UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES

UNIVERSITY OF GHANA MEDICAL SCHOOL

THE BURDEN OF EXTENSIVELY DRUG RESISTANT AND PRE-EXTENSIVELY DRUG RESISTANT TUBERCULOSIS AMONG MULTIDRUG-RESISTANT *MYCOBACTERIUM TUBERCULOSIS* PATIENTS IN GHANA.

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FUFILMENT OF THE REQUIREMENT FOR THE AWARD OF MPHIL MEDICAL MICROBIOLOGY DEGREE

DEPARTMENT OF MEDICAL MICROBIOLOGY

NOVEMBER 2022

DECLARATION

This is to certify that this thesis is as a result of an independent research undertaken by Stephen Ofori Yirenkyi under the Supervision of Prof. Japheth A. Opintan, Prof. Mercy Newman and Dr. Gloria Ivy Mensah towards the award of Master of Philosophy Degree in Medical Microbiology at the Department of Medical Microbiology, University of Ghana Medical School, College of Health Sciences, University of Ghana

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ABSTRACT

Background

The majority of people around the world are at risk because of the rise of extensively drug-resistant (XDR) and pre-extensively drug-resistant (Pre-XDR) tuberculosis (TB), especially in developing countries like Ghana. Extensively Drug Resistant Tuberculosis, a considerably more difficult-to-treat type of MDR tuberculosis, had been reported in at least 46 countries as of February 2008. By 2017, 77 countries had reported 11 000 infections of XDR-TB with a treatment success rate of only 34%. In Ghana, the first case of XDR-TB was published in 2018. However, there is insufficient information on the prevalence of XDR-TB, pre-XDR-TB, and their associated resistant mutations in Ghana. The study sought to provide a baseline data on the burden of pre-XDR-TB and XDR-TB in Ghana. It also determined the mutations responsible for pre-XDR/ XDR-TB, for clinical and programmatic management of pre-XDR/ XDR-TB in Ghana.

Aim of Study: The main aim of the study was to determine the proportion of extensively and pre-extensively Drug Resistant *Mycobacterium tuberculosis* among multidrug-resistant *M. tuberculosis* complex patients in Ghana

Methods: One hundred and seventy-one (171) archived clinical isolates of MDR-TB collected from patients across Ghana between, January 2016 to December, 2020 were retrieved. The isolates were retested to confirm their phenotypic and genotypic drug susceptibility to the first- and second-line anti-TB drugs using the BACTEC MGIT system and MTBDR*plus* 96, MTBDR*sl* 96-line probe assays respectively

Results: Of the 171 archival isolates collected, most of the isolates came from 7 regions, Eastern region having the highest (39.5%) followed by Greater Accra (19.8%). Majority of the samples were from males (78.9%). Of the 171 archival isolates collected, 81 (47.4%) were

confirmed to be MDR, 6 (7.4%) were Pre-XDR-TB and no XDR-TB was detected. The *katG S315T1* (73.3%) and *rpoB S531L* (42.5%) were the common mutations observed among isoniazid and rifampicin resistant isolates respectively. Majority of the mutations and amino acid changes that caused pre-XDR-TB were *gyrAWT3+gyrAMUT3A* and *gyrAMUT3A* (D94A) (50.0%) for fluoroquinolone and (*rrsWT1)* C1402T (50.0%) for aminoglycosides. The other detected mutations with their amino acid changes were *gyrA* MUT1 (A90V), *gyrA*WT3+*gyrA* MUT3C (D94G) and *gyrA* MUT2 (S91P) (16.7%) each for fluoroquinolones and *rrWT2* (position 1484) (33.3%) and *rrs* MUT2 (G1484T) (16.7%) for the aminoglycosides.

Conclusion: Pre-XDR-TB prevalence was marginally higher amongst MDR-TB patients in Ghana and no XDR-TB was detected. Nonetheless, a sustained surveillance of pre-XDR-TB and XDR-TB is advocated. The most common flouroquinolone mutation associated with pre-XDR-TB was D94A

DEDICATION

This work is dedicated to all patients with MDR-TB who died of Pre-XDR –TB and XDR-TB before the introduction of Pre XDR-TB and XDR-TB diagnosis in Ghana. It is also dedicated to Mr. Jonas Ofori Yirenkyi, my late father who always urged me to pursue this degree, my supportive wife, Lydia Merley Laryea, my mother, Victoria Agyemfrah and my children, Seth Ofori Yirenkyi, Stephanie O. Ofori Yirenkyi and Steve Laryea Ofori Yirenkyi.

ACKNOWLEDGEMENT

My profound gratitude goes to the Almighty God in Heaven the Creator, for His abundant grace and making this work a possibility.

I also express my profound gratitude and acknowledgement for the immense contributions of my supervisors, Prof. Japheth A. Opintan, Prof. Mercy Newman and Dr. Gloria Ivy Mensah, for their diligent guidance to see this study through. The Lord Jesus Christ bless you to continue to impact into generations.

My heartfelt appreciation to Mr. Felix Sorvor of the National TB control Programme for providing me with reagents, materials, and data towards this work.

I am also grateful to Head of laboratory of the Eastern Regional Hospital, Koforidua and Mr. Roger Laryea of the Eastern Regional Hospital, and Mr. Amo Omar (Head of Chest Clinic Lab.), Korle Bu Teaching Hospital and Mr. Honesty Ganu for their immense support especially for granting me access to the isolates for this work. I wish to thank Mr. Roger Laryea, Mr. Felix Sorvor, Mr. Osmaun Taufik and Mr. Honesty Ganu for their various contributions in laboratory work, data analysis and proof reading of the write up of this work.

I say thank you all and the Lord Jesus Christ bless you abundantly.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AFB	Acid Fast Bacilli
ADR	Adverse Drug Reactions
AM	Amikacin
BDQ	Bedaquiline
CAP	Capreomycin
Cfz	Clofazimine
Cs	Cycloserine
Dlm	Delamanid
DOT	Directly Observed Therapy
DR	Drug Resistance
DR-TB	Drug Resistance Tuberculosis
DST	Drug Susceptibility Testing
EQA	External Quality Control
E	Ethambutol
Eto	Ethionamide
FLD	First-Line Anti TB medicines
GLI	Global Laboratory Initiatives
HIV	Human Immunodeficiency Virus
INH	Isoniazid
hH	High-dose Isoniazid
Imp-Cln	Imipenem-cilastatin
LPA	Line Probe Assay
Lfx	Levofloxacin
Lzd	Linezolid
MDR-TB	Multi Drug Resistance Tuberculosis
MGIT	Mycobacterium Growth Indicator Tube
MTB	*Mycobacterium tuberculosis*

MTBC	*Mycobacterium tuberculosis* Complex
Mfx	Moxifloxacin
Mpm	Meropenem
NTP	National TB Programme
NTM	Non-Tuberculous Mycobacterium
Pre-XDR-TB	Pre- Extensively Drug Resistance Tuberculosis
PANTA	Polymycin B, Amphotericin B, Nalixidic Acid, Trimetoprim and Azloxillin
Pto	Prothionamide
PAS	*p*-amoxicillin acid
PCR	Polymerase Chain Reaction
QRDR	Quinolone Resistance Determining Region
RIF	Rifampicin
RR	Rifampicin Resistance
RRDR	Rifampicin Resistance Determining Region
SLD	Second-line anti TB Drugs
SLI	Second-Line Injectables
S	Streptomycin
SIRE	Streptomycin, Isoniazid, Rifampicin and Ethambutol
TB	Tuberculosis
Trd	Terizidone
WHO	World Health Organization
XDR	Extensively Drug Resistance
ZN	Ziehl-Neelsen
Z	Pyrazinamide

CHAPTER ONE

1.0 INTRODUCTION

Despite intensive efforts to manage it, tuberculosis (TB) continues to be a serious medical issue with a significant public health impact, particularly in low- and middle-income nations (Cao et al., 2013). Tuberculosis is still one of the most prevalent communicable diseases in the world, much like in Ghana where DOTS (Directly Observed Treatment Short Course) is still utilized to administer drugs to treat the illness (Acheampong et al., 2018).

Globally, national TB control programmes (NTP) are finding it increasingly difficult to control and manage TB cases. Significant causes include the increase in drug-resistant strains of the TB pathogen, *Mycobacterium tuberculosis*, as well as inferior disease treatment outcomes. Poor medical management of patients, lack of directly observed treatment, limited, or interrupted drug supplies, poor drug quality, widespread availability of anti-tuberculosis drugs without prescription, and lack of uniformity between the public and private health sectors regarding drug resistance may all contribute to the development of drug-resistant TB and subsequent transmission of drug-resistant strains in the community (Lambregts-van et al., 1998).

The spread of resistant strains, particularly multidrug-resistant (MDR) TB strains, which challenge national control efforts, increases the burden of this contagious deadly infection (Vaziri et al., 2019). Evidence demonstrates that multidrug-resistant tuberculosis (MDR-TB), which poses a severe danger to global TB prevention and burdens developing nations with costly and toxic therapies, worsens the tuberculosis epidemic (Liu et al., 2021). The tuberculosis epidemic has gotten worse over the past ten years due to the progression of drug resistance in failing MDR-TB treatment regimens towards pre-extensively drug resistance (pre-XDR) and extensively drug resistant tuberculosis (XDR-TB) (Oudghiri et al., 2021). According

to the World Health Organization, MDR-TB is defined as *mycobacterium tuberculosis* strain which is resistant to Rifampicin and Isoniazid, pre-XDR-TB is defined as MDR-TB strain and also rifampicin-resistant with resistance to fluoroquinolone (FQ), on the other hand, extensively drug-resistant (XDR)-TB denotes MDR-TB strain with further resistance to any FQs [ofloxacin levofloxacin or moxifloxacin] and other group A drugs (Bedaquilline, and Linezolid) (Sinha et al., 2017; Chakaya et al., 2022).

Extensively drug-resistant (XDR)-TB, a considerably more difficult-to-treat type of MDR-TB, was initially recorded in 46 countries in 2008, however, 77 countries reported XDR-TB cases with an efficacy rate of only 34% as reported by WHO in 2017 (WHO, 2018).

With the detection of a possible link between mutations influencing expression and functioning of chromosome-encoded targets and medication resistance to TB, superior techniques such as molecular technologies have replaced traditional methods for sensitive, and more reliable diagnosis of TB and assessment of the mycobacterial resistance status (Oudghiri et al., 2018). Rifampicin (RIF), Isoniazid (INH), Fluoroquinolone (FQ), and second-line injectable anti-TB medicine resistance have all been extensively demonstrated over the years in a variety of studies. (Angelina et al., 2021; Oladimeji et al., 2022; Ssengooba et al., 2016). Molecular research also suggested that point mutations in the *rpoB* gene are mostly responsible for RIF resistance, while mutations in the *katG*, *ahpC*, and *inhA* genes are responsible for INH resistance (Ali et al., 2011; Oudghiri et al., 2018; Rana et al., 2022). Furthermore, it is hypothesized that point mutations in the genes encoding the two DNA subunits, *gyrA* and *gyrB*, are the main source of FQs resistance. (Province et al., 2019). Other earlier publications indicated that the majority of the mutations causing FQ resistance are concentrated in a brief region of the *gyrA* gene known as the Quinolone Resistance Determining Region (QRDR) (Mujuni et al., 2022; Province et al., 2019). In general, it is understood that mutations in the

rrs genes are frequently known to confer resistance to injectable medicines, however mutations in the *tlyA* gene and *eis* gene have also been mentioned (Ali et al., 2011; Diriba et al., 2022).

1.1 Problem Statement

The advent of *M. tuberculosis* strains which are resistant to powerful anti-tuberculosis medications has endangered global attempts to speed up the END TB Strategy by 2030 adopted by UN in the Sustainable Development Goals. (Goyal et al., 2017; WHO, 2020). The proportion of MDR-TB patients who have XDR-TB or pre-XDR-TB has seen considerable increase over the past few decades in most countries especially high burden TB countries and developing countries like Ghana and continues to rise steadily (WHO, 2019). In 2018, it was projected that 6.2% of MDR-TB infections had extensively drug resistance (Id et al., 2020). Hence, estimating the burden of XDR-TB/ pre-XDR-TB among MDR-TB holds immense epidemiological and preventive importance especially in endemic areas like Ghana,

Finding drug resistance-causing mutations in *M. tuberculosis* in a specific geographic area is crucial for epidemiological purposes (Diriba et al., 2022). In light of this, several studies have been carried out worldwide mostly in high TB burden countries to determine burden and mutations within the *M. tuberculosis* genome especially within the hot spot regions of genes associated with XDR and pre-XDR TB (Ali et al., 2011; Mujuni et al., 2022; Salvato et al., 2019). However, in Ghana, there is inadequate data on the burden of XDR and pre-XDR-TB with the associated gene mutations and the patterns of the mutations responsible for this deadly form of already devastating disease condition. If this is not properly studied and utilized could undermine the END TB strategy being undertaken by the National Tuberculosis Control Programme (NTP). It is against this background that the present study is designed to investigate the burden of extensively drug resistance and pre-extensively drug resistance tuberculosis among multidrug-resistant *mycobacterium tuberculosis* patients in Ghana.

1.2 Justification

According to a recent global T.B report, 3.3% of new TB diagnosis and 18% of TB diagnoses that had previously been treated globally developed MDR/ RR-TB out which 17.9% had pre-XDR/ XDR-TB (Diriba et al., 2022; WHO, 2022). In Africa, in 2017 it was reported that for both newly diagnosed cases and patients that had already been treated, the rates of RR/MDR-TB were 2.7%and 14%, respectively with 727 XDR-TB cases and in 2021, 20000 cases of MDR-TB were reported in Africa with 5.5% pre-XDR/ XDR-TB (WHO, 2018; WHO, 2022). Osei-Wusu and colleagues reported Ghana's first case of XDR-TB in 2018 (Osei-Wusu, et al, 2018).

To execute the necessary measures to reduce risk episodes and related consequences in MDR-TB patients, it is crucial to provide a baseline description of the burden of extensively and pre-extensively drug resistant tuberculosis. The study will provide a baseline data on the burden of pre-XDR-TB and XDR-TB in Ghana and will further provide information on the mutations that are associated with pre-XDR/ XDR-TB and the mutational patterns of these strains in the country. The data would set the platform for policy reforms in clinical and programmatic management of MDR-TB, and XDR-TB/ pre-XDR-TB in Ghana to the benefit of drug resistant TB patients and the general populace.

1.3 Aim of Study

The main aim of the study is to determine the proportion of Pre-XDR/ XDR-TB among MDR-TB patients in Ghana

1.4 Specific Objectives

The specific objectives are to:

i. To determine phenotypic drug resistance of MDR-TB isolates to first line anti-TB drugs
ii. To identify genotypic drug resistance of first- and second-line anti-TB drugs and the mutations associated to pre-XDR/ XDR *M. tuberculosis.*

CHAPTER TWO

LITERATURE REVIEW

2.1. History of Tuberculosis

One of the first infectious diseases that man is aware of is tuberculosis, which first appeared around 3400 BC. It was the root of the "White Plague" that afflicted Europe in the seventeenth and eighteenth centuries. During this time, over 100% of the European population was sick, and TB was responsible for a quarter of all adult deaths (Todar K, 2009;Schiffman, 2009). Tuberculosis (TB) is an ancient infection that has been recorded for thousands of years, from studies of ancient human bones. Its origin remained unknown until March 24, 1882, when Dr. Robert Koch announced his discovery of the causative bacillus, later known as *Mycobacterium tuberculosis* (Nakaoka et al., 2006;Baddeley, 2020). At the time its etiologic agent was discovered in 1882, about one-seventh of all fatalities in Europe were caused by TB. Today, it persists as one of the commonest infections and the leading cause of mortality caused by a single infectious agent (Nakaoka et al., 2006;WHO, 2020). Drug Resistant TB (DR-TB) is giving rise to lots of challenges comparable to those faced prior to the development of chemotherapy, such as the inability to cure TB, high mortality and morbidity, unabated transmission posing a major public health threat and unsustainable treatment cost (Demile et al., 2018).

2.2 Global Burden of Tuberculosis

According to WHO (WHO, 2020) in its 2020 report, approximately more than a quarter of the world population are living with *M. tuberculosis* and despite all the efforts by National T.B control programmes with the END TB strategy adopted by WHO, there was an estimated 10.6 million TB incidences in 2021 with 1.4 million deaths of HIV-negative people and 187, 000

deaths of HIV-positives people. The number of new cases of drug-resistant TB diagnoses in 2021 was about 450 000.(WHO, 2022).

Worldwide, more than 10 million persons with an incidence of both new and relapsed TB infections were diagnosed in 2021 and reported to WHO through various national programs, and other relevant organisations. A rise from 5.8 million in 2020 (WHO, 2022)

A great portion of the global TB cases (Fig. 1) are concentrated in developing and low-income nations.

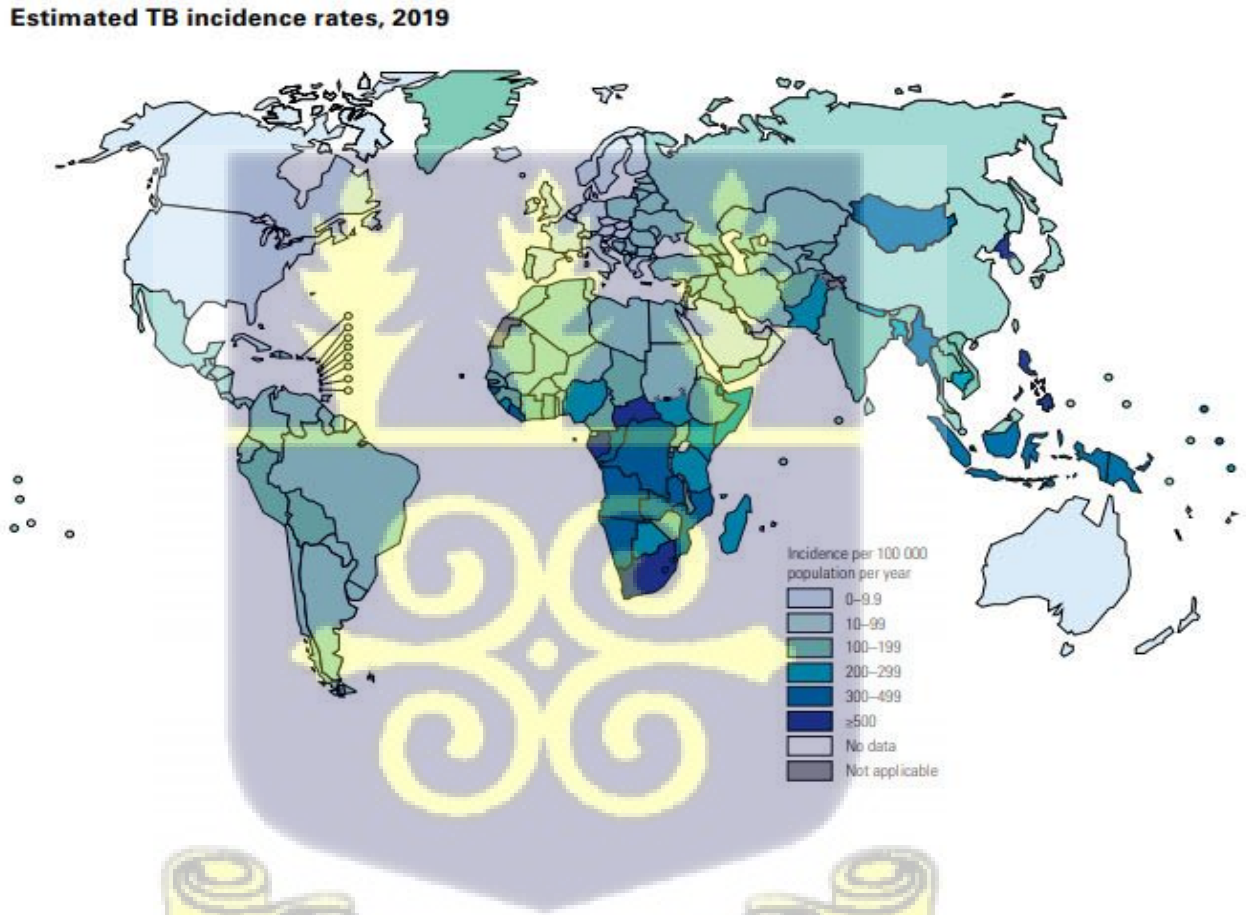


Figure 1. Global estimated tuberculosis incidence rate, 2019. (Source: Baddeley, 2020; Shana, 2021).

The highest rates of TB cases in 2019 were seen in the WHO regions of South-East Asia (44 percent), Africa (25 percent), and the Western Pacific (18 percent). The lowest rates were found in the Americas (2.9%), Europe (2.5%), and the Eastern Mediterranean (8.2%). Eight countries,

including Bangladesh (3.6%), India (26%), Indonesia (8.5%), China (8.4%), the Philippines (6.0%), Pakistan (5.7%), Nigeria (4.4%), and South Africa (3.6%), accounted for two thirds of the global total (Baddeley, 2020; Chakaya et al., 2021).

2.2.1 Tuberculosis in Ghana

A serious concern to public health in Ghana and around the world is tuberculosis (Bonsu et al., 2020) Based on current data, tuberculosis (TB) is a highly prevalent disease in Ghana, contributing significantly to both major disability and death rates in the nation (Addo et al., 2018). According to a recent national TB prevalence survey, 111 (95% CI: 76–145) adults had smear-positive TB per 100,000 people. 356 (95% CI: 288–425) cases of bacteriologically proven tuberculosis were reported for per 100,000 people (Angelina et al., 2021: Chakaya et al., 2022). MDR-TB has been documented to have emerged in Ghana, according to several research. The first cases of extensively drug-resistant (XDR) tuberculosis were reported in 2018 (Angelina et al., 2021). According to the WHO global TB 2022 report (WHO, 2022), the burden of TB in Ghana was 136 per 100 000 population, total incidence of MDR/RR-TB was 3.6 per 100 000 population whiles there was only one (1) reported case of XDR-TB

2.3 Epidemiology of Tuberculosis and Drug Resistant Tuberculosis

TB continuous to be among the top ten causes of mortality globally and the leading cause of mortality from a single microbial agent since 2007 and ranks higher than HIV/AIDS. In 2021, approximately 10.6 million persons were diagnosed with tuberculosis (TB) worldwide and approximately 1.6 million people died of the infection (Helwig et al., 2022). Of the estimated 10.6 million persons diagnosed with TB, 5.5 million (56%) were men, 3.2 million (32%) were women, 1.2 million (12%) were children (Smith, 2021). Two-thirds (2/3) of the global burden were in eight countries; Indonesia, India, China, Nigeria the Philippines, Democratic Republic of Congo, Pakistan, and Bangladesh (WHO, 2022; Smith, 2021). Tuberculosis is found all over

the world, however it is more prevalent in underdeveloped and emerging countries (Baddeley, 2020). In 2019, the World Health Organization (WHO) areas of South-East Asia (44%), Africa (25%), and the Western Pacific (18%) had the most TB cases, with lesser numbers in the Eastern Mediterranean (8.2%), the Americas (2.9%), and Europe (2.5%) (Baddeley, 2020). In Africa, Southeast Asia, the Middle East, Latin America, and Eastern Europe, tuberculosis is widespread. The prevalence of tuberculosis varies greatly depending on the nation, age, race, gender, and socioeconomic position of the individual. Infection with the human immunodeficiency virus (HIV) is a substantial risk factor for active tuberculosis. HIV-positive people are 400 times more likely than HIV-negative people to get tuberculosis (Baddeley, 2020). People living with HIV accounted for 8.2% of all TB cases (Chakaya et al., 2021). The most current global tuberculosis report states that in 2018 3.4% of new TB infections and 18% of cases of TB were diagnosed as Rifampicin Resistant (RR)/MDR-TB. In 2021, around 450000 people globally acquired RR/MDR-TB (WHO, 2022).

