

Variables	DR-TB n=50 (%)	P-Value	Non-resistant TB n=50 (%)	NTM n=50 (%)
Sex				
Male	2 (4%)	0.127	5 (10%)	15 (30%)
Female	2 (4%)	0.127	8 (16%)	18 (36%)
Age (years)				
20-30	2 (4%)	0.127		3 (6%)
31-40	2 (4%)	0.127		4 (8%)
41-50			3 (6%)	18 (36%)
51-60			5 (10%)	5 (10%)
≥ 61			5(10%)	3 (6%)
Education level				
No education				3 (6%)
primary				2 (4%)
Secondary	1 (2%)	3.246	2 (4%)	2 (4%)
Tertiary	3 (6%)	0.042	11 (22%)	26 (52%)
Occupation				
Unemployed			8 (16%)	4 (8%)
Self-employed	4 (8%)	0.023	4 (8%)	9 (18%)
Employed			1 (2%)	20(40%)

The study investigated healthcare-seeking behaviour and community responses among individuals diagnosed with tuberculosis. Most of the participants (28%) who developed resistance and those with normal TB, sought medical care at healthcare centres after three weeks of illness, as compared to NTM majority (56%) who did so within two weeks of disease onset. Interestingly, tuberculosis vaccination coverage was observed in all (8%) those who were resistant and those who were having NTM participants (Table 4.3). Upon receiving a positive diagnosis, only 2% from those who were resistant disclosed their condition to their families. Very few (6%) of active and resistant TB patients disclosed their TB status to colleagues, friends and community members with only 4% getting support (Table 4.3). Notably, a substantial proportion of participants (80%) refrained from disclosing their TB status primarily due to the pervasive stigma associated with TB.

Table 4.3 Understanding Knowledge, Social Behaviour and Stigma among resistant, Non-resistant and NTM patients

Variables	DR-TB n=50 (%)	Non-resistant TB n=50 (%)	NTM n=50 (%)
After how long did you go to the health care center for consultation? **<Three weeks** **≥Three weeks**	1 (2%) 3 (6%)	2 (4%) 11 (22%)	28 (56%) 5 (10%)
Have you ever had a vaccine to prevent TB	Yes, 4 (8%) No, 0	10 (20%) 3 (6%)	33 (66%)
When you tested positive for TB, did you tell your family?	Yes, 1(2%) No, 3 (6%)	9 (18%) 4 (8%)	33 (66%)
Does your family seem to be supportive?	Yes, 0 No, 4(8%)	3 (6%) 10 (20%)	32 (64%) 1 (2%)
Did you inform your colleagues at work, community members or friends after tested positive to TB?	Yes, 2 (4%) No, 2 (4%)	1 (2%) 12 (24%)	7 (14%) 26 (52%)
Do they seem to be supportive in terms of encouraging you to go to the health care centers?	Yes, 1 (2%) No, 3 (6%) N/A, 0	1 (2%) 0 12 (24%)	2 (4%) 0 31 (62%)

The results reveal that minority (6%) of NTM of participants sought consultation from traditional healers during their illness with approximately 2% relying on medication given during consultation (Figure 4.2). Moreover majority (20%) of those who developed resistance and those who did not but still with tuberculosis had consulted traditional healers during their illness and about 6% and 18% took medication given respectively. According to Figure 4.2, majority (32%) of these participants had been using self-medication including pharmacy self-medication before visiting the health care facility for diagnosis. Such behaviour has been reported by Zai et al.(2017) as it complicates disease management leading to treatment failure or development of resistant strains. Among the participants, 30% reported having history of TB treatment before with only 2% of those found to have NTM. Some (24%) among the participants mentioned that there was once one person who had drug-resistant TB before within their families.

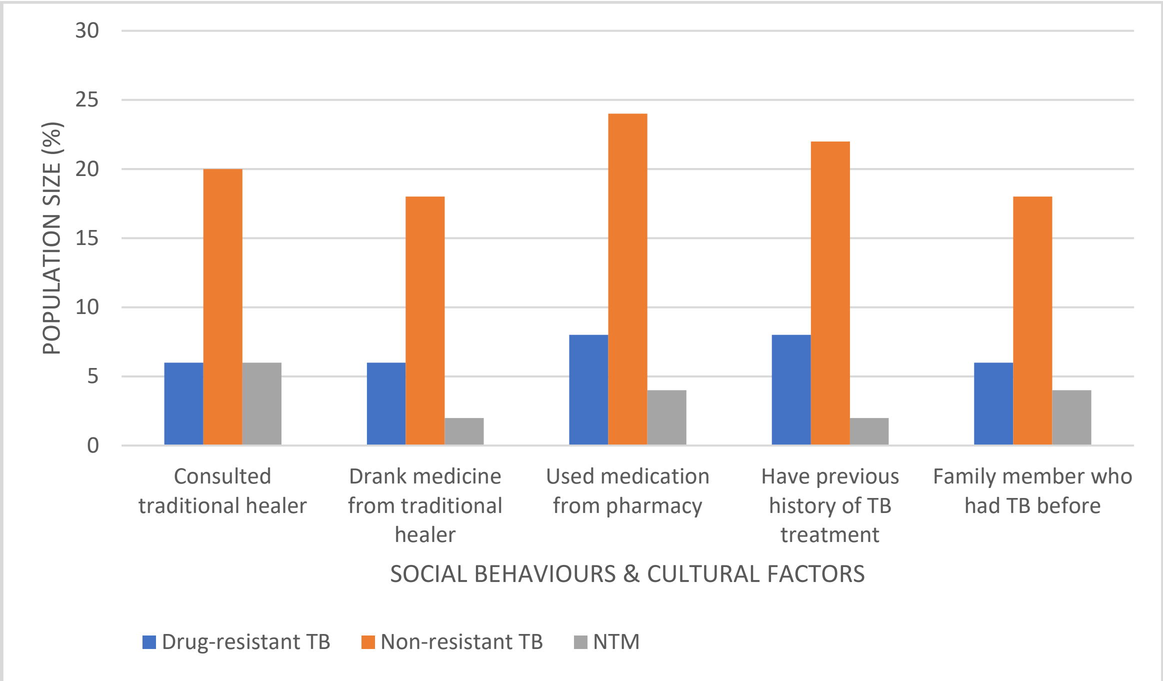

Figure 4.2 Social behaviour and cultural factors among resistant, non-resistant and NTM patients (n=50).