A total of 450 000 MDR/RR-TB cases were diagnosed and reported globally in 2021, an increase of 10% from 186 883 cases in 2018 (WHO, 2020)

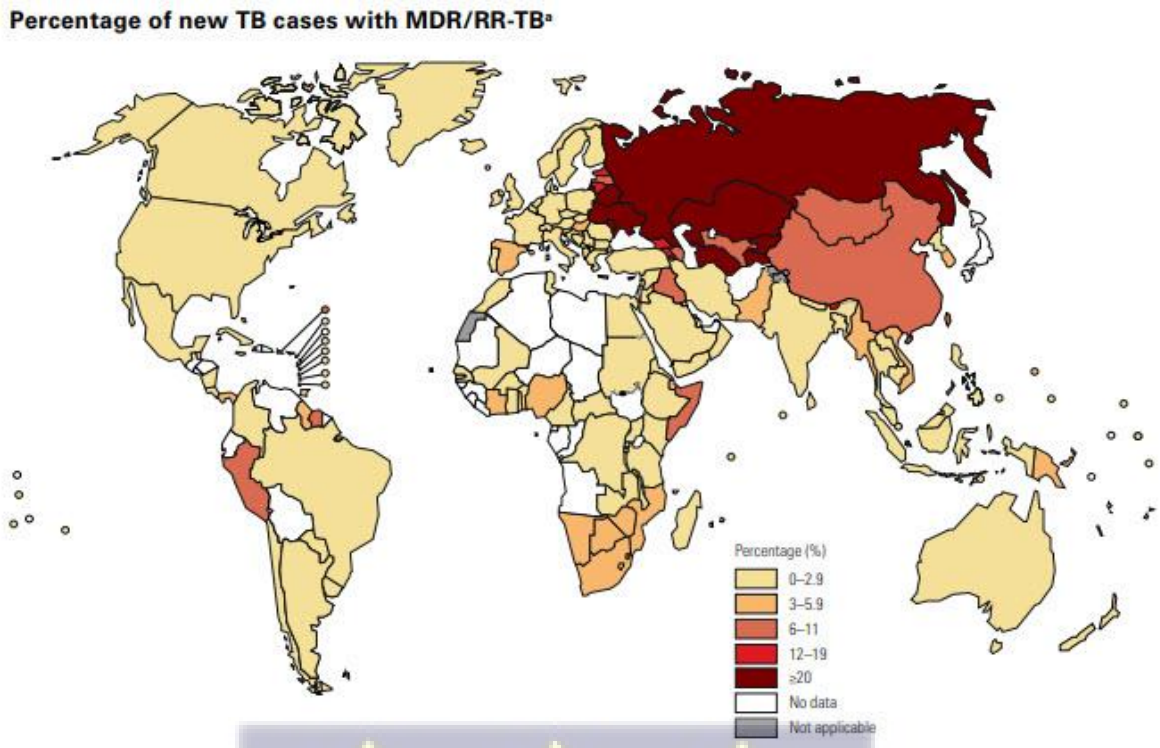


Figure 2. Global Distribution of new TB cases with MDR/ RR-TB (Source, WHO, 2020; Smith, 2021).

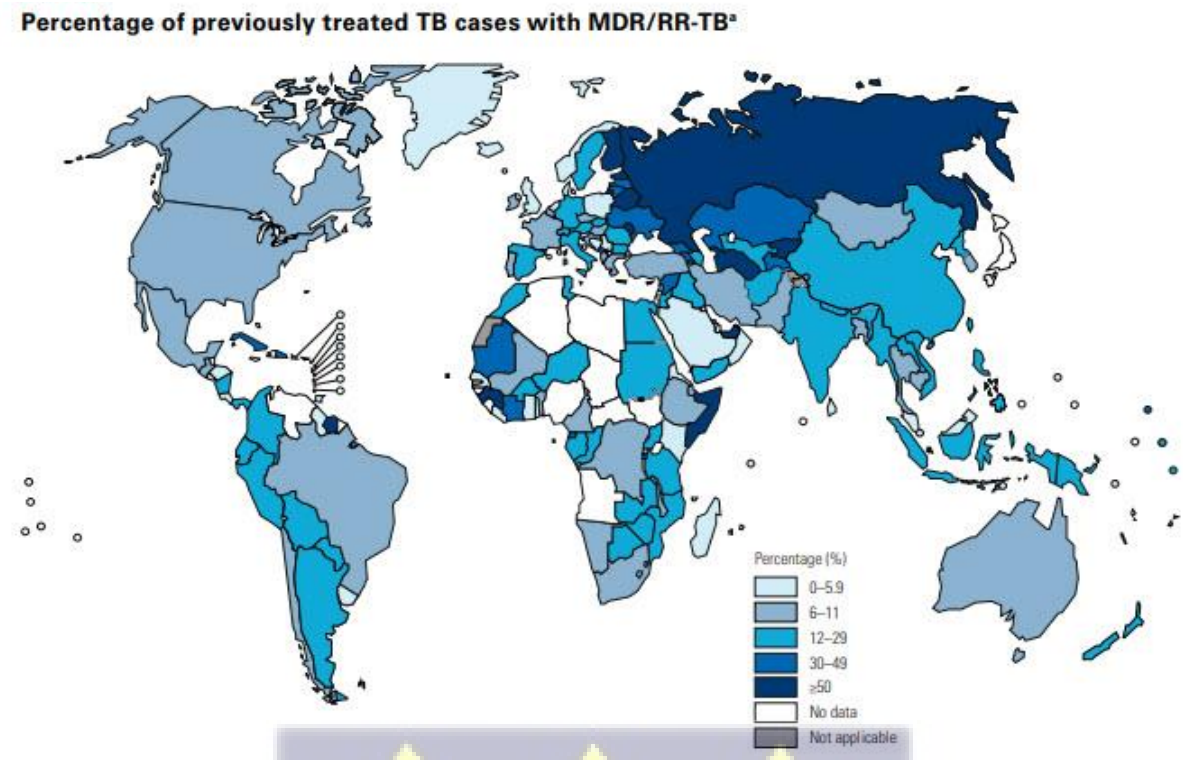


Figure 3. Global Distribution of previously treated TB cases with MDR/RR-TB (Source, WHO, 2020; Smith, 2021).

In 2021, it was estimated that 17.9% of MDR-TB cases worldwide had XDR-TB (Id et al., 2020: WHO, 2022). In Africa it was reported that the rates of RR/MDR-TB for fresh cases and those who had already had treatment were 2.7%and 14%, respectively with 727 XDR-TB cases and in 2021 there were 20 000 cases of MDR-TB with 5.5% pre-XDR/ XDR-TB (WHO, 2018; WHO, 2022). The first XDR-TB was isolated in Ghana in 2018 (Osei-wusu et al., 2018).

TB, HIV-TB positive, MDR/RR-TB, and XDR-TB cases have been reported in 2019 worldwide and for each WHO area. The notifications are listed in the table below.

Table 1.0: TB, HIV-TB positive, MDR/RR TB, and XDR TB Notifications, Worldwide and for WHO Regions, 2019.

WHO REGION	TOTAL NOTIFIED	NEW AND RELAPSE[a]	PULMONARY NEW AND RELAPSE		EXTRA-PULMONARY NEW AND RELAPSE (%)	HIV-POSITIVE NEW AND RELAPSE	MDR/RR-TB	XDR-TB[b]
			NUMBER	OF WHICH BACTERIOLOGICALLY CONFIRMED (%)				
Africa	1 436 330	1 400 293	1 191 433	66%	15%	318 238	29 155	618
The Americas	250 341	235 600	199 417	78%	15%	20 122	4 979	138
Eastern Mediterranean	506 641	497 998	377 324	55%	24%	1 705	6 328	73
Europe	243 789	200 322	168 574	66%	16%	25 100	47 936	8 560
South-East Asia	3 641 245	3 378 887	2 728 541	57%	19%	75 366	86 623	2 444
Western Pacific	1 416 592	1 389 744	1 281 527	46%	8%	15 895	31 009	517
Global	7 494 938	7 102 844	5 946 816	57%	16%	456 426	206 030	12 350

(Source: WHO, 2020)

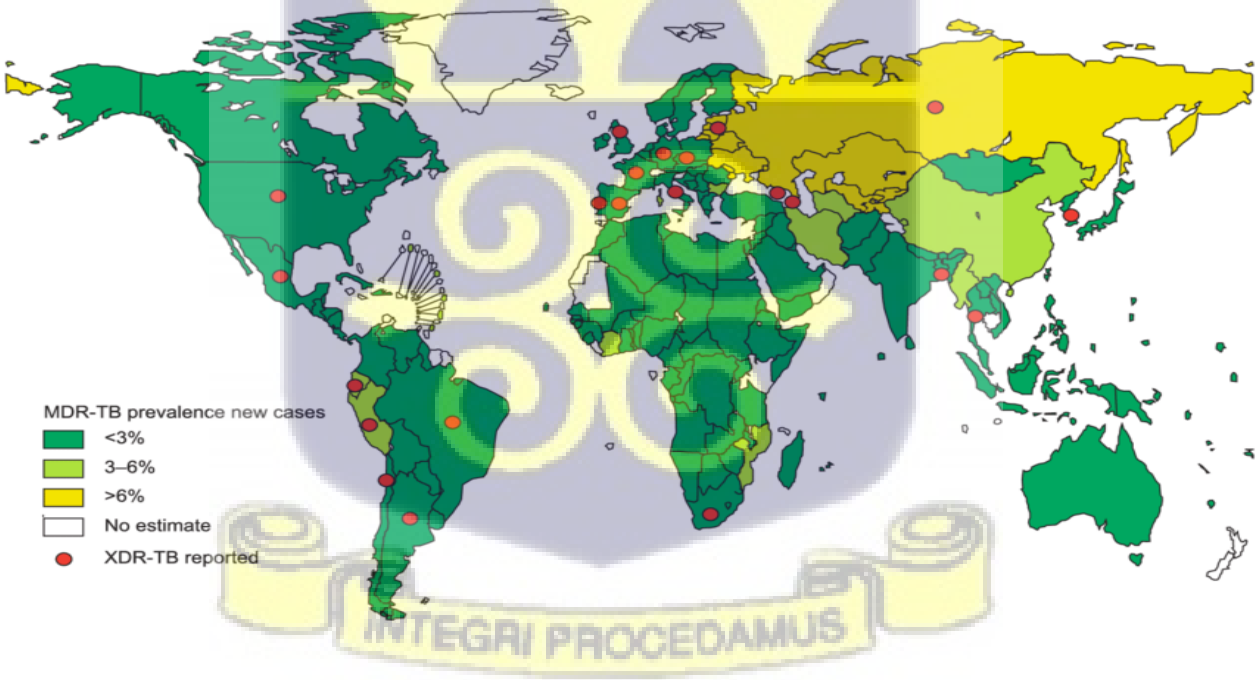


Figure 4. Global distribution of MDR-TB and XDR-TB in 2019 (Source, CDC, 2020)

2.4 Pathophysiology and Pathogenesis of Tuberculosis

The bacillus *Mycobacterium tuberculosis* causes tuberculosis, which most commonly affects the lungs (pulmonary tuberculosis), but can also affect other parts of the body (extrapulmonary TB) (Baddeley, 2020). Alveolar macrophages in the lungs take up *M. tuberculosis*, which are unable to digest it. By inhibiting the bridge molecule early endosomal auto-antigen 1, the bacterium cell wall stops the phagosome from fusing with a lysosome (EEA1). The fusion of vesicles containing nutrients is not prevented by this blockage. As a result, the bacteria grow uncontrollably inside the macrophage and elude macrophage destruction by neutralizing reactive nitrogen intermediates. The bacteria also have the *UreC* gene, which stops the phagosome from becoming acidic ((SS Munsiff *et al*, 2008; Bell, 2005). Tuberculosis may affect almost all the body's organs, and the disease's onset and course might differ from one location to the other. The most frequent and contagious kind of tuberculosis is pulmonary TB. It damages lung tissues, from which *M. tuberculosis* can spread to other organs by blood circulation, causing milliary and extrapulmonary tuberculosis. (Todar, 2009)

Disease development is determined by the *M. tuberculosis* complex strain, previous exposure, immunization, infectious dosage, and the infected host's immunological condition (Todar K, 2009).

There are five phases involved in the development and course of the disease.

Phase 1: Uninfected people inhale small droplet nuclei that can stay airborne for several hours after they are ejected by a person with active TB infection. The nuclei of the inhaled droplets may go to the lungs' air sacs, or alveoli, where infection starts. Alveolar macrophages take up the germs in a general way. However, because they are not active, the macrophages cannot eliminate the intracellular pathogens (Todar K, 2009; Baron et al., 1999).

i. Phase 2 starts between 7 and 21 days after the initial infection. In inactive macrophages, *Mycobacterium tuberculosis* grows almost unchecked until the macrophages explode. To phagocytose the bacteria, more macrophages start to extravasate from peripheral blood into the alveoli, but these are similarly inactivated and cannot kill the germs (Todar K, 2009; Baron et al., 1999; V. Balasubramanian et al, 2004; American Thoracic Society, 2000).

ii. Phase 3: Lymphocytes start to infiltrate alveoli. The *M. tuberculosis* antigen is recognised by lymphocytes, in particular T cells, when it is digested and presented in the presence of MHC molecules. T-cells become activated as a result, and cytokines such gamma interferon (IFN-) are released (Ganu, 2016; Todar K, 2009; Baron et al., 1999). The release of IFN- γ results in the activation of macrophages, making them able to eradicate *M. tuberculosis*. At this point, the person tests positive for tuberculin (Ganu, 2016; Todar K, 2009; Baron et al., 1999). The host's robust cell-mediated immune response is what causes this positive tuberculin reaction. To control an infection, a cell-mediated immune response must be mounted. An antibody-mediated immune response will not assist in the prevention of MTB disease since this organism is intracellular and, if extracellular, is resistant to complement killing because of the high lipid concentration in its cell envelop. (Ganu, 2016; Todar K, 2009). Even though a cell-mediated immune response is essential for preventing TB infection, it is also largely to blame due to the pathology connected to TB. The release of lytic enzymes and reactive intermediates by activated macrophages may promote the emergence of immunological disease tuberculin (Ganu, 2016; Todar K, 2009; Baron et al., 1999).

TNF, gamma IFN, and Interleukin 1 (IL-1) are cytokines that are secreted by activated macrophages and T cells and may contribute to the emergence of immunological disease (Ganu, 2016; Todar K, 2009). The creation of tubercles starts at this point. The

"caseous necrosis" that gives the tubercle its semi-solid or "cheesy" consistency is what gives it its tubercular centre. The anoxic environment and low pH of these tubercles prevent *Mycobacterium tuberculosis* from growing there. However, the bacteria can survive for a long time inside these tubercles (Ganu, 2016; Todar K, 2009; Baron et al., 1999).

iii. Phase 4: Although there are a lot of activated macrophages around the tubercles. Many other macrophages are still inactive or only marginally activated. These macrophages are used by *Mycobacterium tuberculosis* to reproduce, which causes the tubercle to develop (Ganu, 2016; Todar K, 2009; Baron et al., 1999). A bronchus may be invaded by the developing tubercle. If this takes place, *M. tuberculosis* infection may spread to different lung regions. The tubercle may also encroach on an artery or other blood vessels in a similar manner. Extra pulmonary tuberculosis may develop through the haematogenous spread of *M. tuberculosis*, systemic or milliary tuberculosis (Ganu, 2016; Todar K, 2009). Although secondary TB lesions can develop practically everywhere in the body, they typically affect the lymph nodes, genitourinary system, joint, peritoneum and bones. Exudative and granulomatous lesions are the two categories. The build-up of polymorphonuclear neutrophils (PMNs) around MTB causes exudative lesions (Ganu, 2016; Todar K, 2009; Baron et al., 1999). A "soft tubercle" develops as a result of the bacteria reproducing here with almost no resistance. When the host develops tuberculoprotein hypersensitivity, granulomatous lesions take place. A "hard tubercle" develops as a result of this circumstance (Ganu, 2016; Todar K, 2009; Baron et al., 1999).

iv. Phase 5: The tubercles' caseous centres liquefy for an unidentified reason. Because of how well-suited this fluid is essential for the growth of *M. tuberculosis*, the bacterium quickly multiplies extracellularly (Ganu, 2016; Todar K, 2009; Baron et al., 1999).

After a while, the high antigen load causes the adjacent bronchial walls to burst and necrotize. Cavity creation follows from this. Additionally, *M. tuberculosis* can travel quickly to different lung regions and contaminate adjacent airways, as only 10% of *M. tuberculosis* infections result in illness, and an even lower percentage proceed to an advanced state (Ganu, 2016; Todar K, 2009; Baron et al., 1999).

Usually, the infection will eventually be brought under control by the host. The initial lesion becomes fibrous and calcified as it recovers. When this happens, the lesion is referred to as the Ghon complex. The Ghon complex might never go away, depending on how big and severe it is. On a chest X-ray, the Ghon complex is typically easily noticeable (Ganu, 2016; Todar K, 2009).

Most patients only have one main lesion. Bacilli spread through the pulmonary lymphatics as the initial lesion grows, eventually reaching the lymph nodes, which can expand. During the intracellular bacilli's growth, the lymph nodes enlarge. allowing bacilli to escape from the leaky, larger lymph node (Graham R. Stewart, 2003; Ganu, 2016). It's common to refer to TB as progressive primary TB if it arises straight from the primary disease's parenchymal or lymph node component (V. Balasubramanian et al, 2004; Ganu, 2016). The apical parts of the lung are where post-primary illness most frequently develops, even though original lesions can occur anywhere in the lung (Graham R. Stewart, 2003; Ganu, 2016).

Small metastatic foci that have few *M. tuberculosis* cells may also calcify. These foci will typically contain live creatures, nevertheless. These points are known as Simon foci. Chest X-rays can also show the Simon foci, which are frequently where the disease reactivates (Ganu, 2016; Todar K, 2009; Baron et al., 1999).

2.5 Clinical Manifestation of Tuberculosis

The organ involved, the bacterium's features, the surroundings, the host, and also interactions between both host and the organism, all have an enormous effect on the TB symptoms. (American Thoracic Society, 2000; Ganu, 2016). Sometimes symptoms do not appear until the disease has progressed significantly far. Initial symptoms could be attributed to other illnesses that have the same signs as TB. The most symptomatic form of TB, pulmonary, is marked by a chronic cough with progressive phlegm production, chest pains, exhaustion, dyspnea, weight loss, and hemoptysis. Other signs include fever, appetite loss, nocturnal sweats, chills, and paleness (WHO, 2016: Ganu, 2016). Most patients with pulmonary TB also have an abnormal chest X-ray, anaemia, and an elevated erythrocyte sedimentation rate (ESR) (Bonsu et al., 2016); Ganu, 2016).

Extrapulmonary TB has fewer symptoms and is more challenging to identify. Affected bones may enlarge and hurt from tuberculosis of the bones and spine, but the vertebrae may collapse and cause paralysis. If the joints are affected, you can have symptoms similar to arthritis. While intestinal TB causes abdominal swelling and soreness as well as pain that is similar to an appendicitis, bladder and renal TB can induce painful micturition and haematuria. Typically, pain is caused by organ damage at the affected areas (Ganu, 2016; Todar K, 2009).

Isocitrate lyase (ICL), an enzyme necessary for the metabolism of fatty acids, was found to increase *M. tuberculosis* persistence in host tissues in a mouse research by (McKenny *et al*., 2000; Ganu, 2016). In immune-competent mice, ICL gene disruption decreased bacterial persistence and virulence without influencing bacterial growth during the acute phase of infection. The regained pathogenicity of delta ICL bacteria in interferon-gamma (IFN) knockout mice demonstrated a relationship between the necessity for ICL and the immunological state of the host. At the level of the infected macrophages, this connection was obvious. ICL expression was increased when infected macrophages were activated, and the

delta ICL mutant was significantly reduced for survival in active macrophages but not in resting macrophages (McKenny *et al*., 2000; Ganu, 2016). This suggests that the host's reaction to infection has a significant impact on *M. tuberculosis* metabolism in vivo, which has substantial implications for the management of chronic TB (Ganu, 2016).

The *M. tuberculosis* complex strain, prior exposure, immunisation, infectious dose, and the immunological condition of the infected host all have a role in disease progression (Ganu, 2016; Todar K, 2009).

2.6 Transmission of Tuberculosis

Through the intake of air contaminated with droplet nuclei bearing *M. tuberculosis* bacilli with a diameter of 1 to 5µm, tuberculosis is spread from one person to another. The fluid evaporating from these tiny droplet nuclei causes the live tubercle bacillus to float for a considerable amount of time until being breathed (ATS, 2000; Sonal *et al*., 2008). Drug-resistant tuberculosis can spread by primary direct transmission or secondary transmission as a result of inadequate TB treatment over a protracted period of time. (Id et al., 2020; Palmero D. et al, 2015).

2.7 Laboratory Diagnosis of Tuberculosis and Drug Resistant Tuberculosis

The clinical sample used to diagnose pulmonary tuberculosis is sputum. In the study of extra-pulmonary tuberculosis, stools, urine, cerebrospinal fluid (CSF), and aspirates from bone and joints may be utilized to identify *M. tuberculosis*. Microscopy, culture procedures, nucleic acid amplification methods, and immunological tests such as the tuberculin skin test can all be used in the laboratory to diagnose tuberculosis. *M. tuberculosis* may be detected as acid-fast bacilli (AFB) using the Ziehl-Neelsen and fluorescence microscopy procedures for the presumptive

diagnosis of tuberculosis (Frieden *et al*., 2003; Sonal *et al.*, 2008). Only half of all pulmonary tuberculosis patients have Acid Fast Bacilli (AFB) in their sputum (Madigan et al., 2005). Microscopy requires an excess of 10,000 bacilli per ml of sputum to identify *M. tuberculosis* (Todar, 2009). The slightly curved long acid – fast bacilli are stained bright red in the regularly used Ziehl-Neelsen staining procedure, which contrasts sharply against a blue background. If accessible, fluorescent microscopy with Auramine staining is a somewhat more sensitive approach than Ziehl-Neelsen and could be used if available (Flowers, 1995; Ganu, 2016).