Among the respondents, host-related risk factors and comorbidities were assessed, revealing a substantial co-occurrence of HIV infection within 54% of the participants, alongside lower prevalence rates of Asthma (2%), hypertension (10%) and diabetes (12%) as shown in Figure 4.3

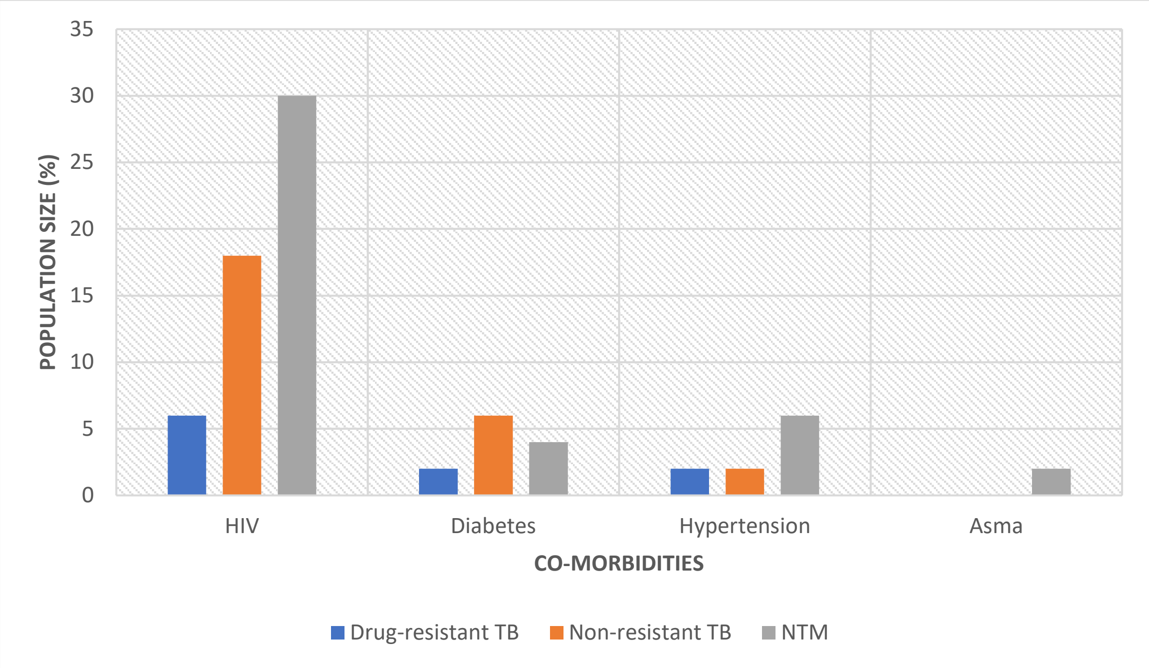

Figure 4.3 Specific factors and co-morbidities among patients who participated in the study (n=50).

4.2.3 Environmental and behavioural characteristics observed among the respondents in the study

Table 4.4 demonstrate the identified environmental and behavioural characteristics of TB patients in Vhembe region, were majority (58%) of patients with less than five family members within their households were those who got NTM as compared to other respondents. About 12% and 10% of normal TB patients had between 5-11 family members within their households, while about and 2% respondents were staying in a household of 5-10 family members and about 2% and 6% of drug-resistant TB patients had about 5- 10 family members respectively.

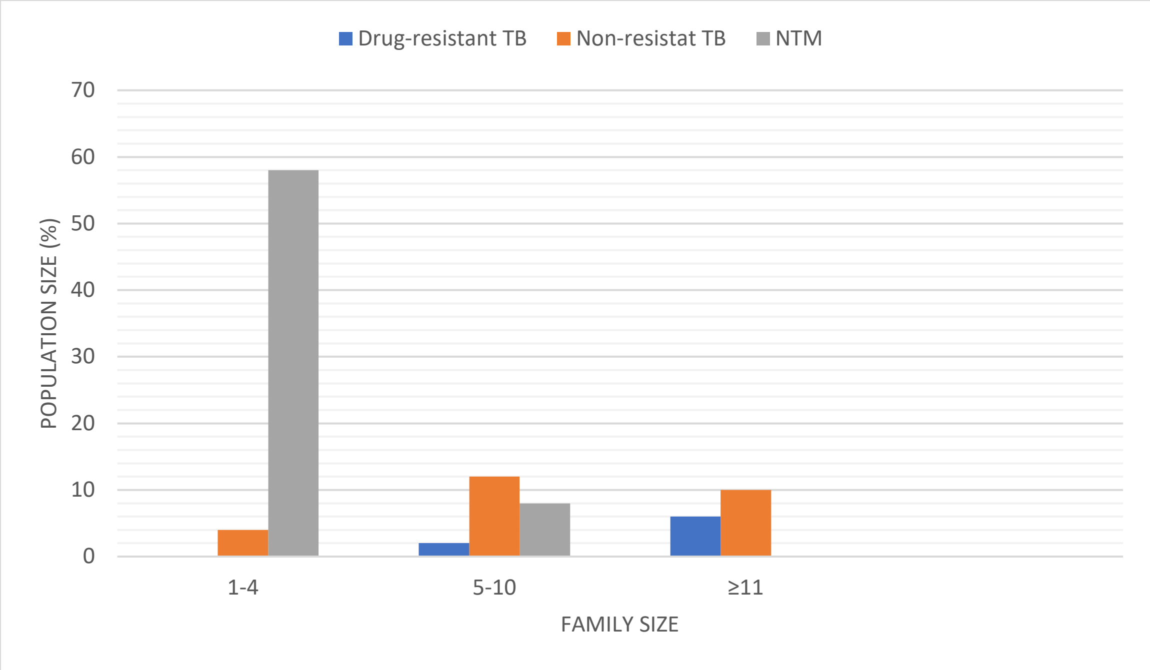

Figure 4.4 Family size among patients who participated in the study (n=50).

About 20% of respondents with normal TB and all (8%) of respondents with drug-resistant TB were sharing their bedrooms with other family members. All (8%) respondents who were resistant reported that their houses had windows, however only 2% were opening windows frequently compared to majority (6%) who never did (Table 4.4). Thirty eight percent (38%) of respondents in the current study indicated that they have travelled to different countries before diagnosis of tuberculosis. About 8% of drug-resistant TB respondents and 20% of active TB respondents within the study population were smokers while 4% of resistant patients drank alcohol. A behaviour of non-adherence to medication in this study was observed among (20%) of those who had resistant TB and those who had normal TB (Table 4.4). All (8%) patients who developed drug resistant TB, reported that they once had contact with a drug-resistant TB patient before diagnosis.

Table 4.4. Environmental and behavioral characteristics among the study respondents

Variables	DR-TB n=50 (%)	P-Values	Non-resistant TB n=50 (%)	NTM n= 50 (%)
Do you share your bedroom with someone else?	Yes, 4 (8%) No, 0	0.023	10 (20%) 3 (6%)	5 (10%) 28 (56%)
Does your house have windows?	Yes, 4 (8%) No, 0	0.023	11 (22%) 2 (4%)	33 (66%)
Do you open your windows?	Yes, 1 (2%) No, 3 (6%)	3.246	3 (6%) 10 (20%)	32 (64%) 1 (2%)
Before diagnosis, did you travel to other countries?	Yes, 2 (4%) No, 2 (4%)	0.127	10 (20%) 3 (6%)	7 (14%) 26 (52%)
Do you wear protective mask?	Yes, 0 No, 4 (8%)	0.023	1 (2%) 12 (24%)	14 (28%) 19 (38%)
Do you smoke?	Yes, 4 (8%) No, 0	0.023	10 (20%) 3 (6%)	9 (18%) 24 (48%)
Do you drink alcohol?	Yes, 2 (4%) No, 2 (4%)	0.127	10 (20%) 3 (6%)	22 (44%) 11 (22%)
Have you ever skipped taking medication?	Yes, 3 (6%) No, 1 (2%)	0.042	7 (14%) 6 (12%)	3 (6%) 30 (60%)
Have you been in contact with someone who has drug-resistant TB?	Yes, 4 (8%) No, 0 Not sure, 0	0.023	4 (8%) 8 (16%) 1 (2%)	3 (6%) 15 (30%) 15 (30%)

About 20% of respondents in this study were nurses in different health care facilities in Vhembe district. Most (4%) drug resistant TB patients in the study were farmers while some (4%) were construction workers (building houses, paving, tiling and ceiling) (Figure 4.5). Most of these respondents worked in dusty environments and majority (70%) never wore protective masks during working hours.

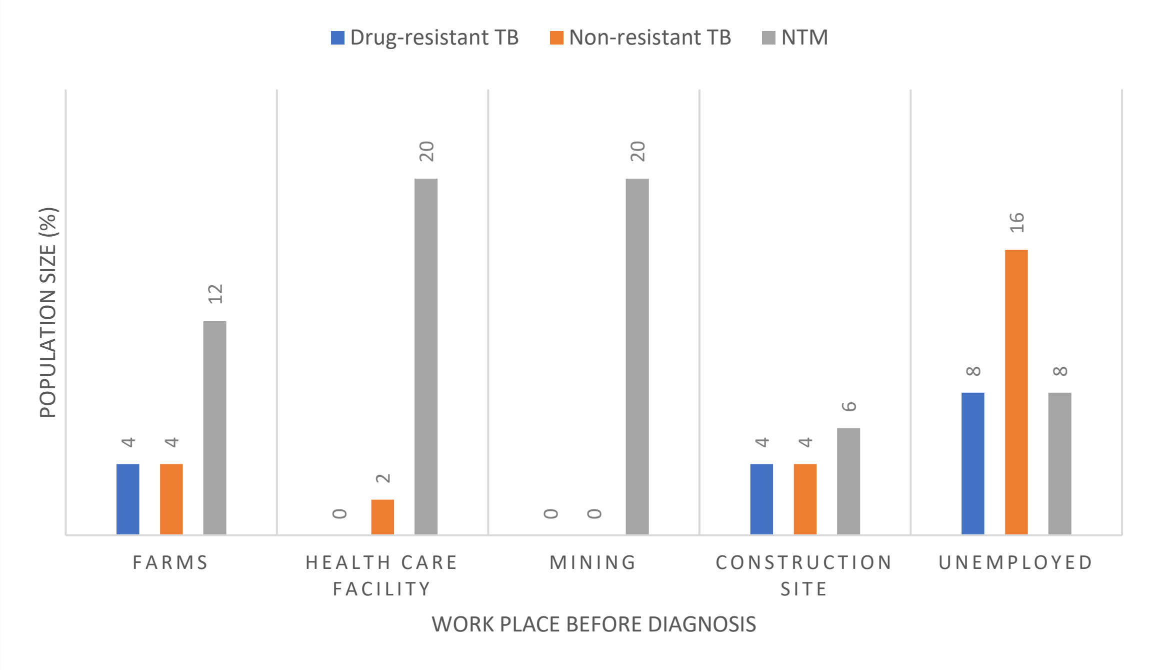

Figure 4.5 Different environments where participants worked before and after diagnosis (n=50)