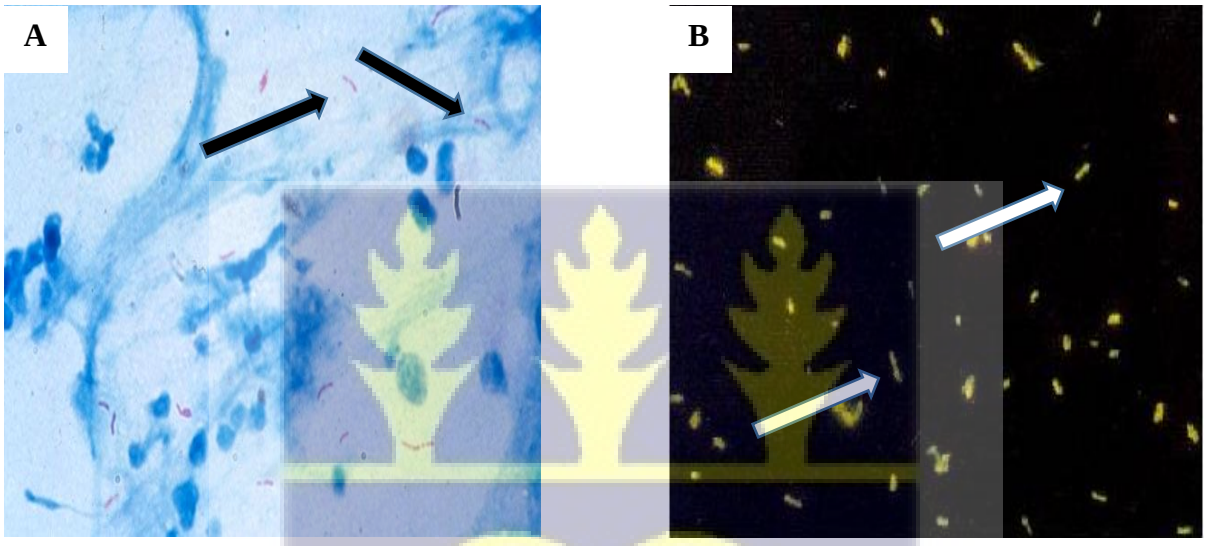


Figure 5: AFBs (arrowed) in Ziehl-Neelsen stained smear (A) and Auramine O stained Smear (B) (Source; Ganu, 2016)

2.7.1 *BACTEC MGIT 960 Liquid Culture System/ Phenotypic DST*

Isolation of *M. tuberculosis* on egg-based selective culture medium such as Lowenstein-Jensen and Ogawa media, which form cream-colored cauliflower-like colonies, can be used to provide a definitive diagnosis. However, because the bacterium takes 6 to 8 weeks to develop, this procedure is rather slow. Broth media, such as Middlebrook medium, and the BACTEC MGIT 960 automated culture system now provide a speedier outcome for both isolation and phenotypic drug susceptibility testing (Chien, 2004). The sensitivity of *M. tuberculosis* culture

techniques is 10 bacilli/ml of sputum sample. *M. tuberculosis* develops cream coloured colonies that are dry with rough surfaces resembling cauliflower on solid medium. They create flakes or floccules in the liquid media of broth cultures (Sonal *et al*, 2008; Ganu, 2016; Bonsu *et al*, 2012).

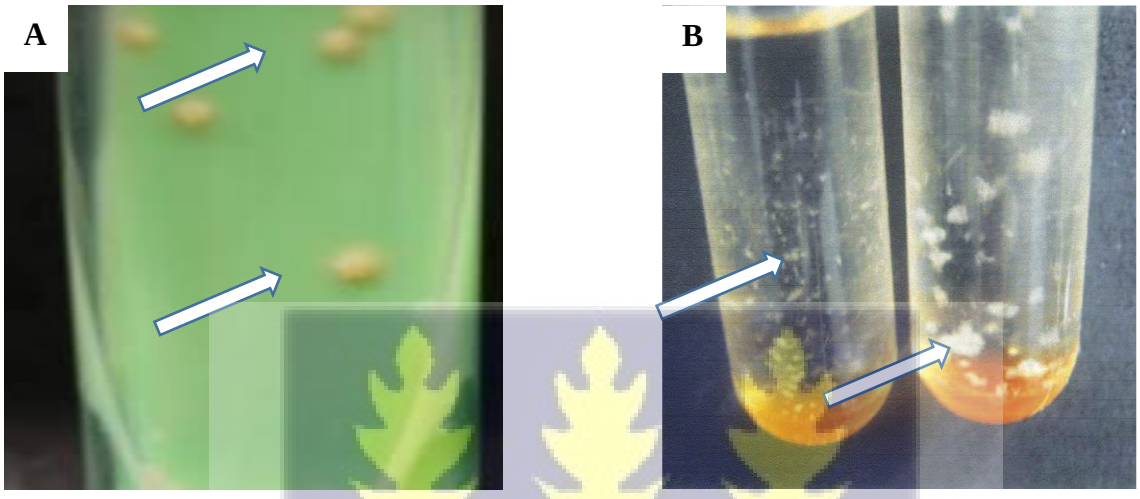


Figure 6: *Mycobacterium tuberculosis* growth on Lowenstein Jensen media (A) and MGIT broth (B) (Source: Bonsu, 2013; (Ganu, 2016)

The MGIT 960 System is a liquid, non-radiometric approach for isolating *M. tuberculosis* and determining its treatment susceptibility. It is an incubation and automated growth detection method that isolates mycobacteria using Middlebrooks 7H9 broth with additional enrichments (Ganu, 2016; WHO, 2020).

Based on how much oxygen is used inside the tube, growth is detected. At the bottom of the tube, silicon gel contains an oxygen-quenched flourochrome, tris-4, 7-diphenyl-1, 10-phenonthroline ruthenium chloride pentahydrate. Free oxygen is used by the bacteria as they develop and is exchanged for carbon dioxide. Since the flourochrome is no longer inhibited by the lack of free oxygen, there is fluorescence under ultraviolet (UV) light, at which at a

determined threshold it is interpreted as positive growth (MGIT Manual). The MGIT system uses qualitative percentage testing to evaluate if isolates of *M. tuberculosis* are susceptible to anti-TB medicines at specific critical concentrations. Before inoculating the growing medium with a suspension of the bacteria isolate, drug solutions are added. Drugs that are effective against *M. tuberculosis* isolates prevent their growth, which reduces the amount of fluorescence in those tubes. In contrast, drug-free tubes (growth control) allow isolates to proliferate unhindered (Bonsu, 2013; Ganu, 2016)

In the isolation of *M. tuberculosis* from clinical specimens followed by DST, several researchers have compared the BACTEC Mycobacterial growth indicator tube (MGIT) 960 method with other liquid radiometric methods and solid media such as Lowenstein-Jensen medium and 2% Ogawa egg medium and found the MGIT 960 method to be a better method (Chien et al., 2000; Goloubeva et al., 2001; P. Idigoras et al, 2000; Jayakumar et al, 1998; Lee et al., 2005; Ganu, 2016). When comparing the nonradiometric techniques available, a study that was included in the Journal of Clinical Microbiology's January 2006 issue found that the MGIT 960 was the most effective method for MTB isolation and drug susceptibility testing (Piersimoni et al, 2006; Ganu, 2016).

2.7.2 *Molecular Diagnosis of Drug Resistant Tuberculosis*

Many molecular technologies currently exist that allow for the quick and simultaneous identification and typing of *M. tuberculosis* in clinical specimens, as well as the detection of treatment resistance genes, shortening the period between suspicion and confirmation of the disease from months to hours (Kamerbeek *et al.*,1997). The common ones widely used and recommended by WHO are GeneXpert, Line Prop Assay and Whole Genome Sequencing (WHO, 2020)

2.7.3 *The MTB/RIF Ultra* and *Xpert MTB/RIF*

A completely automated real-time PCR assay for semiquantitative diagnosis of *M. tuberculosis* and detection of rifampicin resistance is available from Cepheid in Sunnyvale, California, USA. It is called the Xpert MTB/RIF assay. The sensitivity of Xpert MTB/RIF Ultra has increased for the detection of *M. tuberculosis* complex in mixed infections and smear-negative cases. This cartridge-based technology detects frequent mutations in both sputum smear-positive and negative samples in the *M. tuberculosis* codon positions 428 and 452 of the *rpoB* gene's Rifampicin Resistance Determining Region (RRDR)..(WHO, 2020)

2.7.4 *Line Probe Assay*

Traditionally, *M. tuberculosis* strains have been grown in liquid or solid media to test their antibiotic susceptibility. Although it is capable of identifying RIF or INH resistance, second-line anti-TB medicine resistance testing is less precise and complex. (Length, 2021). The World Health Organization currently recommends using line probe assays (LPA) besides the standard culture and antimicrobial susceptibility testing technique, there are several ways to quickly identify first- and second-line drug resistance (Ali et al., 2011; WHO, 2008; Ling D. et al., 2008). DNA extraction, preparing the master mix, performing the PCR, and reverse hybridization are all steps in the LPA process for both first and second line anti-TB drugs using Genotype MDRTB*plus* and Genotype MDRTB*sl*, respectively (Addo et al., 2017). The MTBDR*plus* detects mutations for first line drugs in the *rpoB* and *KatG*, *inhA* hot spot regions for Rifampicin and Isoniazid respectively. *The* MTBDR*sl* line probe assay detects second-line drug resistance quickly and detects mutations in the *gyrA*, *gyrB*, and *rrs* and *eis* hotspot regions for flouroquinolones and second line injectables (that is the aminoglycosides) respectively (Length, 2021). This is shown in Figure 7.

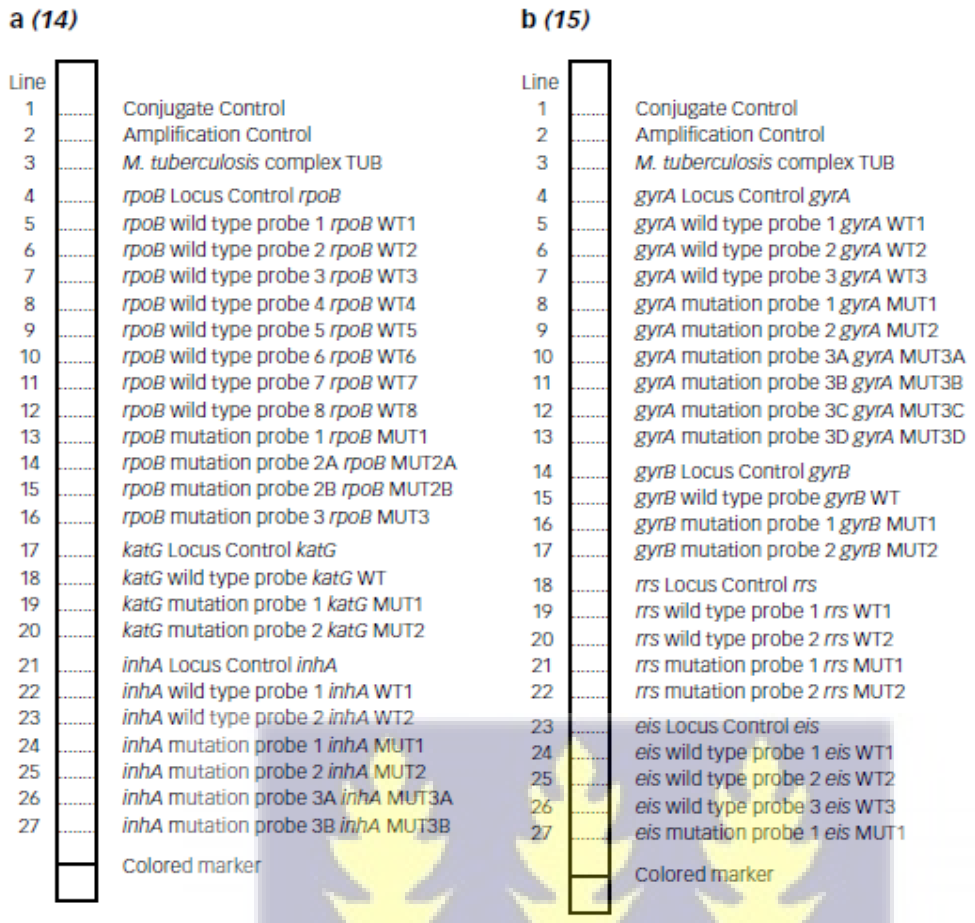


Figure 7: Pattern of the strips for the Genotypye MTBDR*plus* V2 (a) and Genotype MTBDR*sl* V2 (b) (Source: *Line Probe Assays for Drug- Resistant Tuberculosis Detection*, www.stoptb.org/wg/gli)

The *rpoB* gene, which includes four *rpoB* mutant probes [*rpoB MUT1* (D516V), *rpoB MUT2B* (H526D), *rpoB MUT2A* (H526Y), and *rpoB MUT3* (S531L) mutations] and eight *rpoB* wild-type probes, was found to have the most significant alterations, which allowed for the identification of RIF resistance (Ganu, 2016; Bang *et al*., 2006).

To validate the testing processes, the GenoType® MTBDR*plus* V2 strip includes 22 probes in addition to amplification and hybridization controls as well as control probes for gene loci for *rpoB*, *katG*, and *inhA*. The 20 probe Genotype® MTBDR*sl* V2 strip also includes control probes for the *gryA*, *gyrB*, *rrs*, and *eis* gene loci, as well as controls for pcr and hybridization

to confirm the test processes. The TUB probe identifies strains of the MTB complex. 8 *rpoB* wild-type probes (probes WT1 to WT8) cover the area of the *rpoB* gene expressing amino acids 505 to 533 to determine RIF resistance. The most prevalent mutations that confer RIF resistance are particularly targeted by four probes (*rpoB* MUT1 D516V, *rpoB* MUT2A H526Y, *rpoB* MUT2B H526D, and *rpoB* MUT3 S531L). AGC-to-ACC (S315T1) and AGC-to-ACA (S315T2) mutations are assessed by two additional probes (*katG* MUT1 and MUT2), while the wild-type S315 region of *katG* is covered by one probe for the detection of INH resistance. Additionally, the *inhA* gene's promoter region includes areas between locations -15 and -16 for the *inhA* WT1 probe and positions -8 for the *inhA* WT2 probe. With the *inhA* MUT1, MUT2, MUT3A, and MUT3B probes, four mutations (-15C/T, -16A/G, -8T/C, and -8T/A) can be targeted. Once more, evidence of a resistant strain was the presence/staining of one or more mutant probes, or the absence of one or more wild-type probe(s) (Figure 8), (Ganu 2016).

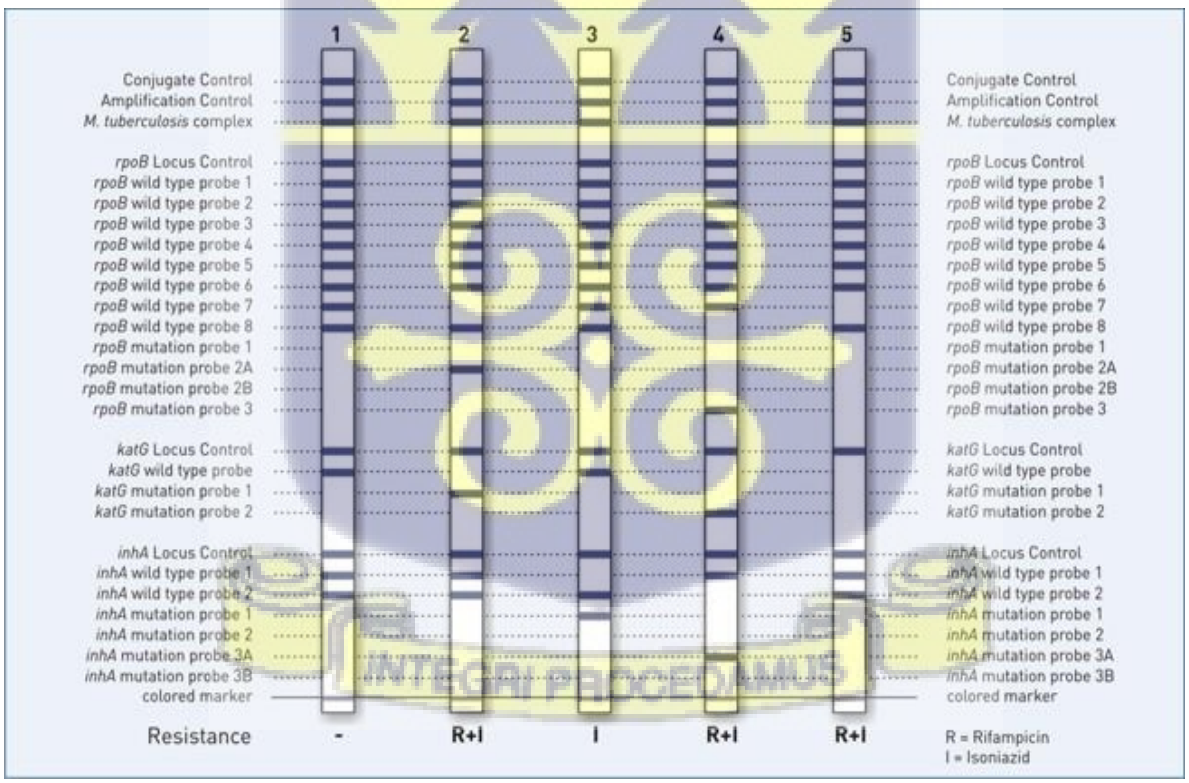


Figure 8: Standard MTBDR*plus* Assay for the detection of MTBC and RIF and INH related mutations (Source: Ganu 2016)

The Genotype® MTBDR*sl* Version 2 contains the quinolone-resistance determining region (QRDR) of the genes *gyrA* (from codon 85 to 96) and *gyrB* (from codon 536 to 541) (16) for detecting fluoroquinolone resistance, as well as the *rrs* (nucleic acid positions 1401, 1402, and 1484) and the *eis* promoter region (from -37 to -2 nucleotides upstream) for resistance to SLI medicines. Eight probes (*gyrA* MUT1 A90V, *gyrA* MUT2 S91P, *gyrA* MUT3A D94A, *gyrA* MUT3B D94N, D94Y *gyrA* MUT3C D94G, *gyrA* MUT3D D94H, *gyrB* MUT1 N538D (N499D) and *gyrB* MUT2 E540V (E201V)) target the most prevalent mutations that confer fluoroquinolone resistance. In order to identify aminoglycoside resistance, *rrs* MUT1 A1401G and *rrs* MUT2 G1484T are the common mutations responsible, one mutation for the *eis* promoter region *eis* MUT1 C-14T is responsible for low-level kanamycin resistance. And again, evidence of a resistant strain was either the absence of one or more wild-type probe(s) or the appearance of one or more mutant probes (Figure 9).

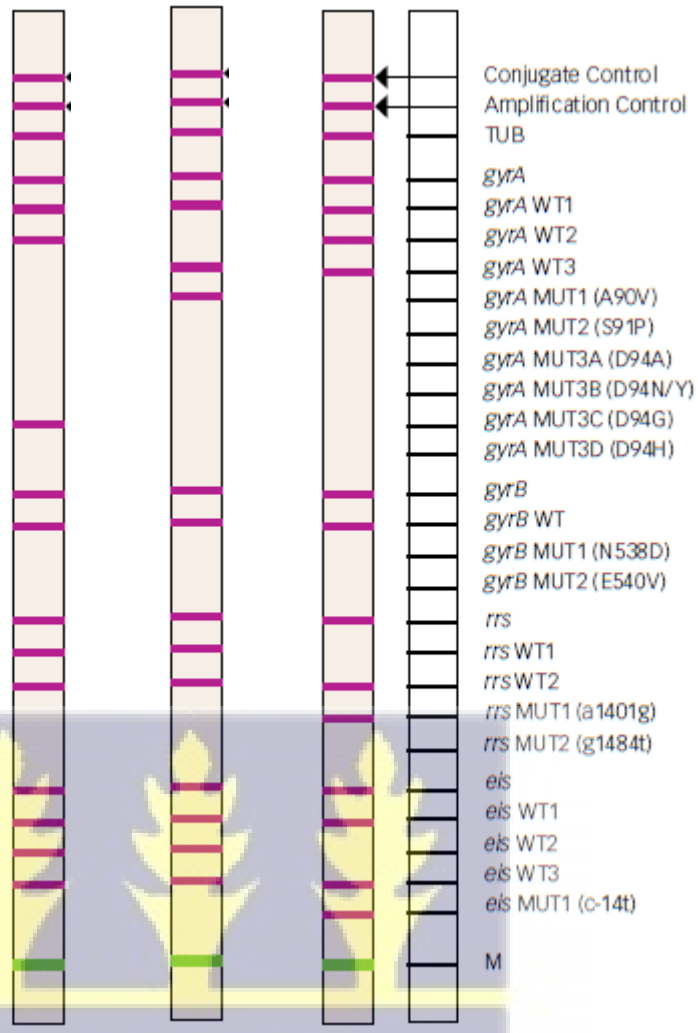


Figure 9: Standard MTBDR*sl* Assay for the detection of MTBC and FQ and SLI related mutations (Source: gli LPA interpretation)

2.8 Treatment/ Prevention of Drug Resistant Tuberculosis

Treatment plans for MDR/RR-TB patients now available are by no means sufficient. Compared to treatments for drug-susceptible TB strains, these regimens need a longer course of treatment, a larger tablet dosage , and the use of drugs with a higher toxicities. Additionally, patients may have severe adverse events and have less favorable treatment outcomes. About 15% of MDR/RR-TB patients still die from the condition, and 26% of those deaths are inflicted on by XDR-TB patients, despite increased treatment outcomes internationally. (WHO, 2020).

Group A drugs (clofazimine, linezolid, high-dose INH, bedaquiline, and delamanid) are frequently used to treat pre-XDR and XDR-TB patients since they are frequently resistant to the majority of potent therapies and have subpar clinical outcomes (Id et al., 2020; Palmero D. et al, 2015).

When a patient's drug resistance profile is not available, a typical treatment plan—which may differ from nation to nation depending on the drug resistance survey (DRS) data for the region—may be chosen. When the DST findings are known, this may be revised to an empirical or a unique regimen (Ganu, 2016).

The following tactical principles are used to choose the medications that will be part of a treatment plan (WHO, 2020; Guideline for MDR-TB, 2019).

i. The patient's history of anti-TB drug use, the country profile of drug resistance and/or the DST profile of the patient.
ii. A minimum of four new core drugs different from ones previously used that are known or expected to be effective
iii. The Injectable drug forms the backbone of the 4 core drugs and should be used in the intensive phase
iv. An effective fluoroquinolone should be selected.
v. Include a first-line medication that the strain is responsive to.
vi. Cross-resistance may occur between drugs of the same group and this is taken into consideration.
vii. Drugs are administered daily under strict directly observed treatment (DOT) course throughout the injectable and continuation phases (Guideline for MDR-TB, 2019; Ganu, 2016).

Two phases make up the Pre-XDR/XDR-TB treatment regimen: the intensive phase and the continuation phase. The drugs used are chosen based on the type of resistant strain being treated. The duration of the treatment is usually twenty (20) months. The course of treatment of Pre-XDR/XDR-TB is summarised in the tables below for adult and children respectively (Guideline for MDR-TB, 2019).

Table 2. The adult regimen for Pre and XDR-TB

TYPE OF RESISTANCE	PHASE OF TREATMENT		TOTAL DURATION
	INTENSIVE	CONTINUATION	
Pre-XDR resistant to SLI	6Lfx-Bdq-Lzd-Cfz-Cs-Z	14Bdq (6)*-Lfx-Lzd-Cfz-Cs-Z	20 months
Pre-XDR resistant to FQs	6Am-Bdq-Lzd-Cfz-Cs-Z	14 Bdq (6)*-Lzd-Cfz-Cs-Z	20 months
XDR-TB	6Dlm-Bdq-Lzd-Cfz--Cs-Z	14 Bdq (6)*-Lzd-Cfz-Cs-Z	20 months

Bdq (6)= Bedaquiline to be given for 6 months in the continuation phase*

(Source; Guideline for MDR-TB, 2019).