In this study, the use of public transport was observed among 22% of normal TB respondents and among 8% of drug-resistant TB patients, with majority (44%) of those who had NTM owning cars (Figure 4.6).

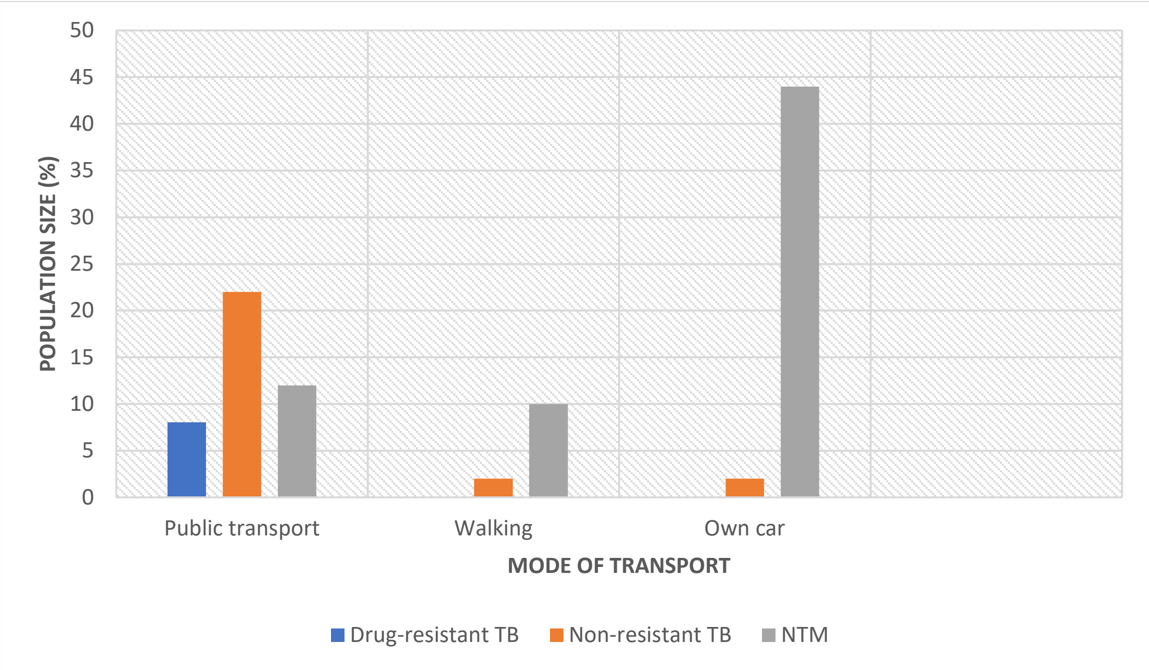

Figure 4.6 Different mode of transport which were used by participants (n=50)

4.2.4 Drug-resistant TB and associated risk factors among the TB patients

Statistical analysis yielded significant associations, notably between previous history of anti-TB treatment and drug-resistant TB status (p=0.023) among the participants. In addition, significant associations between drug-resistant tuberculosis and having contact with drug-resistant TB patient (p=0.023) were observed among the participants, as well as cohabitation with a family member who had tuberculosis before (p=0.042). Smoking behaviour and non-adherence to TB medication showed a strong association with p-values (p=0.023) and (p=0.042) respectively (Table 4.5). Furthermore, these analyses revealed a substantial statistical difference across these groups regarding variables gender, age, educational level, and household size. However, no significant distinctions emerged among the study participants regarding their health-related behaviours.

A significant association between HIV infection and drug-resistant TB was observed in this study with p-value=0.042. The appearance of other chronic diseases such as hypertension, diabetes, and asthma, highlight the importance of considering these factors in TB management. Although not statistically associated addressing them could play a greater role in TB management and preventing unsuccessful treatment outcome.

Table 4.5 Risk factors found to be associated with drug-resistant tuberculosis

Variables	DR-TB n=50 (%)	P-value
Unemployment	4 (8%)	0.023
Previous history of anti- TB treatment	4 (8%	0.023
Family member who had tuberculosis before	3 (6%)	0.042
HIV	3 (4%)	0.042
Smoking	4(8%)	0.023
Contact with a drug-resistant TB patient	4 (8%)	0.023
Non adherence to treatments	3(6%)	0.042

4.3 DISCUSSION

4.3.1 Background

Tuberculosis is more common in certain parts of the world, particularly in low and middle-income countries with limited healthcare resources. However, its prevalence varies significantly by region and country. This section discusses the results of the study based on the literature. The similarity, contradictions as well as inferences are deliberated.

4.3.2 Prevalence of tuberculosis in the Vhembe district

This study reflected low prevalence of TB (13/50; 26%) and drug-resistance TB (4/50; 8%) with majority (33/50; 66%) of patients having Non tuberculous mycobacteria (NTM). As these patients were on treatment, medication might have eradicated the bacterium resulting in such high numbers of NTM cases. According to Patil et al. 2019, a patient who has been on treatment for at least 3-4 months, their symptoms start to reduce and their sputum at this stage might be negative. However, the patient needs to complete the full course treatment period which is 6 months to avoid, re-occurrence of the disease or development of drug-resistance.