Imipenem/clavulanic acid may be substituted for Linezolid for a period of six months in XDR-TB patients who have Adverse Drug Reactions (ADRs) from Linezolid (Lzd). (Guideline for MDR-TB, 2019; WHO, 2020)

Table 3. Pre-XDRTB and XDRTB regimen in children

CATEGORIES	AGE GROUP	REGIMEN	DURATION
Children Confirmed or presumed RR/ MDR-TB	<3 years	Lfx-Lzd-Cfz-Cs	9 to 11 months*
	3 to <6 years	DLM-Lfx-Lzd-Cfz-Cs	
	>6 years	BDQ-Lfx-Lzd-Cfz-Cs	
Pre-XDRTB resistant to SLI	<3 years	Lfx-Lzd-Cfz-Cs	18 months
	3 to <6 years	DLM-Lfx-Lzd-Cfz-Cs	
	>6 years	BDQ-Lfx-Lzd-Cfz-Cs	
Pre-XDRTB resistant to FQs	<3 years	Lzd-Cfz-Cs-Eto	18 months
	3 to <6 years	DLM-Lzd-Cfz-Cs-Eto	
	>6 years	BDQ-Lzd-Cfz-Cs-Eto	
XDRTB	<3 years	Lzd-Cfz-Cs-Eto	18 months
	3 to <6 years	DLM-Lzd-Cfz-Cs-Eto	
	>6 years	BDQ-Lzd-Cfz-Cs-Eto	

*if the child meet others criteria for a shorter regimen. If not the duration should be 18 months

(Source: Guideline for MDR-TB, 2019).

Infection prevention and control, as well as immunization of children with the bacille Calmette-Guérin (BCG) vaccine, are the main methods of preventing *M. tuberculosis* transmission (WHO, 2020).

2.9 Genome of *M. tuberculosis*:

Circular chromosomes of *M. tuberculosis* have a base pair count of 4,411,532 and a Guanidine + Cytosine composition of roughly 65% (Quellet et al, 2010; The Sanger Institute, 2014; Ganu, 2016). Only 41% of the over 4000 genes in the bacterial genome have been described, and 44% of these genes have speculated functions (The Sanger Institute, 2014; Ganu, 2016). The lipid metabolism of the bacteria, which is crucial for their survival, occupies about 8% of their genome (Mohn et al., 2008; The Sanger Institute, 2014; Ganu, 2016).

Recent research suggests that some drug resistance genes and intergenic areas may be implicated in resistance to many drugs, as well as new linkages and drug resistance genes that were not previously related (Zhang et al., 2013; Ganu, 2016). The RNA synthesis, catalase-

peroxidase activity, and cell wall synthesis genes, respectively, have been linked to the *rpoB* gene, the *katG* gene, and the *inhA* gene for Rifampicin and Isoniazid respectively (Zhang *et al*., 2009). By altering ribosome structures at the 16S rRNA, the second-line injectable anti-TB medicines Kanamycin (KM) and amikacin (AM), a KM derivative, inhibit protein synthesis. (Zhang *et al*., 2009). High levels of resistance to KM and AM are associated with mutations at 16S rRNA (*rrs*) position 1400. Variable cross-resistance among KM, AMK, Capreomycin (CPM) or viomycin (VM) may be seen. The *rrs* gene may have either a C1402T or a G1484T mutation in individuals who are resistant to CPM, KM, and VM. (Zhang *et al.*, 2009). Fluoroquinolones (FQs) cause the death of microorganisms by inhibiting DNA gyrase (topoisomerase II) and topoisomerase IV. *GyrA* and *GyrB*, which encode the A and B subunits, are present only in *M. tuberculosis*. The conserved genes *gyrA* (320 bp) and *gyrB* (375 bp), which have the quinolone-resistance-determining region (QRDR), have been shown to have a significant part in *M. tuberculosis* developing FQ resistance (Zhang *et al.*, 2009; Laurenzo *et al*, 2011).

2.10 Mechanism of Resistance of FLDs

2.10.1 *Rifampicin molecular resistant mechanism and the rpoB gene*

First-line anti mycobacterial treatment involves the semi-synthetic rifampin (RIF), a rifamycin derivative. Because of its highly efficient bactericidal action, the medication is a central part of anti-TB therapy. Mycobacterial genes are translated and expressed by the enzyme ribonucleic acid (RNA) polymerase (*rpoB*), which is bound by RIF. This inhibition of bacterial transcription activity results from *rpoB*'s binding to RIF (Ahmad *et al.*, 2014). Mutations have been discovered in the *M. tuberculosis* resistant strains of the *rpoB* gene, which codes for the b-subunit of RNA polymerase. Rifampicin resistance testing is a helpful surrogate sign for MDR-TB because resistance to rifampicin alone is quite uncommon (Ahmad *et al.*, 2014;

Zhang *et al.*, 2009). RIF inhibits the synthesis of mRNA in *M. tuberculosis* by physically limiting the formation of the phosphodiester bond in the RNA backbone and impeding extension of RNA. It does this by attaching to the b-subunit of the RNA polymerase (Zhang *et al.*, 2009; Laurenzo David, 2011; Ahmad *et al.*, 2014).

The rifampicin resistance determining region, or RRDR, of the *rpoB* gene (codons 507–533), which codes for 27 amino acids, has 35 different point mutations or small insertions/deletions, according to data from several studies. Nearly 95% of epidemiologically unrelated rifampicin-resistant clinical *M. tuberculosis* isolates carry these mutations (Ahmad *et al.*, 2014; Zhang *et al.*, 2009)

2.10.2 *Isoniazid molecular resistant mechanism and the katG and inhA gene*

A first-line synthetic drug isoniazid (INH), which is primarily used to treat infections caused by *M. tuberculosis* complex members (*M. tuberculosis*, *M. bovis*, *M. africanum*, and *M. microti*) because all other mycobacteria and other prokaryotes are resistant to it (Ahmad *et al.*, 2014; Laurenzo *et al.*, 2011).

Four different *M. tuberculosis* genes—*katG*, which encodes catalase peroxidase, *inhA*, which encodes the enoyl acyl carrier protein (ACP) reductase, *kasA*, which encodes b-ketoacyl ACP synthase, and *ahpC*, which encodes alkyl hydroperoxide reductase—are the source of the more complex molecular basis for Isoniazid resistance (Ahmad *et al.*, 2014; Zhang *et al.*, 2009; Laurenzo *et al.*, 2011). According to genetic and biochemical evidence, changes in the *ahpC* gene that result from catalase/peroxidase activity decrease are compensatory and do not directly contribute to isoniazid resistance (Ahmad *et al.*, 2014).

The two primary molecular causes of INH resistance are *katG* and *inhA* gene mutations, or its promoter region. *KatG* and *inhA* gene mutations account for more than 95% of all INH resistances (Maurya et al., 2013; Vijdea et al., 2008; Al-Mutairi et al., 2019; Guo *et al*., 2006).

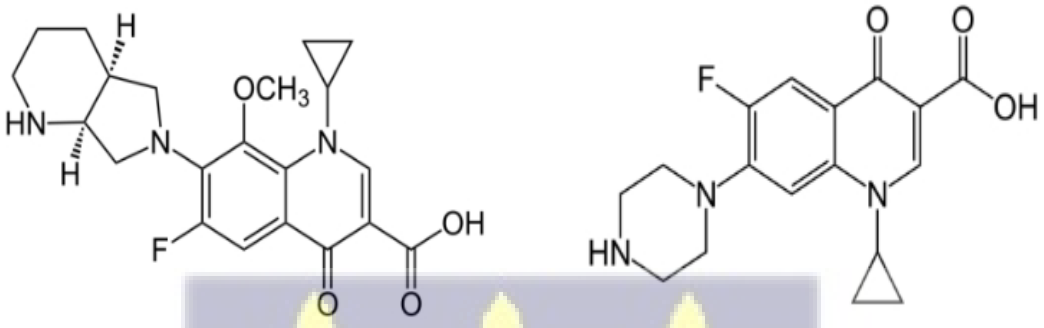
Isoniazid resistance is produced by mutations in *inhA* that occur in the upstream regulatory region, which leads to increased *inhA* protein production and elevated drug target levels via titration mechanisms resulting to low level resistance. There have also been reports of low – level isoniazid-resistant *M. tuberculosis* isolates with mis-sense mutations in the *inhA* structural gene, which lower the enzyme's affinity for decreased nicotinamide adenine dinucleotide (NADH). Mycolic acid biosynthesis is stopped as a result (Ahmad *et al.*, 2014; Laurenzo *et al.*, 2011). Small deletions, insertions, or mis-sense or nonsense mutations within the *katG* gene are mostly responsible for clinically significant high level resistance to isoniazid. The codons 315 and 463 of the *katG* gene are the locations of the most frequent genetic mutations in strains that are resistant to isoniazid (Ahmad *et al.*, 2014; Palomino *et al.*, 2014).

2.11 Mechanism of Resistance of SLDs

2.11.1 *Mechanism of Molecular Resistance of Fluoroquinolones and gyrA and gyrB genes*

As second-line TB medications, the fluoroquinolones (moxifloxacin, gatifloxacin, sparfloxacin, levofloxacin, ofloxacin, and ciprofloxacin) are bactericidal antibiotics with high activity against *M. tuberculosis* (Kolyva *et al.*, 2012). Treatment of MDR-TB and XDR-TB involves the use of 3rd generation fluoroquinolones such a Levofloxacin, moxifloxacin and gatifloxacin. When compared to other tuberculosis medications, fluoroquinolones have a relatively low side-effect profile, and their potent bactericidal effects have caused their use to increase recently. The resistance to fluoroquinolones has, regrettably, increased concurrently

(Laurenzo *et al*, 2011). The action of mycobacterial DNA gyrase, which is encoded by *gyrA* and *gyrB*, is inhibited by all fluoroquinolones in a manner that is similar. The quinolone resistance determining region (QRDR) in *gyrA* has been identified as the major location of mutations that lead to quinolone resistance. 60–70% of MTB strains that are resistant to quinolones have alterations in the *gyrA* QRDR region. (Laurenzo *et al*, 2011; Zhang *et al.*, 2009).



Moxifloxacin Ciprofloxacin

Figure 10: Chemical Structure of Fluoroquinolones (Source: Kolyva *et al.*, 2012).

The specific amino acid substitution in the QRDR and the quantity of resistance mutations determine the level of fluoroquinolone resistance. Therefore, whereas a single *gyrA* mutation may provide low-level resistance, numerous *gyrA* mutations or concurrent *gyrA* and *gyrB* mutations are typically needed to offer high-level resistance to fluoroquinolones. (Kolyva *et al.*, 2012; Zhang *et al.*, 2009).

A study conducted by (Laurenzo *et al*, 2011), the majority of mutations occur at codon 94 (around 60%), then at A90V, S91P, or G88C substitution substitutions (24% , 11% and 3% clinical isolates, respectively). In a different research by (Ahmad *et al*, 2014), the results demostrated that, of the 97 isolates, 73 (73/97, or 75.3%) had mutations in the *gyrA* gene, namely in codons 89, 90, 91, and 94. The codon 94 mutation, which was responsible for 49.5% (48/97) of the isolates, had four distinct variants of the amino acid, including D94G (23/97,

23.7%), D94A (6/97, 6.2%), D94Y (8/97, 8.2%), and D94N (11/97, 11.3%).The second most common mutation was the A90V, which was present in 22.7 percent (22/97) of isolates.

2.11.2 *Mechanism of Molecular Resistance of Aminoglycosides; rrs and eis genes*

The first significant advancement in TB chemotherapy came with the discovery of streptomycin (an aminoglycoside) in the early 1940s. Kanamycin and amikacin are two other aminoglycosides that exhibit strong antimycobacterial properties. Aminoglycosides are being utilised as second-line medications primarily to treat MDR-TB/ XDR-TB (Kolyva *et al.,* 2012; Laurenzo *et al.,* 2011).

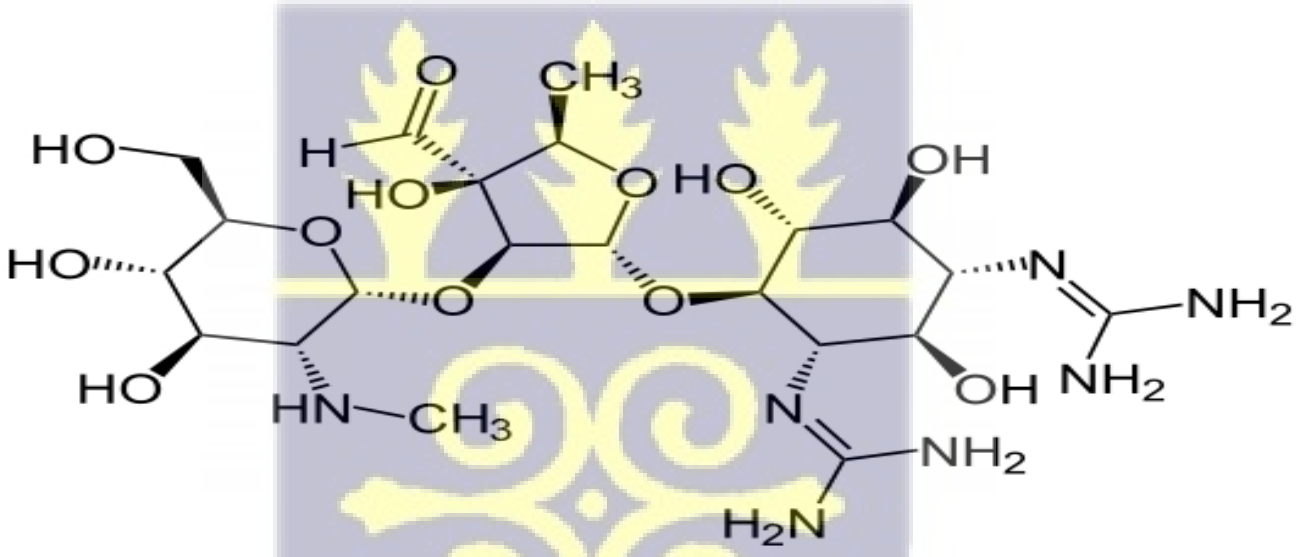


Figure 11. Chemical Structure of Streptomycin (Aminoglycoside) (Source: Kolyva *et al.,* 2012)

Similar to other bacteria, mycobacterial species are susceptible to the mode of action of aminoglycosides through their binding to the 30S ribosomal subunit, which affects polypeptide synthesis and, in turn, inhibits translation (Kolyva *et al.*, 2012; Zhang *et al.*, 2009).

Amikacin and Kanamycin resistance arises from changes in *rrs* similarly to streptomycin resistance. The main contributors to resistance to these drugs have been determined to be two mutations, A1400G and A1401G (Laurenzo *et al*, 2011; Alangaden *et al*., 1998). In a survey by (Alangaden *et al*., 1998), 13 clinical isolates (10 distinct strains) having MICs at concentrations of 0.256 mg/mL, they observed that all 13 isolates carried the A1400G mutation.

Amikacin, kanamycin, and capreomycin resistance are all caused by alterations in the 16S rRNA gene (rrs), particularly at locations 1484, 1402, and 1401, which result in CAP, KAN, and AMK resistance, respectively (Ali et al., 2011;Cui Z. et al, 2011; Alangaden G. J. et al, 1998). High levels of resistance to KM and AM are associated with mutations at 16S rRNA (*rrs*) position 1400. Variable cross-resistance between KM, AMK, Capreomycin, or viomycin may be seen (VM). The *rrs* gene may have either a C1402T or a G1484T mutation in individuals who are resistant to CPM, KM, and VM. (Zhang et al., 2009).

In a different research by (Province et al., 2019), the most prevalent mutation was found in 33.3 percent (21/63) of the 63 SLI Drug resistant isolates, changing the *rrs* gene's location 1401 from A to G. Three other isolates had mutations in the *rrs* gene at positions T1491C, G1454A, and A1499G. G10A (3/63, 4.8%) with C14T (2/63, 3.2%) were two amino acid changes found in the *eis* promoter region. However, only CAP resistant isolates had mutations in *tlyA* that resulted in the amino acid changes A119E (1/63, 1.6%), K69E (1/63, 1.6%), and K189N (1/63, 1.6%). Each of the isolates A1128G, A1138G, C1209T, and C1483T is one., out of 193 SLID isolates, were found in *rrs*. Additionally, only one isolate had a shift from G to T at position 37 of the *eis* promoter region.

2.12 Pre-XDR-TB and XDR-TB

The emergence of pre-extensively (Pre-XDR) and XDR-TB is threatening management of MDR-TB patients for most TB control programmes worldwide, and especially developing countries like Ghana.

Fluoroquinolone (FQ) is the most efficient second-line anti-tuberculosis medicine, and it's mostly used for the treatment of MDR-TB patients (Id et al., 2020; Malik S. et al., 2012). Treatment for MDR-TB patients is compounded further by fluoroquinolone resistance (Pre-XDR-TB), which leads to prolonged treatment times, fewer treatment alternatives, and a poor outcome. Early diagnosis of Pre-XDR/ XDR-TB would go a long way to help clinicians adapt their MDR-TB treatment regimens to include effective medications and avoid treatment failure (Kerléguer et al., 2021; S.E. Smith et al., 2015).

FQ resistance genetic changes in the 320- and 375-bp hypervariable areas of the *gyrA* and *gyrB* genes, which encode DNA gyrase, are responsible for 50 to 90% of phenotypic FQ-resistant isolates.

Genetic changes in the 16S *rRNA* gene (*rrs*), notably at locations 1401, 1402, and 1484, are likely to be linked to resistance to the antibiotics capreomycin and amikacin and kanamycin, resulting in AMK, KAN, and CAP resistance, respectively (Ali et al., 2011;Cui Z. et al, 2011; Alangaden G. J. et al, 1998).

M. tuberculosis strains are typically grown in liquid or solid media to test their susceptibility to various drugs, though this is capable of detecting RIF or INH resistance, it is less accurate and complex when it comes to second-line anti-TB medications (Length, 2021). In order to quickly identify first-line medicine resistance, the World Health Organization currently advises employing line probe assays (INNO-LiPA Rif and MTBDRplus) in addition to the standard culture susceptibility testing approach (Ali et al., 2011; WHO, 2008; Ling D. et al., 2008). The

MTBDR*sl* line probe assay detects second-line drug resistance quickly. These tests all look for genetic changes in the *rpoB, katG, inhA, gyrA, gyrB, rrs* and *eis* hotspot regions (Length, 2021)

Several studies have been carried out worldwide especially in high burden TB countries to determine burden and mutations within the *M. tuberculosis* genome especially within the hot spot regions of genes associated with XDR-TB and pre-XDR TB. In Pakistan, 4.5% of XDR-TB was reported among MDR in 2009, where 49 of 50 isolates had mutations affecting *rpoB*'s 4 amino acid codons, 531 (68%), 516 (24%), 526 (4%), and 513 (2%) (Id et al., 2020). In Myanmar, 13.5% XDR-TB and 27% pre-XDR-TB with two mutations in *gyrB* (T500P and E5014A) together with mutations in *gyrA* as well as mutations in *rrs* (A1401G) in aminoglycosides were reported (Ei et al., 2018).

In a work done by Gehre *et al.*, 2016, to determine the rise of pre-extensively drug-resistant TB in West Africa, there were no XDR-TB found and 2 pre-XDR-TB strains and 11 pre-XDR-TB were found among new MDR-TB patients and retreatment MDR-TB patients respectively.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design:

The study was a retrospective and cross-sectional experimental research design. Archived clinical samples of MDR-TB strains of *M. tuberculosis* complex collected between January 2016 to December, 2020 were used for the study.

3.2 Chemical and Reagents

All materials used are listed in Appendix I and the details of all reagent preparation procedures and composition of commercially acquired kits are in Appendix II.

3.3 Study Site and Sample Collection

One hundred and seventy-one (171) archival clinical isolates of *M. tuberculosis* complex collected from MDR-TB patients visiting the two major TB diagnostic laboratories involved in MDR-TB diagnosis in Ghana (ie, Chest Clinic TB Laboratory, Korle Bu Teaching Hospital, Accra and TB Laboratory, Eastern Regional Hospital, Koforidua) between 2016 and 2020 and kept in Tryptophan soy glycerol broth at -20°C at these laboratories were obtained.

Chest Clinic T.B Laboratory of the Korle Bu Teaching Hospital, Accra, and the TB Laboratory of the Eastern Regional Hospital, Koforidua were the two main TB laboratory networks in the diagnosis of Drug Resistance TB in Ghana Health Service. Samples from these regions (Bono, Ahafo, Bono East, Northen, North East, Savannah, Upper East, Upper West, Volta, Oti and Greater Accra) were sent to Chest Clinic TB Lab, Korle Bu Teaching Hospital, whiles samples from Western, Western North, Ashanti and Eastern Regions were sent to Eastern Regional Hospital T.B Lab. However, on some occasions samples from other regions were also sent to

the Eastern Regional Hospital T.B lab depending on the functionality of the Korle Bu Teaching Hospital, Chest Clinic T.B lab.

3.3.1 Patient Data Collection

Patient treatment category and demographic data were extracted from the laboratory patient registration book at both sites. This included age, sex, Region of referring health facility, mycobacterial culture and DST results.

3.3.2 Inclusion and Exclusion Criteria

Only isolates with confirmed phenotypic and genotypic resistance to at least rifampicin/ rifampicin and isoniazid were selected for the study. Selected isolates that failed to grow on subculture or were not truly MTB complex were excluded from the study. Only isolates that were confirmed to be MDR strains of *M. tuberculosis* complex after phenotypic and genotypic DST retesting were included in the studies.

3.4 Ethical Approval

Ethical clearance for this study was sought from and duly approved by the Ethical and Protocol Review Committee (EPRC) of the College of Health Sciences, University of Ghana.

3.5 Sample Size Determination

The minimum sample size for this study was determined using the Cochran (1963:75) formula,

$n= Z^2pq/e^2$

Using a 95% confidence interval, 0.14 is the proportion of the prevalence rate, and a ±5% precision; where

n = Minimum sample size

Z = Z Score

p = An estimated proportion of an attribute that is present in the population

e = Level of precision

q = 1-p

$n = Z^2pq/e^2 = (1.96)^2(0.14)\ (0.5)/(0.05)^2 = 108.0$

The minimum sample size (n) for this study was 108.0 archived MDR-TB isolates.

171 archived MDR-TB isolates were collected for this study from a total of 230 samples, using simple random sampling. Simple random sampling was done by assigning numbers to all of the isolates identified. The numbers were ranked and isolates selected from a table of random numbers, giving equal chance for an isolate to be selected and minimize bias.

3.6 Laboratory Procedures

The laboratory analysis was carried out at the Eastern Regional Hospital, Koforidua TB Laboratory. All biosafety recommendations regarding the handling of *M. tuberculosis* were followed. Isolates from the Korle Teaching Hospital Chest Clinic TB lab. were transported to Eastern Regional Hospital TB lab in a cold chain.