The patients were all taking the same anti-TB drugs and all being on their fourth month of treatment period. However, among these patients, (13/50; 26%) were still showing the presence of *Mycobacterium tuberculosis* on their sputum with some (4/50; 8%) even showing the development of resistance strains. These patients also reported that their symptoms were not getting any better and also experiencing some side effects. This can be a cause of concern and very alarming as they are not getting any better and medication seems not to be working for them. It is then suspected that the reason why these patients are not getting any better might be due to their living life styles and some behaviors which might be increasing the likelihood of developing drug-resistant TB.

4.3.3 Resistance status in the Vhembe district

In this study, drug-resistant-TB was predominant in the patients of 20-40 years age groups. These shows that young age patients were more prone to drug-resistant TB than old age group, which calls for some concrete steps to be taken to combat this disease in order to save the economically productive population. This finding is in accordance with reports in Ethiopia (Hirpa et al., 2018; Wahab et al., 2015). Similar findings have been reported in other studies which have estimated that 22% and 56% of patients were found in this age group (Patil et al., 2013; Bhat et al., 2010). This age group is mostly exposed to open cases of TB which may be the reason to make this age group more vulnerable. Some studies indicated that there is an increase in mortality and morbidity in young adult population with drug-resistant TB, mostly between 15-44 years of age (Nicholson et al., 2023). On the contrary, a study in another setting found that drug-resistant TB was more prevalent among older age groups (Caminero, 2022).

4.3.4 Risk factors

With regard to gender and DR-TB, both males and females were equally affected by drug-resistant TB in the current study showing that both genders are vulnerable to resistance. However, only males were affected by Extensively drug-resistant TB (XDR-TB) than females. These findings contradict WHO (2021) findings, which showed that in most countries, more males than women are diagnosed with tuberculosis, and men have a higher death rate from drug-resistant TB.

The study participants had a significantly high prevalence literacy level. Only 3 out of 50 individuals had no formal education, while 16/50 (32%) had completed secondary school, and 26/50 (52%) had completed at least tertiary education. A high level of literacy has been reported to positively impact on slowing development of DR-TB because a great degree of literacy has been positively associated with good health perception (Seloma et al., 2023; Pronyk et al., 2020).

Different studies have reported that poverty pushes people to live in overcrowded environments with poorly ventilated rooms, conditions known to favour increased TB prevalence (Chen et al., 2016; Wahab et al., 2015). This study revealed that all (4/50; 8%) participants who developed resistance were unemployed, (2/50; 4%) engaged in peasant farming or some form of self-employment while some (2/50; 4%) were in construction (brick layering, tiling, ceiling). These patients have reported that they inhale a lot of dust during working hours as they work with no protective mask on. Occupational exposure to dust including silica dust, has been well-recognized as a potential risk factor that increases the chances of drug-resistance development among TB patients (Mlangeni et al., 2023).

Moreover, regarding socio-economic factors, majority of patients who developed resistance were from low socioeconomic status. This might be due to poor compliance with TB treatment often due unemployment, poor living and housing condition and limited access to medical treatment and health care services which might facilitate the spread of infectious bacilli (Chen et al.,2016; Wahab et al., 2015). There was a significant positive correlation between unemployment status/ poverty and DR-TB (p= 0.023) that was observed.

This shows that poor / low socio-economic individuals experience a higher prevalence of resistant tuberculosis due to their low income and social status, increasing the risk that such households may fail to adhere to suitable and effective TB treatment. As a result, efforts to prevent and manage drug-resistant tuberculosis should focus on these lower socioeconomic groups first. According to various studies, low socioeconomic level causes overcrowding, slum growth, poor nutrition, and limited access to health care, all of which contribute to the rising tuberculosis burden (Ricks et al., 2017; Zai and Mehmood, 2017; Velásquez et al., 2011). Daniel and Osman (2019) also highlighted that poor socioeconomic conditions contribute to non-adherence to TB treatment, resulting in the development of DR-TB.

Factors such as a history of contact with a known DR-TB patient, history of TB treatment, and smoking have been found to be strongly associated with DR-TB infection. However, factors such as age, gender, and educational status were not statistically associated with DR-TB in this study. The absence of a statistically significant association between these factors and DR-TB in this study does not imply that they are not important factors influencing the incidence of DR-TB. These findings could be attributed to changes in the disease's epidemiology as well as the community's socioeconomic and health status. It is important to highlight that the current findings do not imply that these factors are not related to DR-TB.

An association between history of close contact with DR-TB patients in this study conforms to other studies (Coelho et al., 2019; Ricks et al., 2017; Velásquez et al., 2011). This association would be attributable to acquiring of primary drug resistant bacteria from other patients in the community. History of previous TB treatment was shown to have a strong association with DR-TB in this study, which is in line with findings of (Marahatta et al., 2018; Andrews et al., 2013). This implies that the usual practice of re-treating TB patient with first-line anti-TB drugs is not an effective approach and may contribute to the persistence and continued spread DR-TB strains in the community (Andrews et al., 2013). All (8%) patients who developed resistance in this study had history of TB. A study by Marahatta et al., (2018) had shown a very significant correlation between past history of TB and development of DR-TB, the experience of incompleteness of previous treatment was recorded as a serious predictor of DR-TB in this study.