3.6.1 *Isolate Recovery*

MDR-TB Isolates were sub-cultured to revive and recover pure viable isolates with the use of the BACTEC™ MGIT™ liquid culture system from Becton, Dickinson (BD) Company for phenotypic antimicrobial resistance retesting. The manufacturer's instructions were strictly followed. Positive cultures were examined for mycobacterial growth by the appearance of white flakes and by microscopic examination using Ziehl-Neelsen stained smears from the broth culture. Purity check was performed on confirmed mycobacterial growths by sub culturing on blood agar plate streaked with the positive broth culture and incubated at 37°C for up to 48 hours. Pure mycobacterial colonies were tested for *M. tuberculosis* complex using BACTEC™ MGIT™ TBc Identification kit from BD to exclude Non-Tuberculous Mycobacteria (NTM). Using a sterile Pasteur pipette, a drop of the positive broth was put in the well of the TBc Identification kit after which the kit was incubated for 15mins. After 15 minutes the TBc identification kit was read; a red line at the test zone and control zone indicating the presence of the MPT64 antigen of the *M. tuberculosis,* hence positive for MTB. The kit manufacture's protocol was strictly followed. Those confirmed to be MTBCs were prepared for phenotypic DST and aliquots used for DNA extraction for the molecular procedures.

3.6.1.1 *BACTEC MGIT 960 Inoculation and Incubation.*

The MGIT PANTA vial containing five lyophilized antibiotics (Polymyxin B, Amphotericin B, Nalidixic Acid, Trimethoprim and Azlocillin) was reconstituted by adding 15ml of

supplemented medium called OADC (Oleic acid, Albumin, Dextrose, and Catalase) (see Appendix II). Eight hundred microliters (800 µl) of the resultant solution was added to Mycobacteria Growth Indicator Tube (MGIT) containing 7ml improved Middlebrook 7H9 broth base to complete the growth medium. The PANTA suppress growth of contaminating bacteria and makes the medium selective for mycobacterial growth. The growth supplement is a source of nutritional growth requirement for *M. tuberculosis* and other *Mycobacteria* species.

The MDR- TB isolates were removed from storage, thawed and allowed to reach room temperature. Five hundred microliters (500µl) of the bacteria suspensions were transferred to reconstituted MGIT media and the tubes were cupped tightly, labelled, disinfected and loaded into an automated BACTEC MGIT 960 instrument by scanning the tube barcodes for incubation at 37°C. The instrument monitored the tubes for growth at 15-minute intervals and flagged tubes with growth as positive (+) indicated on a computer monitor, instrument drawer and tube stations. The BACTEC MGIT 960 equipment uses fluorescence detection to identify growth. After forty-two (42) days of incubation, tubes that had not grown were marked negatively (-). For verification of the instrument result, all negative tubes were visually inspected.

3.6.1.2 *Confirmation of Mycobacterial Growth*

Instrument positive MGIT tubes were unloaded by scanning them out of the BACTEC ®MGIT® 960 machine and examined for mycobacterial growth. All positive tubes were visually inspected for mycobacterial growth. *M. tuberculosis* growth and other mycobacterial growths in MGIT appears as flakes or floccules in a clear broth with non-homogenous turbidity. Contaminating bacteria produce a hazy or cloudy turbidity whiles fungal contaminations are seen as cotton ball- like growths in the broth.

Drops of material from the positive tubes were transferred onto clean grease-free slides, air dried and stained by the Zeihl-Neelsen (ZN) staining method (see Appendix III). The ZN-stained slides were examined microscopically for AFBs and the presence of serpentine cord formation if AFBs were present and results documented. The presence of AFBs confirmed mycobacterial growth and serpentine cord formation (Figure 9), provided a presumptive identification of MTBC.

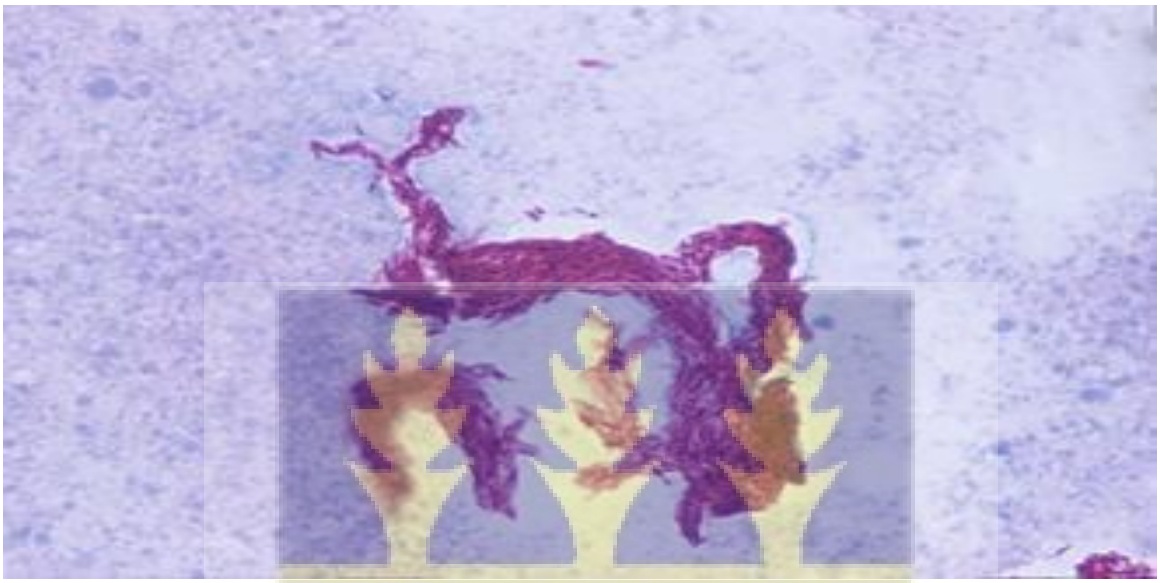


Figure 12: Serpentine cord of *M. tuberculosis* of culture –positive specimen smear stained by Ziehl- Neelsen staining technique (Source: Ganu, 2016).

3.6.1.3 *Mycobacterial Growth Purity Check*

All the MGIT positive cultures were sub-cultured onto Blood Agar plates and incubated at 37˚C ±1˚C for up to 48 hours and examined for the growth of contaminating bacteria. Contaminated cultures were decontaminated using the NaOH – NALC procedure (Appendix IV) and inoculated into fresh MGIT growth media for re-incubation.

3.6.1.4 *Mycobacterium Tuberculosis* Complex Identification

To distinguish *M. tuberculosis* complex from non-tuberculous mycobacteria, the MPT64 antigen of *M. tuberculosis* complex was identified using the BD MGIT MTBc Identification kit, a lateral flow immunochromatography assay. Sterile transfer pipettes were used to transfer 100ul (one drop) of broth from the instrument positive tube (after 15 seconds vortexing) into the sample wells of the test cassettes. The cassettes were incubated at room temperature for 15 to 20 minutes and the results were read. Only tubes with pure uncontaminated mycobacterial growths were tested by this method for MTBC. *M. tuberculosis* complex produces MPT-64 antigens captured as a reddish-pink band on test region (T) of the cassette with an internal control band (C) which validates the test (Figure 10). Test Positive External Quality Control strains were included as internal quality controls. Five hundred microlitres (500 µl) of bacteria suspension from tubes confirmed to be MTBC were transferred into 1000 µl Eppendorf vails for DNA extraction and the remaining bacteria suspension used for phenotypic drug susceptibility testing.

Figure 13: Immunochromatographic cassettes for *M. tuberculosis* identification (Source: Ganu, 2016)

3.6.1.5 *Phenotypic Antimicrobial Susceptibility Testing*

Pure colonies of *M. tuberculosis* complex were tested for their antimicrobial susceptibility to the first-line anti-TB drugs (Streptomycin, Isoniazid, Rifampicin and Ethambutol) at critical concentrations by the broth dilution method using BACTEC™ MGIT™ SIRE® kit on the BACTEC™ MGIT™ system from BD. The instrument and kit manufacturer instruction was observed. Drug-free growth control tubes were also included. The BACTEC™ MGIT™ 960 SIRE Growth Supplement was added (800µl) to the MGIT media. Four milliliters of sterile, distilled water were used to reconstitute each lyophilized drug and 100µl of each transferred to the reconstituted media labelled for each drug. The test bottles' ultimate medication concentrations were as follows: 1.00µg/ml for Streptomycin, 0.10µg/ml for INH, 1.00µg/ml for Rifampicin and 5.00µg/ml for Ethambutol. 500µl of the bacteria suspension each was transferred to each of the drug tubes. Five hundred microlitres (500µl) of a 100-fold dilution of the bacteria inoculum were added to the growth control tube (GC). The tubes were arranged in 5-set DST carrier in the order Growth Control, Streptomycin, INH, RIF and Ethambutol. The BACTEC MGIT 960 instrument was loaded with the DST sets by scanning the carrier set barcode for incubation and automated reading of DST results for 13 – 14 days. Results were interpreted as follows; the Growth Unit (GU) of the drug-free growth control tube was observe; a ≥400 GU of the growth control tube was compared with the drug containing tubes. The drug-containing tube with a GU ≥100 was resistant, where GU of a drug-containing tube ≤100 was susceptible. Tested Positive External Quality Control (EQA) panel strains were included as quality controls.

Isolates with confirmed resistance to RIF only, and RIF and INH was tested for their susceptibility to second-line anti-TB medications (Fluoroquinolones and aminoglycosides) using the Line Probe Assay from Hain Lifescience (Nehren, Germany)

3.6.2 Molecular Method

In order to identify mutations linked to rifampicin, isoniazid, and fluoroquinolone as well as mutations linked to aminoglycosides and fluoroquinolones in MDR TB isolates using DNA-STRIP technology, this study used the Hain's Test, a line probe test. Pure isolates of the bacteria were used to extract the DNA of *M. tuberculosis*, which was then amplified by multiplex PCR using biotinylated primers for specific target regions of the *rpoB, katG*, and *inhA* genes as well as the *gyrA, gryB, rrs*, and *eis* genes. Then, using reverse hybridization and an enzymatic colour reaction, the PCR products were located on a membrane strip.

3.6.2.1 *DNA Extraction*

The GenoLyse extraction kit Version 2.0 (Hain Life Science, Germany) was used to extract DNA from the M. tuberculosis isolates using the heat-alkaline procedure. 1 ml aliquots of the bacteria suspension were concentrated by centrifugation (at 10000rpm) for 15mins. after which the supernatant was decanted to leave the pellet of the bacteria cells. The bacteria pellet was suspended in a 100µl alkaline Lyse solution (A-LYS) and incubated in a heat block at 95°C for 5 min to rupture the bacteria cell and consequently release the bacteria nucleic acid material into solution. The reaction was neutralized with a 100µl neutralizing buffer (A-NB), the mixture was centrifuged at higher speed for five minutes, and the DNA extract from the bacterium lysate in the supernatant was used for the PCR process and stored at -20°C.

3.6.2.2 *PCR Amplification*

The GenoType® MTBDR*plus* Version 2 LPA kit was used to target four (4) genes of interest; TUB, *rpoB, katG* and *inhA* genes and the Genotype® MTBDR*sl* Version 2 LPA kit was used to target five genes of interest; TUB, *gryA, gryB, rrs* and *eis.* The TUB identified MTBC whereas the *rpoB, katG* and *inhA* gene mutations related with Rif, high and low level INH resistance respectively and *gyrA, gryB, rrs*, and *eis* gene changes related to fluoroquinolone, aminoglycoside and low level Kananycin resistance respectively.

The Genotype MTBDR*plus* VER 2.0® and Genotype MTBDR*sl* VER 2.0® LPA is a multiplex PCR method that uses 22 different primer sets in the primer nucleotide mix. (PNM) were used for the DNA amplification. These includes primers to the TUB gene, eight (8) *rpoB* wild-type (WT 1 to WT8) gene locus and four (4) common *rpoB* mutant (*rpoB* MUT1, MUT2A, MUT2B and MUT3) gene regions. Three primers were included to target *katG* mutations for *katG* WT1 locus, *katG* MUT1 and *katG* MUT2 mutant genes. Six (6) *inhA* gene mutation targets were also included; *inhA* WT1, *inhA* WT2, *inhA* MUT1, MUT2, MUT3A and MUT3B for the first line anti- TB drugs (Rifampicin and Isoniazid). The primers for the second line anti-TB drugs (fluoroquinolone and aminoglycosides) also included TUB gene and three (3) *gryA* wild-type (WT1 to WT3) gene locus and six (6) common *gryA* mutant (*gyrA* MUT1, MUT2, MUT3A, MUT3B, MUT3C and MUT3D) gene regions, one (1) *gryB* wild-type and two (2) *gyrB* mutant (*gyrB* MUT1, and MUT2). Primers for *rrs* locus two (2) *rrs* wild-type (WT1 to WT2) and two *rrs* mutant (*rrs* MUT1 and MUT2) were included. Primers for *eis* locus, three (3) *eis* wild-type (WTI to WT3) and one (1) mutant (*eis* MUT1) were also included.

The total PCR volume was 50µL; 10µl amplification mix A/AM-A (amplification buffer containing 2.5mM MgCl2 and Hot Start *Taq* DNA polymerase) was pipetted into a pcr reaction tube, after which 35µl amplification mix B /AM-B (PNM containing 200 nmol/L of each primer, dNTPs [dATP, dCTP, dGTP and dTTP]) was added to in a DNA hood to obtain a master mix. 5µl of the bacterial lysate containing the target DNA was then pipetted into the master mix after which the pcr solution was loaded into the themocycler.

The PCR process was hot started. Prior to the start of the PCR cycle, primmer antibodies that limit the activity of Hot Start DNA polymerase were inactivated by an initial lengthy 95°C

incubation period lasting 15 minutes. This was done in order to rule out non-specific primer binding to the DNA template and the creation of primer dimers prior to PCR.

The GTQ Cycler® 96, a thermal cycler, was used to perform the PCR. The amplification protocol consisted of 15 minutes of denaturation at 95°C, followed by 10 cycles comprising 30s at 95°C and 120s at 58°C; an additional 20 repeated cycles comprising 25 seconds denaturation of dsDNA at 95°C, 40 seconds annealing of forward and reverse primers at 53°C and 40 seconds, DNA *Taq* polymerase mediated elongation or extension by the incorporation of dNTPs (nucleotides) at 70°C (see Appendix III). The *Taq* DNA polymerase transcription of the strands by the incorporation of dNTPs was aided by the PCR buffer and 2.5mM MgCl2 as a source of magnesium (Mg2+), a co-factor for the polymerase. The amplicons were further held at the 70°C extension temperature after thermal cycling for 8 minutes and stored at 4°C until post PCR processing. The kit manufacture's product instruction was strictly adhered to.

3.6.2.3 *Line Probe Assay*

After PCR, the GT Blot 48® machine was used for the line probe assay; a reverse hybridization process. The amplification products of biotin-labelled dsDNA amplicons of the genes of interest were denatured by pipetting 20µl of the amplification product into a trough and adding a 20µl NaOH denaturation solution (DEN) to break the hydrogen bonds between the paired nucleotides after which Deoxyribonucleic acid (DNA) strip (labelled with sample ID) with probes (reaction zones) of unlabelled complementary sequences that were bands immobilized on a nitrocellulose membrane strips that are positively charged, and they were suspended in the amplification product and DEN mixture. 1000µl hybridization solution (HYB) pre-warmed at 45°C was added and incubated at 45°C for 30 minutes for hybridization to occur. The hybridization solution was replaced with 1000µl alkaline stringent solution (STRN) and

incubated at 45°C for 15 minutes after which stringent washing was done for the removal of unbound or non-specifically bound DNA and further rinsed at room temperature.

Following hybridization of the biotin-labelled amplicons to the reaction zones, the strips were treated with 1000µl streptavidin-alkaline phosphatase enzyme conjugate for 20mins. Band sites on the strip where hybridization had happened are where binding takes place because the ligands biotin and streptavidin have a high affinity for one another. A 1000µl hydrogen peroxide substrate solution was added after three washing stages, and it was left to incubate for 20 minutes at room temperature. In order to detect the bound biotin-streptavidin combination colorimetrically, the streptavidin-phosphatase enzyme conjugate reacts with the hydrogen peroxide substrate. Two washings were done and all the strips air-dried after which the strips were attached to the assessment sheets provided with the kits. Visual inspection of the strips that all contained internal controls for amplification and conjugation procedures, including loci controls for the *rpoB, katG*, and *inhA* genes and the *gyrA, gyrB, rrs*, and *eis* genes, were used to find the precise gene sections (wild-type or mutant) that are present in the target ssDNA's heterogeneous mixture. The hybridization procedure was repeated for strips with uninterpretable and/or very faint bands. Only samples with readable results were analysed.

3.7 Quality Assurance

Quality check of MGIT reagents and all other reagents was done before use. *Mycobacterium tuberculosis* External quality control strains, obtained from Korle Bu Teaching Hospital Chest clinic, Laboratory, was included in the MGIT liquid culture procedures and the molecular procedures.

Incubator temperature was monitored daily to ensure that the optimum incubation temperature was maintained. The BD MGIT MTBc Identification kit and LPA DNA strips had internal positive control bands. Growth media, NaOH-NALC solution and buffers used were sterilized.

All procedures except Z-N staining procedure were performed aseptically and in DNA-free environment where applicable to exclude contaminations. Stains were filtered before use to avoid the deposition of artefacts. Acid fast bacilli positive and negative control slides were included in each batch of staining procedure as controls.

3.8 Biosafety

All biosafety procedures recommended by the Centre for Disease Control (CDC) Atlanta, USA, for the handling of infectious materials and Mycobacteria were duly observed. Protective clothing and gadgets such as laboratory coats, examination gloves (powder-free where necessary) and N95 respirator were worn where necessary. Procedures involving the opening, closing and mixing of specimen were done carefully to reduce the creation of aerosols. Tubes were tightly capped before centrifugation in air-tight buckets. Opening of specimen tubes, smear preparation, and all inoculation procedures were done in a Class II biological safety cabinet. All contaminated materials were autoclaved before leaving the lab for incineration.

3.9 Data Management and Statistical Analysis

Data was analysed using Statistical Package for Social Sciences (SPSS version 14) SPSS Inc., Chicago, IL and Prism V%.0 (GraphPad Software) and tables and graphs were used to display the results for both first line and second line anti-TB medications, the frequency with the percentages of detected mutations in the MDR-TB, XDR-TB, and pre-XDR-TB isolates were calculated

CHAPTER FOUR

RESULTS

4.1 Sample Selection

A total of 171 archival isolates of MDR strain of *M. tuberculosis* were collected for the study. Twenty-six (15.2%) of the isolates were obtained from the Chest Clinic TB laboratory, Korle Bu Teaching Hospital, and one hundred and forty-five (84.8%) were obtained from the Koforidua regional Hospital TB laboratory.

Out of the 171 archival isolates sub cultured, 90 (52.6%) were recovered to be *M. tuberculosis* complex out of which 81 of the MTBCs were truly MDRs (resistant to at least Rif and INH) after phenotypic drug susceptibility testing to confirm MDR. Nine isolates were resistant to Isoniazid only and thus not MDR and were excluded from the analysis. A total of eighty-one (81) isolates were confirmed to be MDR-TB and hence suitable for analysis and were thus analysed in this study. Figure 14 shows the trend of the confirmed MDR-TB isolates for the study period.

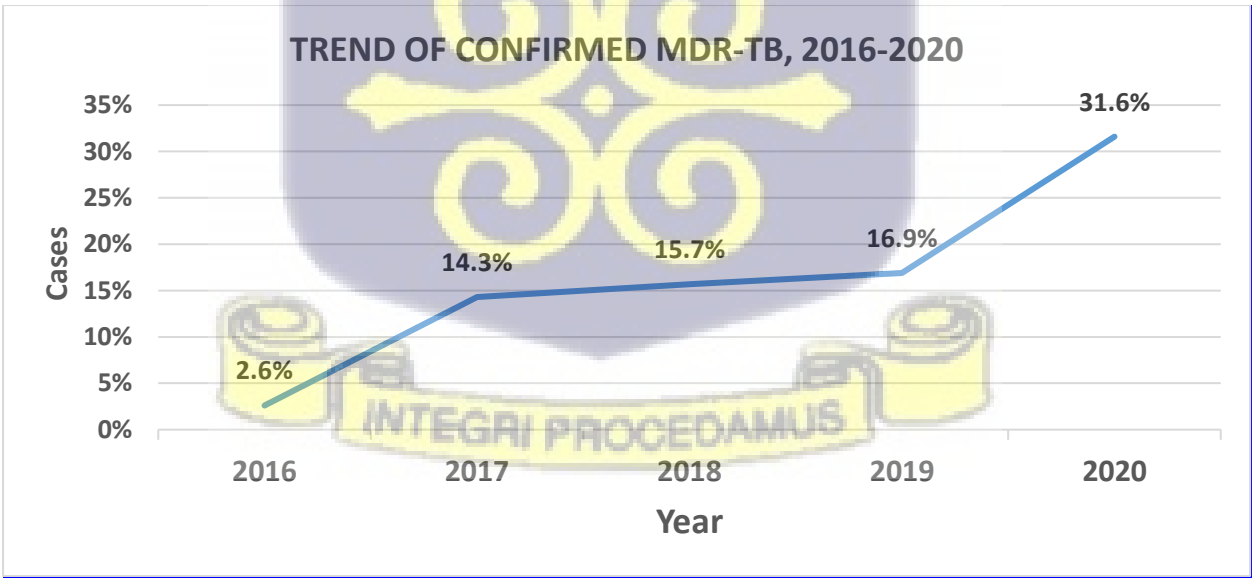


Fig. 14 Trend of Confirmed MDR-TB, 2016 – 2020.

4.2 Patient Demographics

The 81 isolates used for the analysis consisted of 70 (86.07%) males and 11 (14.0%) females. Their ages ranged from 5 years to 84 years with a mean age of 42.7±15.2 years. Age groups of 41-50 years and 31-40 years had a frequency of 26 (32.1%) and 24 (24.7%) respectively, whilst 21-30 years and 51 – 60 years were 12 (14.8%) and 9 (11.1%) respectively. (Fig. 15)

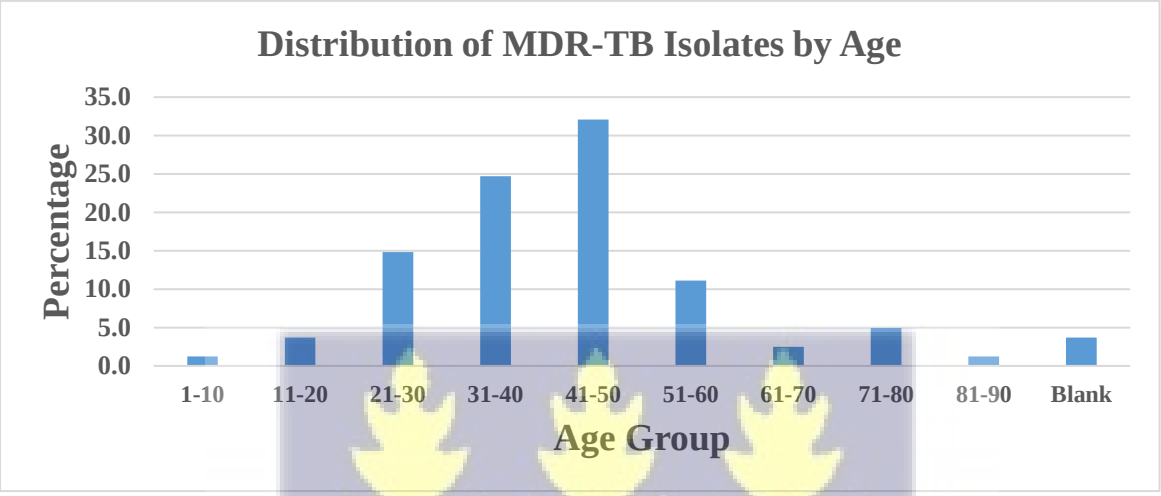


Fig. 15 Distribution of MDR-TB isolates by age

Eastern region had the highest number of patients (39.5%) followed by Greater Accra region (19.8%) whilst the Volta region had the lowest number of patients (1.2%). (Fig. 16)

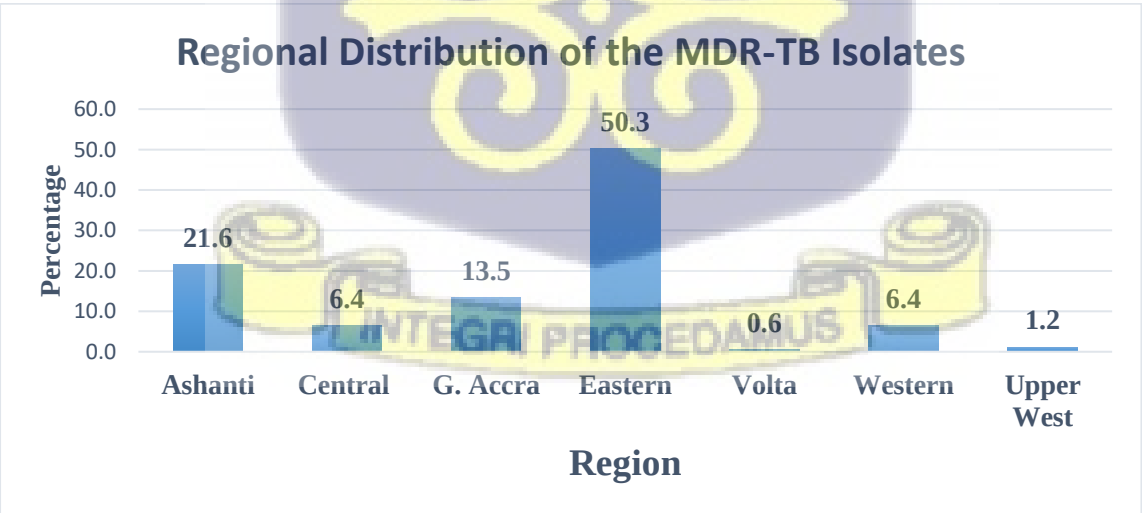


Fig. 16 Regional Distribution of the MDR-TB Isolates

4.3 Phenotypic Drug Susceptibility Testing

In this study, a total of 171 stored MDR-TB isolates were cultured. Out of the 171 isolates, 90 isolates recovered but 81 of them were MDR-TB representing a growth rate of 52.6%. Among the MDR-TB isolates RIF+INH+ Eth and RIF+INH+ Strept. resistance patterns were seen in 18(22.22%) and 21(25.93%) respectively**, (**Table 4).

Table 4: Phenotypic drug resistance patterns

Drug resistance pattern	Frequency	Percentage%
RIF+INH	81	100%
RIF +INH +Eth	18	22.22%
RIF +INH+ Strept.	21	25.93%
RIF+ INH+ Strept+ Eth	9	5.26%

Rif. – Rifampicin, INH – Isoniazid, Strept. – Streptomycin, Eth. - Ethambutol

4.4 Genotypic Drug Resistance Testing

In this study, genotype MTBDR*plus* and MTBDR*sl* line probe assays for first- and second-line anti-TB medications were run respectively on the 81 confirmed MDR-TB samples of which 73 readable results were obtained. The first line drug genotypic susceptibility testing (DST) revealed that 73 isolates showed resistance against Rifampicin whiles 45 isolates were resistant to Isoniazid giving resistance rates of 42.69% and 26.32% respectively. The overall prevalence of multi-drug resistance (MDR) was 26.32%. Also, the second line drug genotypic DST showed that 6 (7.4%) isolates were resistant to Fluoroquinolones (FQ) whiles 4 (2.34%) isolates showed resistance against Aminoglycosides (Km, Cm, VIO and Km, Am, Cm, VIO, 3 and 1 respectively) and 1.2% were aminoglycosides resistance Inferred (Km, Am, Cm, VIO). By the current WHO definitions (WHO, 2022) the study recorded a total of 6 cases of pre-

XDR-TB, representing overall prevalence of 3.5%. However, the study did not record any case of XDR among the isolates. In this study, it was shown that the prevalence of both MDR and pre-XDR were higher among the males as compared to their female counterparts. The age group 20-30 years recorded the highest prevalence of pre-XDR (7.4%) whiles the age group 31-40 years recorded the highest rate of MDR (29.7%). Table 5 shows that Central region had the most cases of MDR and pre-XDR combined with prevalence rates of 50.0% and 8.3% respectively. (Table 5).

Table 5: Genotypic drug resistance of first and second-line anti-TB drugs and prevalence of MDR and pre-XDR among the patients

Parameter	Total	1st line genotypic DST			2nd line genotypic DST		
		RIF	INH	MDR	FQ	AMG	Pre XDR
Total	171(100.0)	73(42.7)	45(26.3)	45(26.3)	9(5.3)	6(3.5)	6(3.5)
Gender							
Male	135(78.9)	63(46.7)	39(28.9)	39(28.9)	8(5.9)	5(3.7)	5(3.7)
Female	36(21.1)	10(27.8)	6(16.7)	6(16.7)	1(2.8)	1(2.8)	1(2.8)
Age group							
<20	10(5.9)	3(30.0)	1(10.0)	1(10.0)	0(0.0)	0(0.0)	0(0.0)
20-30	27(15.8)	14(51.9)	8(29.6)	8(29.6)	2(7.4)	2(7.4)	2(7.4)
31-40	37(21.6)	18(48.7)	11(29.7)	11(29.7)	2(5.4)	2(5.4)	2(5.4)
41-50	53(30.9)	24(45.3)	15(28.3)	15(28.3)	4(7.6)	1(1.9)	1(1.9)
51-60	21(12.3)	8(38.1)	4(19.1)	4(19.1)	0(0.0)	0(0.0)	0(0.0)
Above 60	23(13.5)	6(26.1)	6(26.1)	6(26.1)	1(4.4)	1(4.4)	1(4.4)
Region							
Ashanti	36(21.1)	13(36.1)	9(25.0)	9(25.0)	2(5.6)	2(5.6)	2(5.6)
Eastern	85(49.7)	33(38.8)	24(28.2)	24(28.2)	4(4.7)	2(2.4)	2(2.4)
Central	12(7.0)	10(83.3)	6(50.0)	6(50.0)	2(16.7)	1(8.3)	1(8.3)
Greater Accra	20(11.7)	11(55.0)	6(30.0)	5(25.0)	1(5.0)	1(5.0)	1(5.0)
Volta	4(2.3)	1(25.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Western	12(7.0)	4(33.3)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Upper West	2(1.2)	1(50.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Data is presented as frequency and corresponding percentage in brackets.

4.5 Mutational Patterns Observed

Table 6 shows that among the RIF (*rpoB* gene) mutations the commonly observed mutation patterns among the MDR-TB by LPA were D516V (12.33%), H526Y (6.85%), S531L (42.47%), H526D (12.33%) and Codon 530-533 (12.33%). Of the 45 INH resistance mutations among the MDR-TB isolates by LPA, 39 (86.67%) were *katG* (high level resistance) and 5(11.11%) were i*nhA* (low level resistance). One (2.22%) was of both *katG* and *inhA* type mutations. The prevalent *katG* mutation pattern was S315T1 (73.33%) whiles that of *inhA* was -15 region (6.67%).

Table 6: First line drug mutation patterns observed among MDR-TB by Genotype MTBDR*plus*

Drug	Mutation patterns	Gene mutation profile	Percentage
Rif	**D516V**	*rpoBWT3&4* and *rpoBMUT1*	9(12.3)
	D516Y (513-519)	*rpoBMUT2B* and *rpoBWT3&4*	2(2.7)
	F505L	*rpoBMUT1*	1(1.4)
	H526D	*rpoBWT7* + *rpoBMUT2B*, *rpoBMUT2B*	9(12.3)
	H526Y	*rpoBWT7* + *rpoBMUT2A* and *rpoBMUT2A*	5(6.9)
	S531L	*rpoBWT8* + *rpoBMUT3* and *rpoBMUT3*	31(42.5)
	Codon 503-509	*rpoBWT1*	1(1.4)
	Codon 510-517	*rpoBWT2&3*	2(2.7)
	Codon 526 – 529	*rpoBWT7* and *rpoBWT8*	4(5.5)
	Codon 530-533	*rpoBWT8* and *rpoBWT3&4*+ *rpoBMUT1*	9(12.3)
IHN	**c-15t**	*inhAMUT1*	2(4.4)
	S315T1	*katGWT* + *katGMUT1* and *katGMUT1*	33(73.3)
	t-8c	*katGWT* + *inhAWT2*	1(2.2)
	S315T2	*katGWT* + *katGMUT2*	2(4.4)
	Codon 315	*katGWT*	4(8.9)
	-15 region	*inhAWT1*	3(6.7)

Table 7 shows that, of the 15 second line drugs (FQ and AMG) resistance mutations among the MDR-TB isolates by LPA, 9(60.0%) were of *gyrA* mutations whereas 6(40.0%) were *rrs* associated mutations. The commonest *gyrA* mutation pattern was D94A (26.7%) whiles that of *rrs* was C1402T (20.0%).

Table 7: Second line drug mutation patterns observed among MDR-TB by Genotype MTBDR plus

Drug	Mutation patterns	Gene mutation profile	Percentage
FQ	**A90V**	*gyrAMUT1*	1(16.7)
	D94A	*gyrAWT3+gyrAMUT3A* and *gyrAMUT3A*	3(50.0)
	D94G	*gyrAWT3+gyrAMUT3C*	1(16.7)
	S91P	*gyrAMUT2*	1(16.7)
AMG	**C1402T**	*rrsWT1*	3(50.0)
	G1484T	*rrsMUT2*	1(16.7)
	Position 1484	*rrsWT2*	2(33.3)

The mutations in samples that were resistant to a minimum of one first-line anti-TB medication included *rpoB*, *inhA*, *katG*, and/or combinations of *katG* and *inhA*. Among the samples that were resistant to RIF, 59 samples had no wild-type *rpoB* bands and 56 samples had *rpoB* mutation bands. The most predominant RIF wild type band absent was *rpoBWT8* (61.02%) whiles the most common *rpoB* wild type mutation band present was *rpoBMUT3* (57.14%). The study also showed that among the INH resistant isolates, there were 3(8.57%) samples with absence of *inhA* wild type bands and 31(88.57%) *katG* wild type bands. The most prevalent wild type mutation band present among INH resistant samples was *katGMUT2* (82.93%). See Table 8

The mutations in samples that were resistant to at least one second-line anti-TB medication were either *gyrA* or *rrs*. Among the samples resistant to FQs, there were 3 samples without wild-type *gyrA* bands and 6 samples with *gyrA* mutation bands. There were 6 samples with

absence of wild type *rrs* bands with one *rrs* mutation band present among the AMGs resistant samples. Table 9 shows that the most common *gyrA* mutation band present was *gyrAMUT3A* (20.0%).

Table 8: Observed MTBDR*plus* assay band patterns for first line anti-TB drugs

Drug	Locus	WT band absent	%	Mutation bands present	%
Rif	*rpoB*	*rpoBWT1*	1(1.7)	*rpoBMUT1*	10(17.9)
		rpoBWT2&3	1(1.7)	*rpoBMUT2A*	5(8.9)
		rpoBWT3&4	12(20.3)	*rpoBMUT2B*	9(16.1)
		rpoBWT7	9(15.3)	*rpoBMUT3*	32(57.1)
		rpoBWT8	36(61.0)		
INH	*inhA*	*inhAWT1*	3(8.6)	*inhAMUT1*	3(7.3)
	katG	*katGWT*	31(88.6)	*inhAMUT3A*	1(2.4)
	katG, inhA	*katGWT, inhAWT2*	1(2.9)	*katGMUT1*	34(82.9)
				katGMUT2	3(7.3)

Data is presented as frequency and proportion in brackets

Table 9: Observed MTBDR assay band patterns for second line anti-TB drugs

Drug	Locus	WT bands absent	%	Mutation bands present	%
FQ	***gyrA***	*gyrAWT3*	3 (20.0)	*gyrAMUT1*	1(6.7)
				gyrAMUT2	1(6.7)
				gyrAMUT3A	3(20.0)
				gyrAMUT3B	1(6.7)
AMG	***rrs***	*rrsWT1*	4(26.7)	*rrsMUT2*	1(6.7)
		rrsWT2	2(13.3)		

Data is presented as frequency and proportion in brackets

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Public health initiatives to combat tuberculosis have continued to be threatened by the rising rates of rifampicin (RIF) and isoniazid (INH) resistance as well as multidrug-resistant tuberculosis (MDR-TB) (Ssengooba et al., 2016). MDR-TB has become a major public health concern in both industrialised and developing nations (Green et al., 2010). All tuberculosis patients should get a culture and drug susceptibility testing (DST) done, to see if *M. tuberculosis* is present, whether they are suspected or confirmed cases. The reason behind this is that, drug-resistant TB strain makes it difficult to provide normal TB medication regimens, especially to patients who have already received treatment. (Forson et al., 2018; Steinmetz et al., 2016).

5.1.1 Prevalence of MDR Among Different Gender, Age Group and Region of Residence

Prevalence of both MDR-TB and pre-XDR-TB were higher among the males as compared to their female counterparts and this may be due the fact that men are more prone to intake of alcohol, tobacco and engage in other social risk factors for developing drug resistance TB (Mohammed et al., 2022). Moreover, men are also more likely to be non-complaint to TB treatment than women. Our findings are consistent with other studies done in China and Iraq (Pan et al., 2022; Mohammed et al., 2022). In contrast other studies shows high drug resistance among females than males due to cultural settings in some regions of the world where females tends to nurse males with drug resistant TB in the home (Mohammed et al., 2022; Mesfin et al., 2018).

The age group 20-30 years recorded the highest prevalence of pre-XDR (7.4%) whiles the age group 31-40 years recorded the highest rate of MDR-TB (29.7%). This may be attributed to these age groups being socially active and thus engaging in more predisposing social risk factors like smoking, intake of alcohol and mining activities (Mohammed et al., 2022; Dahal et al., 2019). The result was consistent with research done by (Mohammed et al., 2022) and (Mesfin et al., 2018) who had age groups 15-34 years and 25-34 years respectively with most prevalence of MDR-TB and pre-XDR-TB.

The Central region was the region with the highest burden of both MDR-TB and pre-XDR-TB cases with prevalence rates of 50.0% and 8.3% respectively. This may due to the more rural settings of the region. Studies have shown that TB patients living in rural communities are more likely to develop drug resistant TB as they are less likely to adhere to the treatment regimens and have low access to good health care as compared to the urban regions (Oladimeji et al., 2022; Pan, 2022)

5.1.2 Resistance to first line anti-TB drugs

In the present study, among the MDR-TB isolates RIF+INH+ Eth and RIF+INH+ Strept. resistance patterns were seen in 18(22.22%) and 21(25.93%) respectively. This finding is consistent with the findings of previous report by Forson et al., (2010) among tuberculosis patients population in Accra, Ghana. A rising body of research across writers offers a variety of overarching causes for the rise in drug resistance among TB patients over time. These mostly include improper drug prescription by healthcare professionals, elevated risk of infection transmission due to crowding, inadequate community TB control programmes, significant variability of anti-TB regimens, and patients who do not comply to their prescribed treatment regimens (Goyal et al., 2017; Li et al., 2018).

Insufficient public health resources and inconsistent or selective treatment adherence among patients, according to a different study, lead to *Mycobacterium tuberculosis* being exposed to sublethal levels for insufficiently long periods of time (Otu et al., 2013). Despite this, investigations in Ghana have found that MDR-TB isolates have greater frequencies of streptomycin and ethambutol resistance similar to other studies done in Central Africa (Forson et al., 2018; Bwalya et al., 2021). The high resistance to streptomycin and Ethambutol may be due to selective drug pressure due to excessive use of these drugs in treating other bacterial infections and poor drug adherence and frequent reuse in retreatment cases and management of MDR-TB in the case of Ethambutol (Addo et al., 2018). In contrast, it has also been noted that MDR isolates from Asia have lower frequencies of streptomycin and ethambutol resistance (Sinha et al., 2017).

Over the past three decades, molecular diagnosis of MTB resistance has seen significant usage. These molecular methods are based on the detection of mutations in particular MTB genes that are linked to drug resistance (Momen et al., 2021). The present study characterized mutations conferring resistance to both first- and second-line anti-TB drugs among MDR-TB isolates and the occurrence of pre-XDR-TB and XDR-TB in Ghana of which the study revealed that *katG* gene accounted for majority (86.67%) of INH resistance and this may be due to the fact that mutations in the *katG* retains the activity of catalase peroxidase but reduces the binding of INH to *katG* (Guo et al., 2006). This finding confirms the results of previous reports which shows that *katG* gene mutations account to 40–95% of INH resistance (Chen et al., 2017; Tadesse et al., 2016).

This study found that among the *rpoB* gene mutations the most prevalent observed mutation conferring RIF resistance were S531L (42.47%), D516V (12.33%), H526Y (6.85%), H526D (12.3%) which indicates high resistance to rifampicin and this is due the fact that rifampicin

binds to β-subunit of the RNA polymerase and these mutations usually occurs within the amino acids 507-533 regions of the *rpoB* region(Laurenzo et al, 2011). The finding of the current study compares with previous reports in Africa (Addo et al., 2017; Diriba et al., 2022) and China (Chen et al., 2017). However, this finding is lower than the finding of previous proportions reported from India (Kumari et al., 2016). Likewise, this study found that the most predominant observed mutation conferring INH resistance was S315T1 (73.33%). This finding is in concordance with the earlier report of Addo et al., (2017) which recorded 77.8% S315T1 mutation among all the INH-resistant strains.

5.1.3 Resistance to second line anti-TB drugs

The rise and spread of extensively drug-resistant tuberculosis (XDR-TB) and multidrug-resistant tuberculosis (MDR-TB) poses a serious danger to world health (Momen et al., 2021). For this reason, the present study assessed the resistance of the MDR TB isolates against second line anti TB drugs and further determined the burden of pre-XDR and XDR-TB among multi-drug resistant tuberculosis patients. The study recorded a drug resistance rate of 7.40% for Fluoroquinolones (FQ) and 4.9% and 2.5% Aminoglycosides (AMGs) resistance and aminoglycosides resistance inferred respectively. Injectable second-line medications and fluoroquinolones are both very powerful against MDR-TB. Treatment of MDR-TB will be more challenging if there is resistance to FQ or injectable second-line medicines, for this reason, diagnosing patients with pre-XDR-TB will enable doctors to closely follow these patients and stop the development of XDR-TB, which is more challenging to treat (Zhang et al., 2021).

In this study, cases of pre-XDR-TB as per the new WHO definitions (WHO, 2020), which is MDR-TB with resistance to fluoroquinolones, was 7.4% among the MDR-TB isolates. This finding corroborates with the earlier reports of Goyal et al., (2017) (7.9%) and Shibabaw et al.,

(2020) (5.7%). Compared with our findings, pre-XDR-TB has been found to be more common in China (66.4%), Cambodia (13.6%) and Brazil (15%) (Araújoa et al., 2021; Kerléguer et al., 2021; Yao et al., 2021).

If left unchecked, Ghana's growing number of resistant isolates will make it extremely difficult to keep TB under control. The rise in pre-XDR TB cases highlight the need for proactive and prompt measures, such as ongoing patient monitoring, patient counselling and assistance to increase adherence to treatment, and medication supply management to stop the spread of XDR-TB (Osei-wusu et al., 2018). Previous authors hypothesised that, in addition to its usage in MTB infection, the indiscriminate use of FQs in the majority of common diseases, such as pneumonia and pyrexia of unknown origin, may be the cause of the increased burden of FLQ resistant in pre-XDR-TB patients (Diriba et al., 2022; Tasnim et al., 2018).

Our research identified *rrs* and *gyrA* gene alterations among second-line anti-TB medications. A *gyrA* gene mutation was shown to be the root cause of FQ resistance, while injectable SLD resistance was caused by a *rrs* gene mutation (AMG). According to an earlier study, a mutation in the *gyrA* codon imparts resistance to Levofloxacin and is linked to low-level resistance to Moxifloxacin, while a mutation in the *rrs* region is linked to high-level Amikacin, Capreomycin, and kanamycin resistance (Diriba et al., 2022). In this study, the *gyrA* mutations were found to occur in codons 90, 91, and 94 with A90V, S91P, D94A, and D94G mutations whiles the *rrs* mutations found were C1402T, G1484T and position 1484 mutations. The observation of the current study conform with the reports of several previous studies from different parts of the world in China, Cambodia and Ethiopia (Diriba *et al*., 2022; Kerléguer *et al*., 2021; Yao *et al*., 2021).

It is noteworthy that codon 94 hosted most of the frequently seen mutations among FQ resistant isolates. According to a previous study, codon 94 substitutions are a phenomenon that may be

due to the codon targeting the water-magnesium ion bridge which is a known conserved C3/C4 keto acid of quinolones, which is crucial for stabilizing the molecule binding pocket of quinolones at the position of an amino acid group which amplifies the undesired effect of the interaction between DNA gyrase and most quinolones (Province et al., 2019).

gyrA mutations may serve as a possible diagnostic indicator for MDR and FQ, as well as a potential predictor of pre-XDR or XDR-TB, according to empirical research linking *gyrA* mutations to MDR, FQ, pre-XDR, and XDR-TB. (Province et al., 2019). According to Borrell and colleagues, Variable levels of fitness loss result from the interaction among rifampicin and FQ-resistant mutations in Mycobacterium, which is influenced by epistasis. (Borrell *et a*l., 2013). Therefore, it is proposed that the advancement of MDR to pre-XDR or XDR is as a results of the epistatic interaction of mutations occurring between *gyrA* mutations and the genetic changes in the drug-resistant gene associated with RIF resistance. (Province *et al*., 2019). Hence, this study suggests that additional research be done to evaluate the relationship between *gyrA* mutations and drug resistance gene mutations imparting rifampicin for the prevention of pre-XDR/ XDR-TB in the study group.

5.2 CONCLUSION

Among the MDR-TB isolates RIF+INH+ Eth and RIF+INH+ Strept. resistance patterns were averagely high (>22%). The *katG* gene accounted for over 85% of INH resistance which indicates high prevalence of high-level Isoniazid resistance. The most prevalent mutations conferring RIF resistance among our study population were S531L, D516V, H526Y, H526D.

There was a modest increase in the proportion of pre-XDR-TB amongst MDR-TB patients in Ghana, but no XDR-TB was found. The most common fluoroquinolone mutation associated to

pre-XDR-TB was D94A. Nonetheless, a sustained surveillance of pre-XDR-TB and XDR-TB is highly advocated.