These findings are also consistent with the study by Tadesse (2015), which reported that resistance to anti-TB drugs can arise after a history of incomplete or inappropriate TB treatment regimens lasting at least one month. This could be because previous inadequate anti-TB treatment only suppresses the growth of susceptible bacilli while having no effect on other resistant strains, creating conditions conducive to the dominant multiplication of pre-existing drug-resistant mutants, a rise and fall phenomenon (Shongwe, 2018; Mekonnen et al., 2015).

Poor treatment adherence remains a key barrier in the global fight against tuberculosis, and the reasons for noncompliance are varied (WHO, 2013). Many studies found that poor treatment adherence and irregular treatment were the most significant risk factors for developing drug-resistant tuberculosis (Hirpa et al., 2018; Mesfin et al., 2018; Mulu et al., 2015). This study also revealed that drug resistance TB developed mostly among patients (6%) who did not adhere to proper treatment during the intensive and continuous phases. This could be linked to increased bacterial death and growth cycles, providing more opportunity for individual mutations of several independent genes to accumulate (Lomtadze et al., 2020).

Moreover, majority (30/50; 60%) of the participants who adhered to the treatment, within a period of four months, it was noticed from their sputum that the bacteria is getting eradicated as the sputum showed no presence of *Mycobacterium tuberculosis* as compared to (10/50; 20%) who had poor adherence level to treatment. These findings show that high compliance levels to TB treatment by NTM patients may be due to participants' sufficient understanding of TB treatment and good health education provided by health care workers on the importance of treatment adherence.

According to Osterberg and Blaschke (2016), factors such as feeling better, treatment side effects, substance abuse (cigarette smoking, alcohol consumption, and others), fear of discrimination and stigmatization, cultural beliefs, and social mobility all contribute to failure to take TB drugs as prescribed by the DOT strategy. Furthermore, community members strongly associate TB with HIV/AIDS due to the perceived mode of transmission, and many TB symptoms, such as weight loss, fever, and chronic cough, are associated with AIDS, labelling most HIV-negative TB patients as AIDS cases, resulting in stigma and discrimination (Barroso et al., 2020). Most of these causes can be avoided if the community is equipped with sufficient knowledge (Osterberg and Blaschke, 2016).

Most (15/50; 30%) of the participants in the study were using public transport to travel to health care facilities for medication collection, where they pinpointed the lack of transport money to facilities as a contributory factor for non-adherence to TB treatment. This is in line with a number of studies which linked non adherence to transport (Zegeye, et al., 2019; Mekonnen and Azagew, 2018; Woimo, et al., 2017; Mathew, et al., 2015). Similar findings were also reported among TB patients who mentioned the transport cost to collect their medication as the main reason why they do not finish their medication (Ruru, et al., 2018). Vhembe district is the poorest district in South Africa, and very rural, therefore majority of the patients come from remote parts of the district with poor infrastructures, which makes public transportation relatively expensive (Mlangeni et al., 2023).

Some participants demonstrated a preference for collecting their medication or seeking consultation at healthcare facilities located far from their homes to avoid encounters with acquaintances. This observation aligns with the findings of Chang and Cataldo (2014), who reported a similar pattern among older and younger individuals in India, where older individuals tend to seek care from government facilities while younger ones opted for private facilities. This choice may be driven by young individuals' desire to avoid recognition by community members in their place of residence. As most of them were unemployed, consultation to private facilities and transport was always costly, these can be a reason why such young age group have developed resistance.

For centuries, Tuberculosis has been subjectively related to environmental risk factors associated with poverty, including indoor air pollution, tobacco smoke, starvation, overcrowded living circumstances, and excessive alcohol consumption (Chen et al., 2016). Scientists are now presenting sufficient evidence to support these correlations, prompting some tuberculosis experts to urge that control strategies must address underlying risk factors in order to restrict disease spread and progression (Chen et al., 2016).

The study elaborated that (16/50; 32%) of the participants were not employed and worked occasional jobs of which when ill, they never had support from employers. Munro et al., (2007), also reported on patients who had difficulty in obtaining sick leave for treatment, fear for asking money to purchase TB drugs and fear of losing work or dismissal and some ending up hiding their illnesses from their employers and thus posing difficulties in treatment adherence and increasing chances of TB transmission among other employees. Among the study respondents, (16/50; 32%) were not getting any support either from their families, colleagues, friends or community members. Lack of support from family members or community members influences TB patients not to adhere to their treatments as they feel left out and not being cared for which arise development of resistance. This is consistent with the findings of Deshmukh et al., (2018) who revealed that TB patients were discriminated in communities where they stay and were barred from partaking in social activities with other members of the community.

Alcohol consumption, cigarette smoking and/or taking illicit drugs are other factors that have been pinpointed in literature as risk behaviours that lead to resistance development in TB patients. In other studies, smoking of tobacco was frequently reported as one of the risk factors for drug-resistant TB (Mesfin et al., 2018) and was also a risk factor for default (Barroso et al., 2020). Freiman, et al. (2018) explain that substance abuse among TB patients impairs focus and renders the drugs ineffective which results to resistance. However, the current study revealed that alcohol consumption was not associated with the development of drug-resistant tuberculosis. Several reports, including one from WHO (2013), have found that alcohol use increases the chance of developing drug-resistant tuberculosis due to poor adherence to treatment, reduced immunological responses, and an increased risk of adverse drugs effects. As a result of these conditions, alcohol drinking was identified as a significant population-level risk factor for MDR-TB (Shin et al., 2019). This study contradicts a publication by Zetola et al., (2016), who reported that alcohol use influences the development of drug-resistant tuberculosis.

Furthermore, it was evident from the findings of this study that smoking contributes significantly to the development of DR-TB. This study supports the findings of Gómez-Gómez et al., (2017), who reported that smoking and alcoholism are strongly associated with MDR-TB. However, the current findings infer that smoking has a co-relation with the development of drug-resistant tuberculosis.