5.3 RECOMMENDATIONS

There were reported cases of aminoglycosides resistance Inferred which clinically would have been reported as aminoglycosides resistance. Second line DST should be done with the MGIT in Ghana to ascertain the true resistance of the inferred aminoglycosides resistance. Additionally, whole genome sequence should be considered to confirm the resistance of the inferred aminoglycosides resistance.

Further studies need to be conducted to evaluate the relationship between *gyrA* mutations and drug resistance gene mutations imparting rifampicin for the control of pre-XDR and XDR-TB among the study cohort. Additionally, whole genome sequencing studies to be done on these isolates to better describe the distribution and phylogeny of the polymorphisms within the *gyrA* region.

Due to the high percentage of isolates not being recovered by culture and genotypically, provision of proper storage conditions for storage of MTB isolates within the TB Lab network in Ghana for future research works is highly advocated.

5.4 LIMITATIONS

Time, financial resources and access to sequencer were the limiting factors in this study. More data could have been collected to conduct risk factor analysis as well as deeper phylogeny and polymorphism studies on the isolates.

6.0 REFERENCES

Acheampong, D., Opoku, R., Boye, A., Agyirifo, D., Dadzie, I., Barnie, P., Kwakye-Nuako, G., & Nyandzi, F. (2018). Diagnosis and Treatment Outcome of Smear Positive Pulmonary Tuberculosis: Retrospective study in Kpando Municipal, Ghana. *Journal of Advances in Medicine and Medical Research*, *25*(9), 1–11. https://doi.org/10.9734/jammr/2018/40156

Addo, K. K., Addo, S. O., Mensah, G. I., Mosi, L., & Bonsu, F. A. (2017). Genotyping and drug susceptibility testing of mycobacterial isolates from population-based tuberculosis prevalence survey in Ghana. *BMC Infectious Diseases*, *17*(1), 1–7. https://doi.org/10.1186/s12879-017-2853-3

Addo, K. K., Owusu, R., Bonsu, C., Owusu-Darko, K., Addo, S. O., Mensah, G. I., Newman, M. J., Ofori-Adjei, D., & Bonsu, F. A. (2018). First Nationwide Survey on the Resistance to First Line Anti-Tuberculosis Drugs in Ghana. *Journal of Tuberculosis Research*, *06*(01), 68–80. https://doi.org/10.4236/jtr.2018.61007

Ahmad, S., & Mustafa, A. S. (2014). *Molecular Diagnosis of Drug-Resistant Tuberculosis*. *May*.

Al-Mutairi, N. M., Ahmad, S., & Mokaddas, E. M. (2019). Molecular characterization of multidrug-resistant Mycobacterium tuberculosis (MDR-TB) isolates identifies local transmission of infection in Kuwait, a country with a low incidence of TB and MDR-TB. *European Journal of Medical Research*, *24*(1), 1–13. https://doi.org/10.1186/s40001-019-0397-2

Alangaden, G. J., Kreiswirth, B. N., Aouad, A., Khetarpal, M., Igno, F. R., Moghazeh, S. L., Manavathu, E. K., & Lerner, S. A. (1998). Mechanism of resistance to amikacin and kanamycin in Mycobacterium tuberculosis. *Antimicrobial Agents and Chemotherapy*, *42*(5), 1295–1297. https://doi.org/10.1128/aac.42.5.1295

Ali, A., Hasan, R., Jabeen, K., Jabeen, N., Qadeer, E., & Hasan, Z. (2011). Characterization of mutations conferring extensive drug resistance to Mycobacterium tuberculosis isolates in Pakistan. *Antimicrobial Agents and Chemotherapy*, *55*(12), 5654–5659. https://doi.org/10.1128/AAC.05101-11

American Thoracic Society. (1972). In *The American Journal of the Medical Sciences* (Vol. 263, Issue 2, p. 122). https://doi.org/10.1097/00000441-197202000-00008

Angelina, A. S., Kwarteng, A., Twumasi-, S., Owusu, M., Arthur, R. A., Mawunyo, R. D., Adu-amoah, L., Addofoh, N., Okyere, P. B., Dzata, F., Bonsu, F., Adusi-poku, Y., Kranzer, K., Siroka, A., Gemert, V., Dean, A., & Owusu-dabo, E. (2021). The burden of drug resistance tuberculosis in Ghana ; results of the First National Survey. *PLoS ONE*, 1–14. https://doi.org/10.1371/journal.pone.0252819

Araújoa, L. G., Garciab, M. T., Zaccariottoc, T. R., Moretti, M. L., Levyc, C. E., & Resende, M. R. (2021). Clinical outcomes and molecular characterization of drug-resistant tuberculosis in pre- and extensively drug-resistant disease based on line probe assays. *The Brazilian Journal of Infectious Diseases*, *5*(1), 1–5.

https://doi.org/10.1016/j.bjid.2021.101544

Baddeley, A. (2020). GLOBAL Tb Report 2020. In *World Health Organisation* (Vol. 66).

Bonsu, F., Addo, K. K., Alebachew, Z., Gyapong, J., Gockah, R., Law, I., Tadolini, M., Onozaki, I., & Sismanidis, C. (2020). *National population-based tuberculosis prevalence survey in Ghana , 2013*. *24*(August 2019), 321–328.

Bwalya, P., Yamaguchi, T., Solo, E. S., Chizimu, J. Y., Mbulo, G., Nakajima, C., & Suzuki, Y. (2021). Characterization of Mutations Associated with Streptomycin Resistance in Multidrug-Resistant Mycobacterium tuberculosis in Zambia. *Antibiotics*.

Chakaya, J., Khan, M., Ntoumi, F., Aklillu, E., Fatima, R., Mwaba, P., Kapata, N., Mfinanga, S., Hasnain, S. E., Katoto, P. D. M. C., Bulabula, A. N. H., Sam-Agudu, N. A., Nachega, J. B., Tiberi, S., McHugh, T. D., Abubakar, I., & Zumla, A. (2021). Global Tuberculosis Report 2020 – Reflections on the Global TB burden, treatment and prevention efforts. *International Journal of Infectious Diseases*, *113*, S7–S12. https://doi.org/10.1016/j.ijid.2021.02.107

Chakaya, J., Petersen, E., Nantanda, R., Mungai, B. N., Migliori, G. B., Amanullah, F., Lungu, P., Ntoumi, F., Kumarasamy, N., Maeurer, M., & Zumla, A. (2022). The WHO Global Tuberculosis 2021 Report – not so good news and turning the tide back to End TB. *International Journal of Infectious Diseases*, *124*, S26–S29. https://doi.org/10.1016/j.ijid.2022.03.011

Chen, J., Peng, P., Du, Y., Ren, Y., Chen, L., Rao, Y., & Wang, W. (2017). Early detection of multidrug- and pre- extensively drug-resistant tuberculosis from smear-positive sputum by direct sequencing. *BMC Infectious Diseases*, 1–7. https://doi.org/10.1186/s12879-017-2409-6

Claudio Piersimoni piersim@tin.it, Armando Olivieri, Luca Benacchio, C. S. (2006). Current Perspectives on Drug Susceptibility Testing of Mycobacterium tuberculosis Complex: the Automated Nonradiometric Systems. *Journal of Clinical Microbiology*.

Cooper, S. A. K. J. E. P. K. S. L. G. G. K. B. D. M. J.-G. N. G. F. deSauvage; A. M. (2005). IL-23 Compensates for the Absence of IL-12p70 and Is Essential for the IL-17 Response during Tuberculosis but Is Dispensable for Protection and Antigen-Specific IFN-γ Responses if IL-12p70 Is Available. *The Journal of Immunology*. https://doi.org/175(2), 788-795.

Dahal, S., Banjara, M. R., Khadka, D. K., & Ghimire, G. R. (2019). *Drug Susceptibility Pro fi le of Mycobacterium tuberculosis Isolated from Patients Visiting National Tuberculosis Centre , Nepal*. *5*(1), 63–68.

Demile, B., Zenebu, A., Shewaye, H., Xia, S., & Guadie, A. (2018). Risk factors associated with multidrug-resistant tuberculosis (MDR-TB) in a tertiary armed force referral and teaching hospital, Ethiopia. *BMC Infectious Diseases*, *18*(1), 1–10. https://doi.org/10.1186/s12879-018-3167-9

DiFonzo, N., & Bordia, P. (1998). Reproduced with permission of the copyright owner . Further reproduction prohibited without. *Journal of Allergy and Clinical Immunology*, *130*(2), 556. http://dx.doi.org/10.1016/j.jaci.2012.05.050

Diriba, G., Kebede, A., Tola, H. H., Alemu, A., Yenew, B., Moga, S., Addise, D., Mohammed, Z., Getahun, M., Fantahun, M., Tadesse, M., Dagne, B., Amare, M., Assefa, G., Abera, D., & Desta, K. (2022). Utility of line probe assay in detecting drug resistance and the associated mutations in patients with extrapulmonary tuberculosis in Addis Ababa , Ethiopia. *Open Access*. https://doi.org/10.1177/20503121221098241

Forson, A., Kudzawu, S., Kwara, A., & Flanigan, T. (2010). High frequency of first-line anti-tuberculosis drug resistance among persons with chronic pulmonary tuberculosis at a teaching hospital chest clinic. *Ghana Medical Journal*, *44*(2).

Forson, A., Kwara, A., Kudzawu, S., Omari, M., Otu, J., Gehre, F., Jong, B. De, & Antonio, M. (2018). A cross-sectional study of tuberculosis drug resistance among previously treated patients in a tertiary hospital in Accra , Ghana : public health implications of standardized regimens. *BMC Infectious Diseases*, 4–9.

Ganu, H. (n.d.). *Mutation Patterns in MDR-TB isolates in Ghana*.

Gehre, F., Otu, J., Kendall, L., Forson, A., Kwara, A., Kudzawu, S., Kehinde, A. O., Adebiyi, O., Salako, K., Baldeh, I., Jallow, A., Jallow, M., Dagnra, A., Dissé, K., Kadanga, E. A., Idigbe, E. O., Onubogu, C., Onyejepu, N., Gaye-Diallo, A., … Antonio, M. (2016). The emerging threat of pre-extensively drug-resistant tuberculosis in West Africa: Preparing for large-scale tuberculosis research and drug resistance surveillance. *BMC Medicine*, *14*(1), 1–12. https://doi.org/10.1186/s12916-016-0704-5

Goloubeva, V., Lecocq, M., Lassowsky, P., Matthys, F., Portaels, F., & Bastian, I. (2001). Evaluation of Mycobacteria Growth Indicator tube for direct and indirect drug susceptibility testing of Mycobacterium tuberculosis from respiratory specimens in a Siberian prison hospital. *Journal of Clinical Microbiology*, *39*(4), 1501–1505. https://doi.org/10.1128/JCM.39.4.1501-1505.2001

Goyal, V., Kadam, V., Narang, P., & Singh, V. (2017). Prevalence of drug-resistant pulmonary tuberculosis in India : systematic review and. *BMC Public Health*. https://doi.org/10.1186/s12889-017-4779-5

Graham R. Stewart, B. D. R. & D. B. Y. (2003). Tuberculosis: a problem with persistence. *Nature Reviews Microbiology*. https://www.nature.com/articles/nrmicro749#citeas

Green, E., Obi, C. L., Nchabeleng, M., Villiers, B. E. De, Sein, P. P., & Letsoalo, T. (2010). *Drug-susceptibility Patterns of Mycobacterium tuberculosis in Mpumalanga Province , South Africa : Possible Guiding Design of Retreatment Regimen*. *28*(1), 7–13.

Guo, H., Seet, Q., Denkin, S., Parsons, L., & Zhang, Y. (2006). Molecular characterization of isoniazid-resistant clinical isolates of Mycobacterium tuberculosis from the USA. *Journal of Medical Microbiology*, *55*(11), 1527–1531. https://doi.org/10.1099/jmm.0.46718-0

Helwig, N. E., Hong, S., & Hsiao-wecksler, E. T. (2022). *Global T.B Report 2022*.

Huang, T. S., Kunin, C. M., Lee, S. S. J., Chen, Y. S., Tu, H. Z., & Liu, Y. C. (2005). Trends in fluoroquinolone resistance of Mycobacterium tuberculosis complex in a Taiwanese medical centre: 1995-2003. *Journal of Antimicrobial Chemotherapy*, *56*(6), 1058–1062. https://doi.org/10.1093/jac/dki353

Id, A. S., Gelaw, B., Gebreyes, W., Robinson, R., Wang, S., & Tessema, B. (2020). *The burden of pre-extensively and extensively drug-resistant tuberculosis among MDR-TB patients in the Amhara region , Ethiopia*. 1–13.

Kerléguer, A., Delvallez, G., Sam, S., & Mao, T. E. (2021). *Resistance to Second-Line Anti-TB Drugs in Cambodia : A Phenotypic and Genetic Study*. 1089–1104.

Kumari, R., Tripathi, R., Pandey, A. P., Banerjee, T., & Sinha, P. (2016). Rapid Screening of MDR-TB in Cases of Extra Pulmonary Tuberculosis Using Geno Type MTBDR plus. *PLoS ONE*, 1–10. https://doi.org/10.1371/journal.pone.0159651

Lambregts-van Weezenbeek, H.M. Jansen, J. Ven, |N. J. D. Nagelkerke, M. M. G. G. Sebek, D. van S. (1998). *Origin and management of primary and acquired drug-resistant tuberculosis in The Netherlands: the truth behind the rates*. IUATLD.

Laurenzo David, M. S. A. (n.d.). *Mechanisms of drug resistance in Mycobacterium tuberculosis and current status of rapid molecular diagnostic testing _ Enhanced Reader.pdf*.

Length, F. (2021). *Characterization of Mutations Linked with Second Line Anti-TB Drug Resistance in Pakistan*. *8*(2), 137–142.

Li, Q., Zhao, G., Wu, L., Lu, M., Liu, W., Wu, Y., Wang, L., Wang, K., & Qian, H. (2018). *Prevalence and patterns of drug resistance among pulmonary tuberculosis patients in*. 4–9.

Line probe assays for drug- resistant tuberculosis detection. (n.d.).

Liu, Q., Yang, D., Qiu, B., Martinez, L., Ye, J., Huan, S., Jianming, W., & Zhongqi, L. (2021). Drug resistance gene mutations and treatment outcomes in MDR-TB : A prospective study in Eastern China. *PLoS ONE*, 1–15. https://doi.org/10.1371/journal.pntd.0009068

Maurya, A. K., Singh, A. K., Kumar, M., Umrao, J., Kant, S., Nag, V. L., Kushwaha, R. A. S., & Dhole, T. N. (2013). Changing patterns and trends of multidrug-resistant tuberculosis at referral centre in Northern India: A 4-year experience. *Indian Journal of Medical Microbiology*, *31*(1), 40–46. https://doi.org/10.4103/0255-0857.108720

Mesfin, E. A., Beyene, D., Tesfaye, A., Admasu, A., Addise, D., Amare, M., Dagne, B., Yaregal, Z., & Tesfaye, E. (2018). *Drug-resistance patterns of Mycobacterium tuberculosis strains and associated risk factors among multi drug-resistant tuberculosis suspected patients from Ethiopia*. *110*, 1–16.

Mohammed, K. A. S., Khudhair, G. S., & Al-rabeai, D. B. (2022). *Prevalence and Drug Resistance Pattern of Mycobacterium tuberculosis Isolated from Tuberculosis Patients in Basra , Iraq*. *71*(2), 205–215.

Momen, G., Aainouss, A., Lamaammal, A., Blaghen, M., Messoudi, M., Belghmi, K., Mzibri, M. El, Driss, M., Messaoudi, E., Khyatti, M., & Chaoui, I. (2021). Molecular characterization of mutations associated with resistance to second line drugs in Mycobacterium tuberculosis patients from Casablanca, Morocco. *Open Access*, 1–8.

Mujuni, D., Kasemire, D. L., Ibanda, I., Kabugo, J., Nsawotebba, A., Phelan, J. E., Majwala, R. K., Tugumisirize, D., Nyombi, A., Orena, B., Turyahabwe, I., Byabajungu, H., Nadunga, D., Musisi, K., Joloba, M. L., & Ssengooba, W. (2022). Molecular characterisation of second - line drug resistance among drug resistant tuberculosis patients tested in Uganda : a two and a half - year ' s review. *BMC Infectious Diseases*, 1–10. https://doi.org/10.1186/s12879-022-07339-w

Nakaoka, H., Lawson, L., Squire, S. B., Coulter, B., Ravn, P., Brock, I., Hart, C. A., & Cuevas, L. E. (2006). Risk for tuberculosis among children. *Emerging Infectious Diseases*, *12*(9), 1383–1388. https://doi.org/10.3201/eid1209.051606

No Title. (n.d.). https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Todar%2C+K.+%282009%29.+Todar's+Online+Text+Book+of+Microbiology.+%5BOnline%5D.+Retrieved+July+9%2C+2009+at+4%3A34+pm+from+http%3A%2F%2Fwww.textbookofbacteriology.net%2Findex.html&btnG=#d=gs_cit&t=16

NTP. (1999). Management of drug resistant tuberculosis. *Indian Journal of Medical Sciences*, *53*(5), 220–227.

Oladimeji, O., Othman, Y., Oladimeji, K. E., Atiba, B. P., Adepoju, V. A., & Odugbemi, B. A. (2022). Patterns of Presentation of Drug-Resistant Tuberculosis in Nigeria : A Retrospective File Review. *Microbiology Research*, 609–619.

Organization, W. H. (2020). *Guidance for the surveillance of drug resistance in tuberculosis*. http://apps.who.int/bookorders.

Osei-wusu, S., Omari, M. A., Otchere, I. D., Asare, P., Forson, A., Otu, J., Antonio, M., & Yeboah-manu, D. (2018). *Second-line anti-tuberculosis drug resistance testing in Ghana identifies the first extensively drug-resistant tuberculosis case*. 239–246.

Otu, A., Umoh, V., Habib, A., Ameh, S., Lawson, L., & Ansa, V. (2013). Drug Resistance among Pulmonary Tuberculosis Patients in Calabar , Nigeria. *Hindawi*, *2013*.

Oudghiri, A., Karimi, H., Chetioui, F., Zakham, F., Bourkadi, J. E., Elmessaoudi, M. D., Laglaoui, A., Chaoui, I., & El Mzibri, M. (2018). Molecular characterization of mutations associated with resistance to second-line tuberculosis drug among multidrug-resistant tuberculosis patients from high prevalence tuberculosis city in Morocco. *BMC Infectious Diseases*. https://doi.org/10.1186/s12879-018-3009-9

Oudghiri, A., Momen, G., Aainouss, A., Laglaoui, A., El, M. D., El, M., Id, M., & Id, I. C. (2021). Genotypic diversity of multi- and pre- extremely drug-resistant Mycobacterium tuberculosis isolates from Morocco. *PLoS ONE*, *iii*, 1–10. https://doi.org/10.1371/journal.pone.0253826

P. Idigoras, X. Beristain, A. Iturzaeta, D. V. & E. P.-T. (2000). Comparison of the Automated Nonradiometric Bactec MGIT 960 System with Löwenstein-Jensen, Coletsos, and Middlebrook 7H11 Solid Media for Recovery of Mycobacteria. *European Journal of Clinical Microbiology and Infectious Diseases*. https://doi.org/10.1007/s100960050492

Palomino, J. C., & Martin, A. (2014). Drug resistance mechanisms in Mycobacterium tuberculosis. In *Antibiotics* (Vol. 3, Issue 3, pp. 317–340).

https://doi.org/10.3390/antibiotics3030317

Pan, Y. (2022). *Drug Resistance Patterns and Trends in Patients with Suspected Drug-Resistant Tuberculosis in Dalian , China : A Retrospective Study*. *July*, 4137–4147.

Province, H., Li, Q., Gao, H., Zhang, Z., Tian, Y., Liu, T., & Wang, Y. (2019). *Mutation and Transmission Profiles of Second-Line Drug Resistance in Clinical Isolates of Drug-Resistant Mycobacterium tuberculosis From*. *10*(August), 1–12. https://doi.org/10.3389/fmicb.2019.01838

Rana, V., Singh, N., Nikam, C., Kambli, P., Singh, P. K., Singh, U., Jain, A., Rodrigues, C., & Sharma, C. (2022). Molecular Epidemiology and Polymorphism Analysis in Drug-Resistant Genes in M . tuberculosis Clinical Isolates from Western and Northern India. *Dovepress*, *March*, 1717–1732.

S., A., & C., P. (2012). Old and New TB Drugs: Mechanisms of Action and Resistance. *Understanding Tuberculosis - New Approaches to Fighting Against Drug Resistance*, *May 2014*. https://doi.org/10.5772/30992

Salvato, R. S., Schiefelbein, S., Barcellos, R. B., Praetzel, B. M., Anusca, I. S., Esteves, L. S., Halon, M. L., Unis, G., Dias, C. F., Miranda, S. S., de Almeida, I. N., de Assis Figueredo, L. J., Silva, E. C., Kritski, A. L., Dalla Costa, E. R., & Rossetti, M. L. R. (2019). Molecular characterisation of multidrug- resistant Mycobacterium tuberculosis isolates from a high-burden tuberculosis state in Brazil. *Epidemiology and Infection*.

Schiffman, G. (2009). *Tuberculosis Symptoms. eMedicineHealth*.

Sinha, P., Srivastava, G. N., Gupta, A., & Anupurba, S. (2017). Association of Risk Factors and Drug Resistance Pattern in Tuberculosis Patients in North India. *Open Access*, 139–145. https://doi.org/10.4103/jgid.jgid

Smith, S. (2021). Epidemiology of tuberculosis. *Transactions of the Annual Meeting. National Tuberculosis Association*, *48*, 470–478.

SS Munsiff, D Nilsen, P. F. (2008). *Clinical policies and protocols*. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Sonal+M.+S.%2C+Nilsen%2C+D.%2C+%26+Fujiwara%2C+Tuberculosis+clinical+Policies+and+Protocols%2C+&btnG=#d=gs_cit&t=1697343071663&u=%252Fscholar%253Fq%253Dinfo%253A8ufLX9HUVMkJ%253Ascholar.google.com%252F%2526output%25

Ssengooba, W., Meehan, C. J., Lukoye, D., William, G., Musisi, K., Joloba, M. L., Cobelens, F. G., & Jong, B. C. De. (2016). Whole genome sequencing to complement tuberculosis drug resistance surveys in Uganda. *Infection, Genetic and Evolution*, *40*, 8–16. https://doi.org/10.1016/j.meegid.2016.02.019

Steinmetz, A., Boakye-appiah, J. K., Steinmetz, A. R., & Pupulampu, P. (2016). High prevalence of multidrug-resistant tuberculosis among patients with rifampicin resistance using GeneXpert Mycobacterium tuberculosis / rifampicin in Ghana High prevalence of multidrug-resistant tuberculosis among patients with rifampicin resistance us. *INTERNATIONAL JOURNAL OF MYCOBACTERIOLOGY*, *March*. https://doi.org/10.1016/j.ijmyco.2016.02.004

Tadesse, M., Aragaw, D., Dimah, B., & Efa, F. (2016). Drug resistance-conferring mutations in Mycobacterium tuberculosis from pulmonary tuberculosis patients in Southwest Ethiopia. *International Journal of Mycobacteriology*, *5*(2), 185–191. https://doi.org/10.1016/j.ijmyco.2016.02.009

Tasnim, T., Tarafder, S., Alam, F. M., & Sattar, H. (2018). Pre-Extensively Drug Resistant Tuberculosis (Pre-XDR-TB) among Pulmonary Multidrug Resistant Tuberculosis (MDR-TB) Patients in. *PLoS ONE*, 199–206. https://doi.org/10.4236/jtr.2018.63018

Taylor, G. M., Goyal, M., Legge, A. J., Shaw, R. J., & Young, D. (1999). Genotypic analysis of Mycobacterium tuberculosis from medieval human remains. *Microbiology*, *145*(4), 899–904. https://doi.org/10.1099/13500872-145-4-899

V. Balasubramanian ∗, E.H. Wiegeshaus ∗, B.T. Taylor †, D. W. S. (2004). Pathogenesis of tuberculosis: pathway to apical localization. *Tubercle and Lung Disease*. https://doi.org/10.1016/0962-8479(94)90002-7

Vaziri, F., Kohl, T. A., Ghajavand, H., Kamakoli, K., & Merker, M. (2019). Genetic Diversity of Multi- and Extensively Drug-Resistant Mycobacterium tuberculosis Isolates in the Capital of Iran , Revealed by Whole-Genome Sequencing. *Journal of Clinical Microbiology*, *November 2018*, 1–7.