A notable finding of this study was that most individuals (35/50; 70%) did not use face masks, with only (15/50; 30%) adhering to this infection control measure. Non-adherence to wearing face masks emerged as a significant risk factor, particularly among drug-resistant TB patients (4/50; 8%) who used public transport while ill. This behaviour may be attributed to a lack of knowledge and understanding regarding the importance of wearing face masks in minimizing TB transmission. Furthermore, discomfort associated with mask use could also contribute to non-compliance. It is vital to emphasize the significance of face masks in preventing the spread of infectious diseases, as emphasized by the World Health Organization (WHO, 2017). Surgical masks have effectively reduced the transmission of drug-resistant TB, as demonstrated by a study conducted by Dharmadhikari et al., (2012).

According to the findings of this study, majority of patients consulted traditional healers and pharmacies before visiting healthcare facilities. Mathibela et al., (2015), further indicated that approximately 80% of Africans rely on traditional medicines to meet majority of their health needs. The results of these study revealed that (13/50; 26%) of active and resistant tuberculosis patients first consulted a traditional healer, whereas (16/50; 32%) used self-medication rather than seeking treatment in a clinic or hospital. The study done by Hargreaves et al., (2015) found that patients who consulted traditional healers before seeking health care services were more likely to delay commencing their treatments. In this study, most of the participants (19/50; 38%) visited the health care facility after three or more weeks of illness because they preferred to see traditional healer first. Such activity promotes the spread of tuberculosis among community members.

Overcrowding is a crucial factor in the spread of tuberculosis and resistance strains. In this study, approximately (8/50 ;16%) of the respondents lived in an overcrowded household with more than 10 family members in a four-roomed house. Approximately (19/50; 38%) of these participants shared a bedroom with other family members. A study conducted in Gambia found that TB cases rise when there is overcrowding (Hill et al., 2016). Lygizos et al., (2013) discovered that ventilation caused by opening windows and doors provides a high rate of air exchange, absolute ventilation, and theoretical protection against airborne tuberculosis infection. In this study (14/50; 28%) were not opening their windows, which creates a better chance for the bacterium to be transmitted among other family members in an overcrowded household.

Numerous studies have shown that comorbidities such as HIV have a major impact on both TB treatment outcomes and mortality rates (Hayashi and Chandramohan, 2018; Obore et al., 2020). The risk of progression from active tuberculosis to development of resistance is influenced by several risk factors, among which coexisting diseases are one of the most contributing risk factors.

Among the host-related factors examined in the study, Human Immunodeficiency Virus (HIV) emerged as a prominent risk factor, with a notably high prevalence (54%) compared to other host-related factors such as hypertension, diabetes and asthma. The elevated incidence of TB among HIV-positive individuals can be attributed to the compromised immune systems of HIV-positive individuals, which increase the risk of TB reactivation from latent infection (Kirenga et al., 2015). These findings align with a study conducted in Australia, which also reported a higher prevalence of TB among individuals living with HIV (Coorey et al., 2022). The findings of this study provide critical insights into the complex interplay of various risk factors among TB patients in the Vhembe region. The high prevalence of HIV among TB patients emphasizes the importance of integrated approaches in addressing both diseases.

In the current study, HIV infection was associated with drug-resistant TB, contrary to a study conducted by Marahatta et al., (2018), but it is however similar to another study that have found an association between HIV infection and MDR-TB (Andrews et al., 2013). The difference in these studies is likely to have been attributed to variation in HIV prevalence between this study and other studies. In addition, a study by Khan et al., (2017) have shown that drug-resistance TB risk factors are likely to differ in settings with low and high prevalence of HIV and drug-resistant TB.

Chapter 5
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The aim of this study was to determine the prevalence of drug-resistant TB and associated risk factors among patients in the Vhembe district of the Limpopo province, South Africa. This was achieved by using a detailed questionnaire, running a multiplex real time PCR using Allplex™ MTB/MDR/XDRe detection kit, and also by using the chi-square test statistical tool.

The first objective was to determine the prevalence of drug-resistant TB (MDR/XDR) in patients from health care facilities using Allplex™MTB/MDR/XDR.The results of this study provided evidence of lower prevalence of drug-resistant TB as compared to what have been reported in other provinces. As shown in Figure 4.1 and Table 4.1, out of 50 participants that were enrolled in the study only 8% had drug-resistant TB with 4% of the participants having mono-resistant tuberculosis and the remaining 4% having Extensively drug-resistant tuberculosis (XDR-TB). However, among these participants none of them were found to have Multidrug-resistant TB (MDR-TB).

The second objective was to assess the risk factors associated with drug-resistant TB (MDR/XDR) through a detailed questionnaire. The results of this study provided information about potential risk factors that were found to be associated with the occurrence of drug- resistant TB status. Furthermore, although the prevalence of drug-resistant TB seems low, nevertheless, its potential risk to public health is important.

The third objective was to study the association between risk factors and drug-resistant TB using statistical analysis. This study highlighted unemployment, previous history of TB treatment, non-adherence to TB treatment, smoking habit, and history of contact with DR-TB case were the risk factors associated with DR-TB (Table 4.5). Therefore, risk factors which are rarely emphasized in TB control strategies should be considered when formulating TB control policy to monitor patients with drug resistance TB.

In conclusion, this study revealed the immediate and compelling need for comprehensive efforts aimed at strengthening TB prevention, education, and control strategies in the Vhembe district of Limpopo, South Africa. Addressing these key risk factors, most notably treatment adherence and the prevalent issue of TB-related stigma, while placing vital emphasis on early testing, and community engagement, holds the promise of achieving significant eradication in the burden of TB within the region. These findings highlight the importance of health education, as well as efforts by health care providers to improve community knowledge and awareness of the tuberculosis disease, in order to reduce incorrect perceptions about tuberculosis, its transmission, and further promote early TB diagnosis and infection control in the community.

5. 2 Limitation of the study

- The study took place in a rural setting; for some conditions prevailing in urban settings may be different, therefore the study findings cannot be generalized. However, a full description of the methods was elaborated in detail.
- Unwillingness of people to participate in the study due to stigma and society judgement.

5.3 Recommendations for Health Care facilities and surrounding communities

- More preventative campaigns against HIV infection should be introduced further in order to combat HIV infection since HIV is one of the driving factors behind many newly TB infections.
- More educational campaigns should be conducted to educate the public about TB including its mode of transmission and management.
- Adherence counselling should discourage substance abuse and the patients should be referred to social workers and psychologists for counselling.
- There should be community awareness campaigns on TB such that are supportive to TB patients during treatment periods. Counselling of TB patients to disclose their status to significant others is necessary as it could result in increasing support during TB treatment.

- Community awareness should be conducted targeting those with TB on the risk they should avoid as it could lead to development drug-resistant in future.

5.4 Recommendations for future studies

- In-depth studies should be conducted regularly to explore factors that contribute to the development of drug-resistance TB because the factors vary with time and environment.
- More studies should be conducted which will explore the determinants and risk factors of drug-resistant TB on a large sample size.
- Further research is needed at the Vhembe district to confirm if there is no misdiagnosis of tuberculosis to non-tuberculous mycobacteria and new molecular sensitive tests endorsed by WHO need to be used.

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Chapter 1

GENERAL INTRODUCTION

1.1 Background

Tuberculosis (TB) is a life-threatening disease caused by *Mycobacterium tuberculosis* (Silva et al., 2018; Seung et al., 2015). The bacterium primarily infects the lungs; however, it can also infect other parts of the body such as the kidney, spine, and brain resulting in extrapulmonary tuberculosis (Martinez et al., 2019; Gengiah et al., 2014). The disease can spread easily when an infected person releases the bacterium into the atmosphere, this can be through coughing or sneezing (Datta et al., 2017; Cousins et al., 2016).

The *Mycobacterium tuberculosis* can survive in the host without causing any disease, this can be referred to as latent TB infection (Silva et al., 2018). The World Health Organisation (WHO) defines latent tuberculosis infection as a state of persistent immune response to stimulation by *M. tuberculosis* antigens without evidence of clinically manifested active TB (WHO, 2017). Individuals with latent tuberculosis infection (LTBI) do not show any symptoms but typically respond positively to the tuberculin skin test or TB blood test (Migliori et al., 2020; Lohrasbi et al., 2018; Gilpin et al., 2016). Tuberculosis can progress as a newly acquired infection, or reactivation of latent infection (Martinez et al., 2019; Gengiah et al., 2014). However, the progression of the disease can be influenced by the availability of other chronic diseases within the individual and residing in areas with high TB prevalence (Lohrasbi et al., 2018; Ramaliba et al., 2017; Ejeta et al., 2016).

In 2020, 9.9 million people were reported to be infected with tuberculosis (TB), with 1.1 million children, 3.3 million women, and 5.5 million men (WHO, 2020b). According to the World Health Organization's 2022 Global TB report, 10.6 million cases of tuberculosis infections were reported in the year 2021, and 1.6 million people died from the disease (including 187 000 HIV-positive individuals) (WHO, 2022).

Tuberculosis strategies and associated targets have been identified in the United Nations' (UN) Sustainable Development Goals (SDGs) (for the period 2016-2030) and the World Health Organisation's (WHO) End TB Strategy (for the period 2016-2035). These goals and strategies share a common objective: To end the global TB epidemic.

Appendix 1: Ethical clearance granted by the 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: Letters 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.

Ramalivhana (signature) — 14/07/2023

pp **Head of Department** — **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

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LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF
HEALTH

Ref : LP_2021-11-001
Enquires : Ms PF Mahlokwane
Tel : 015-293 6028
Email : Phoebe.Mahlokwane@dhsd.limpopo.gov.za

Prof Traore AN

PERMISSION TO CONDUCT RESEARCH IN DEPARTMENTAL FACILITIES

Your Study Topic as indicated below;

ADME POLYMORPHISM IN TUBERCULOSIS: PHARMACOGENETIC ANALYSIS OF SAMPLES FROM PATIENTS IN HOSPITALS IN THE VHEMBE DISTRICT OF LIMPOPO, SOUTH AFRICA

1. Permission to conduct 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. This permission is for Vhembe PHC facilities only as selected in the NHRD.
   c. In the course of your study, there should be no action that disrupts the routine services, or incur any cost on the Department.
   d. After completion of study, it is mandatory that the findings should be submitted to the Department to serve as a resource.
   e. The researcher should be prepared to assist in the interpretation and implementation of the study recommendation where possible.
   f. The approval is only valid for a 1-year period.
   g. If the proposal has been amended, a new approval should be sought from the Department of Health
   h. Kindly note that, the Department can withdraw the approval at any time.

Your cooperation will be highly appreciated

Head of Department — **Date** 18/05/2022

pp

Private Bag X9302, Polokwane
Fidel Castro Ruz House, 18 College Street, Polokwane 0700. Tel: 015-293 6000/12. Fax: 015 293 6211.
Website: http/www.limpopo.gov.za

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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 Avhean Marry Mehaphuli

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

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

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Appendix 3: 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**

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

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

Appendix 4: 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.		