Vijdea, R., Stegger, M., Sosnovskaja, A., Andersen, Å. B., Thomsen, V., & Bang, D. (2008). Multidrug-resistant tuberculosis: Rapid detection of resistance to rifampin and high or low levels of isoniazid in clinical specimens and isolates. *European Journal of Clinical Microbiology and Infectious Diseases*, *27*(11), 1079–1086. https://doi.org/10.1007/s10096-008-0548-9

WHO. (2016). Global epidemiology of tuberculosis. *The Lancet*. https://doi.org/10.1016/S0140-6736(06)68384-0

WHO. (2018a). TB burden report 2018. In *World Health Organization* (Vol. 63, Issue 10). https://apps.who.int/iris/handle/10665/274453

WHO. (2018b). *WHO Factsheet*.

WHO. (2019). *Global Tuberculosis report*.

WHO. (2020). WHO consolidated guidelines on tuberculosis. Module 4: treatment - drug-resistant tuberculosis treatment. Online annexes. In *Who*.

WHO. (2022). Global TB Report 2022 Factsheet. *World Health Organization*. https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2022

Yan, J. J., Huang, A. H., Tsai, S. H., Ko, W. C., Jin, Y. T., & Wu, J. J. (2000). Comparison of the MB/BacT and BACTEC MGIT 960 system for recovery of mycobacteria from clinical specimens. *Diagnostic Microbiology and Infectious Disease*, *37*(1), 25–30. https://doi.org/10.1016/S0732-8893(00)00118-8

Yao, C., Guo, H., Li, Q., Zhang, X., Shang, Y., Li, T., Wang, Y., Xue, Z., Wang, L., Li, L., & Pang, Y. (2021). Prevalence of extensively drug - resistant tuberculosis in a Chinese

multidrug - resistant TB cohort after redefinition. *Antimicrobial Resistance & Infection Control*, 1–8. https://doi.org/10.1186/s13756-021-00995-8

Yeboah-Manu, D. (2013). Drug Susceptibility Pattern of Mycobacterium Tuberculosis Isolates From Ghana; Correlation with Clinical Response. *Mycobacterial Diseases*, *02*(03). https://doi.org/10.4172/2161-1068.1000107

Zhang, J., Ren, Y., & Pan, L. (2021). *Analysis of drug resistance and mutation profiles in Mycobacterium tuberculosis isolates in a surveillance site in Beijing , China*. https://doi.org/10.1177/0300060520984932

Zhang, Y., & Yew, W. W. (2009). Mechanisms of drug resistance in Mycobacterium tuberculosis. *International Journal of Tuberculosis and Lung Disease*, *13*(11), 1320–1330.

7 APPENDICES

7.1 Appendix I: MATERIALS

- Vortex mixer
- Timer
- Adjustable Automatic pipettes
- Filtered pipette tips
- Sterile transfer pipettes
- Glass slides
- Disposable bacteriological loops
- Forceps
- Slide rack
- Slide Dryer
- Staining rack
- Slide file
- Bunsen burner
- 5% Phenol disinfectant solution

- 0.1% Hypochlorite
- Beakers
- 1.5ml Eppendorf vials
- PCR reaction vials
- Blood Agar Plates
- BACTEC MGIT
- BACTEC MGIT PANTA
- BACTEC MGIT Growth Suplement
- BACTEC MGIT TBc Identification Kit
- BACTEC MGIT SIRE Kit
- GenoLyse DNA Extraction Kit
- Genotype MTBDR*plus* Kit
- Genotype MTBDR*sl* Kit
- DNA-Free marker
- Hybridization Trough
- BSC Class II
- Micro Centrifuge
- Heat Block
- BD BACTEC MGIT 960
- GTQ Cycler 96
- GT Blot 48

7.2 Appendix II: REAGENT COMPOSITION AND PREPARATION

1. Ziehl-Neelsen staining Solutions (Prepared in-house)

   A. 0.3% Carbol Fuchsin

      a. 3g Basic Fuchsin Powder

      b. 50g Phenol

      c. 100ml Ethanol

      d. 900ml Distilled water

   B. 20% Sulphuric Acid

      a. 200ml Conc. Sulpheric Acid

      b. 800ml Distilled Water

   C. 0.3% Methylene blue

      a. 3g Methylene Blue Powder

      b. 1000ml Distilled Water

2. Sodium Hydroxide N-Acetyl-L-Cysteine (NaOH-NALC) reagent for digestion and decontamination

   i. To make 4% NaOH solution (A), use 100ml of distilled water with 4g of NaOH pellets.

ii. ii. To make a 2.9% sodium citrate solution (B), dissolve 2.9g of sodium citrate with 100ml of distilled water.

iii. Autoclaved the two solutions A and B to sterilize them

iv. Aseptically combine equal parts of sodium citrate solution and NaOH prior to usage (only prepare as much as will be needed in a single day.

v. To reach a final concentration of 0.5%, add NALC powder. (To a 100ml solution of NaOH-Na citrate, add 0.5g of NALC powder)

vi. Use the mixture immediately after thorough mixing. If left unattended for longer than 24 hours, NALC activity is lost.

3. BD BBL™ MGIT™ Culture Media (7ml) Lot: 0113550. Exp: 15th December, 2022

   i. Improved Middlebrook 7H9 broth base - 5.9 g

   ii. Casein peptone - 1.25g

   iii. Sterile Distilled water (DH_2O) - 1L

4. BD BACTEC™ MGIT™ 960 Supplement Kit. Lot: 0087627. Exp:22nd December, 2022

   A. BD BACTEC™ MGIT™ Growth Supplement 15mlx6 Lot: 0087618 Exp:22nd December, 2022

      i. Bovine Albumin - 50.0 g

      ii. Dextrose - 20.0 g

      iii. Catalase - 0.03 gm

      iv. Oleic Acid - 0.1 gm

      v. Polyoxyethyline state (POES) - 1.1 gm

      vi. Distilled water - 15 ml

B. BD BBL™ MGIT ™PANTA™ x6. Lot: 0087621. Exp:22nd December, 2022

i. Polymyxin B - 6,000 units

ii. Amphotericin B - 600µg

iii. Nalidixic Acid - 2400µg

iv. Trimethoprim - 600µg

v. Azlocillin - 600µg

5. BD MGIT TBc Identification Test Kit. Lot: 20035004 Exp: 15 December, 2022

6. BD BACTEC™ MGIT™ 960 SIRE Kit Lot: 0280267 Exp:27 December, 2022

A. Streptomycin - lyophilized drug per vial - 332µg

B. Isoniazid - lyophilized drug per vial - 33.2µg

C. Rifampin - lyophilized drug per vial - 332µg

D. Ethambutol - lyophilized drug per vial -1660µg

E. BD BACTEC™ MGIT™ 960 SIRE Supplement Lot: 5092532

i. Catalase - 0.03g

ii. Oleic acid - 0.6g

iii. Bovine albumin - 50g

iv. Dextrose - 20g

7. GenoLyse Kit® Ver 1.0. Lot: XL00042. Exp: 02 February, 2023

A. A-Lyses (Lyses Buffer): Lot: QK0234. Exp: 02 February, 2023

B. A-NB (Neutralizing Buffer): Lot: QL0234. Exp: 02 February, 2023

8. Genotype MTBDR*plus* Kit Ver 2.0 Lot: OV00244. Exp: 17th October, 2022

A. AM-A (Amplification mix A) *plus*. 0.3mlx4: Lot: OQ0421.1 Exp: 19th November, 2022
B. AM-B (Amplification mix B) *plus.* 1.05mlx4: Lot: OR0421.1 Exp: 19th November, 2022
C. Line Probe Assay Reaction (Strips 48 strips) x2. Lot: OT0320.2A Exp: 22nd February, 2022

9. Genotype MTBDR*sl* Ver. 2.0 Kit Lot: AAX00108 . Exp. 10th October, 2022
   A. AM-A (Amplification mix A) *sl.* 0.3mlx4: Lot: UP0521. Exp: 21st February, 2023
   B. AM-B (Amplification mix B) *sl.* 1.05mlx4: Lot: UQ0521. Exp: 21st February, 2023
   C. DEN (Denaturation solution) 1.2ml x2 Lot: N0221 Exp: 22nd February, 2023
   D. HYB (Hybridization Solution) 120ml.Lot: B0714. Exp: 22nd February, 2023
   E. STR (Stringent Solution) 120ml. Lot: K0714. Exp: 22nd February, 2023
   F. RIN (Rinse Solution) 120x2. Lot: I0714 Exp: 22nd February, 2023
   G. CON-D (Conjugate Diluent) 120ml. Lot: E0714. Exp: 22nd February, 2023
   H. CON-C (Conjugate Concentrate) 1.2ml Lot: PO714B. Exp: 22nd February, 2023
   I. SUB-D (Substrate Diluent) 120ml Lot: GO714 Exp: 6th December, 2022
   J. SUB-C (Substrate Concentrate) 1.2ml Lot: T0121A. Exp: 6th December, 2022
   K. Line Probe Assay Reaction (Strips 48 strips) x2. Lot: ABB0121B176 Exp: 28th February, 2022

7.3 Appendix III: PROCEDURES

A. MGIT Media Inoculation

- Isolates were removed from storage and allowed to thaw to room temperature
- MGIT tubes were labelled with sample codes 0.8 ml of growth supplement/PANTA mixture was added to each media
- 0.5 ml of the isolate suspension was transferred to each tube and incubated in the MGIT machine until they were flagged positive

B. Ziehl-Neelsen staining procedure

- Smears were prepared by placing a drop of broth from positive culture tubes on a clean slide.
- Smears were air dried and heat fixed
- The slides were placed on a staining-rack over a sink and flooded with 0.3 % carbol fuchsin.
- The stain was gently heated until steam begun to appear
- The hot stain was left on the slide for 5 minutes
- The stain was washed off with distilled water and the smear decolorized with 20% sulphuric acid solution for 5 minutes.

- The decolourizer was thoroughly washed off with copius amount of distilled water and 0.3% methylene blue solution was used as a counterstain for one minute.
- For a microscopic examination, the slides were rinsed and air dried.
- The stained slides were systematically screened for AFBs and serpentine cord formation with oil immersion objective using a binocular bright field microscope.

C. BD MGIT TBc Identification

- 0.1 ml of bacteria suspension from pure, AFB positive MGIT positive cultres were transferred to the sample wells of DB MGIT TBc identification test cassettes.
- The cassettes were incubated on the bench for 20 minutes and results were read

D. Phenotypic DST

- 4 ml of sterile distilled/deionised water was used to reconstitute each drug vial
- The mixture was mix thoroughly until the drug is completely dissolved.
- 5 MGIT growth media tubes were labelled: Growth Control, Streptomycin, Isoniazid, Rifampicin and Ethambutol.
- 0.8ml of growth supplement was added to labelled BACTEC MGIT 960 tubes.
- For the medium's medication concentration to reach the following critical level, 100 µl of reconstituted drug solutions were added into their corresponding tubes:

- Streptomycin - 1.0µg/ml of medium
- Isoniazid - 1.0µg/ml of medium
- Rifampicin - 1.0µg/ml of medium

 Ethambutol - 5.0µg/ml of medium

- 0.5ml of inoculum was added to the drug tubes
- 0.5ml of 1:100 dilution of the neat inoculum was added to the drug free (growth Control) tube.
- The tubes were arranged in the MGIT 5-set DST carriers and incubated in the MGIT machine
- The instrument monitored the entered susceptibility test sets to determine susceptibility for up to 13 days.
- Results were qualitative and indicated susceptibility (S), resistance (R), or indeterminacy (X).

E. DNA Extraction

- 500µl of bacterial suspensions were put into a 1.5 ml screw-cap container that was clearly labelled.
- The vials were centrifuged for 15 min at 10, 000 xg in a centrifuge with aerosol tight rotor and supernatants discarded.
- 100µl Lysis Buffer (A-LYS) was added to the deposits and resuspended.
- The mixtures were heated to 95°C in a heat block for 5 minutes with the lids securely fastened.
- 100µl of Neutralisation Buffer (A-NB) was added and vortexed for 5 sec.
- The mixture was centrifuged for 5 min at full speed in a microcentrifuge with aerosol tight rotor
- Supernatants were transfer into a new labelled screw cap tube and store at -20°C for PCR procedure.

F. PCR

- Thaw AM-A and AM-B (taken from GenoType MTBDRplus 2.0) were thawed to room temperature
- 10µl of AM-A was transferred into labelled sterile PCR tubes
- 35µl of AM-B was added to the PCR tubes and thoroughly mixed.
- The PCR tubes were tightly closed and all reagents were returned to the correct storage facility
- 5µl of samples negative and positive control samples were added to their respective PCR tubes containing master mix.
- The PCR reaction tubes were loaded on to a Thermal Cycler and the lid closed.
- On the thermal cycler, the following program for the amplification procedure was selected and run.

Temperature Profile	**Number of Cycles**
95ºC for 15 min	1
95ºC for 30 sec 65ºC for 2 min	10
95ºC for 25 sec 50ºC for 40 sec 70ºC for 40 sec	20
70ºC for 8 min	1
4 ºC for ∞	1

G. Line Probe Assay

- 20µl of denaturation solution (DEN) was transferred into each well of trays

- 20µl of DNA PCR products were added to DEN in each tray and mix by pipetting up/down
- The mixture was incubated at room temperature for 5 minute
- DNA strips were labelled with fine DNA strip marker according to the sample codes
- The labelled strips were placed in each well in the tray corresponding to the sample codes and mixed by tilting the mixture up and down
- 1ml pre warmed Hybridization solution was added, mixed and incubated at 45°C for 30 minutes
- After complete aspiration of the hybridization solution, add 1 ml of the Stringent solution and incubate at 45 °C for fifteen minutes.
- The strips were incubated in 1 ml of the conjugate solution for 30 minutes at room temperature after being washed in 1 ml of the rinse solution for 1 minute.
- The strips were rinsed in 2 changes of 1 ml Rinse solution for 1 minute each and rinsed again in 1 ml distilled water for 1 minute at room temperature
- The strips were treated with 1 ml Substrate solution at room temperature for 10 minutes.
- The strips were rinsed in 2 changes of water, dried and visually inspected for result interpretation

7.4 Appendix IV: Sample results of Genotype MTBDR*plus* Line Probe Assay

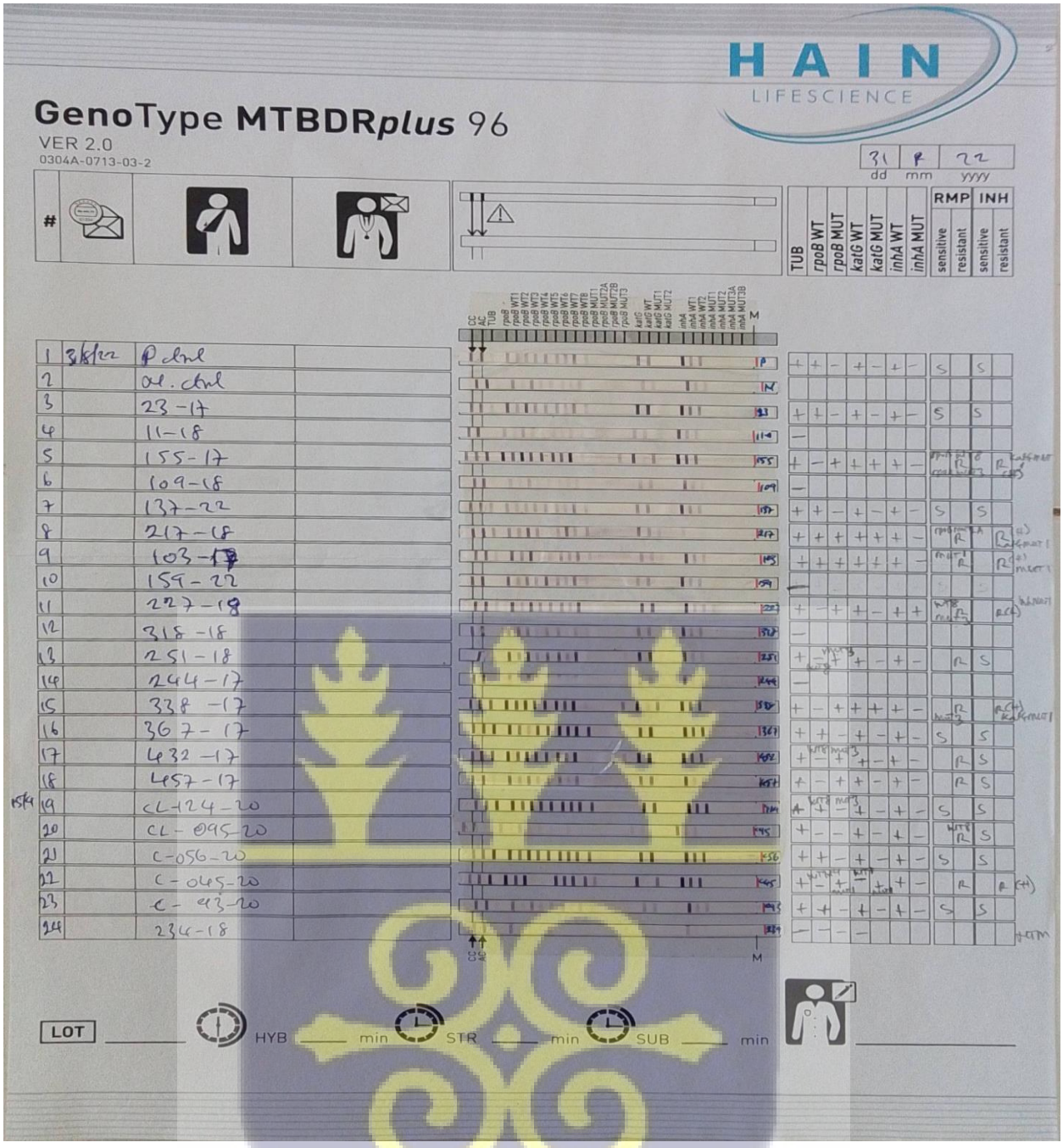


Figure 17: Sample result of Genotype MTBDR*plus* Line Probe Assay

7.5 Appendix V: Sample result of Genotype MTBDR*sl* Line Probe Assay

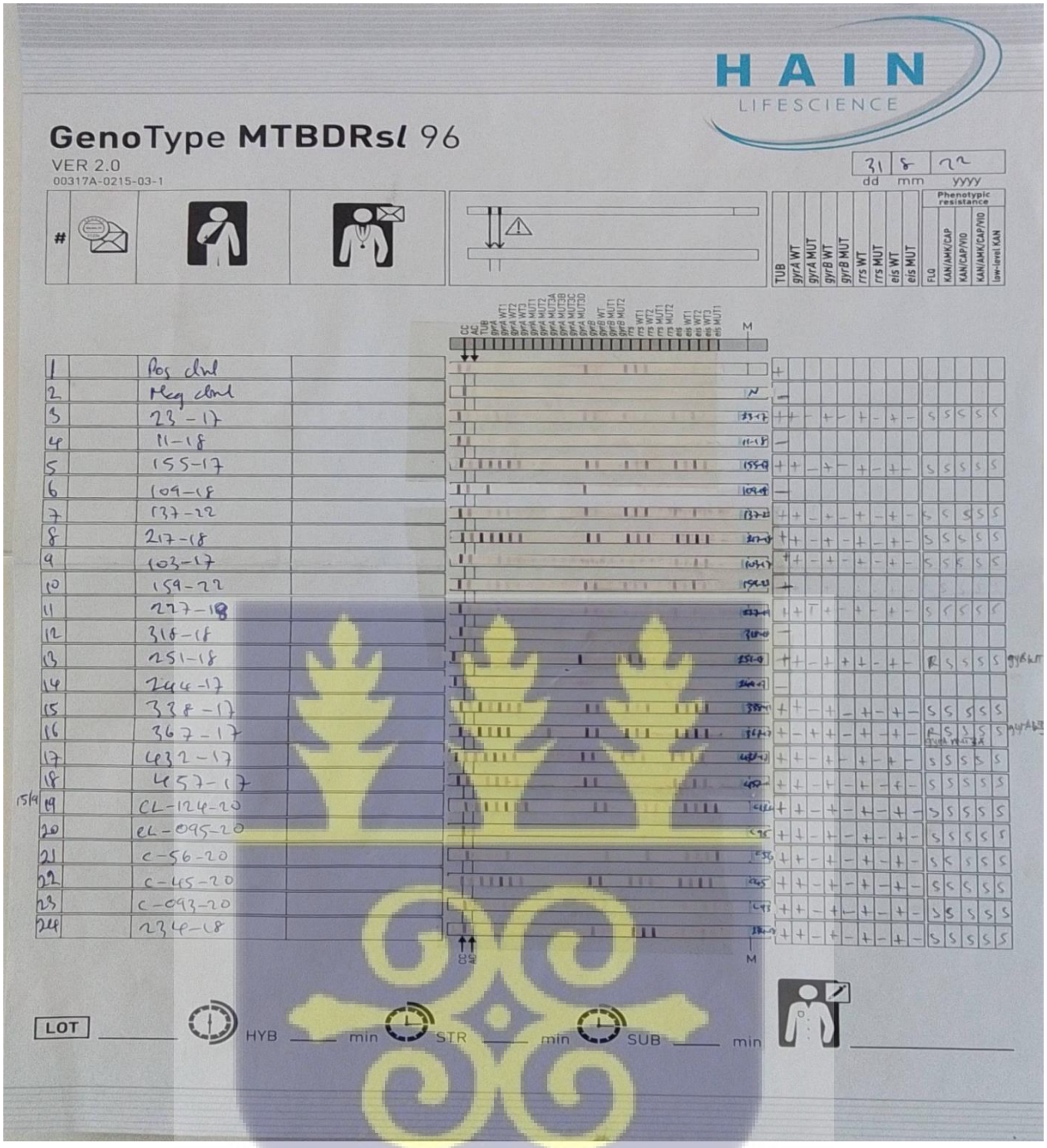


Figure 18: Sample result of Genotype MTBDR*sl* Line Probe Assay

7.6 Appendix VI: Ethical Clearance Approval

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

Ref. No.: EPRC/ APR/2022 April 19, 2022

Mr. Stephen Ofori Yirenkyi
Department of Medical Microbiology
University of Ghana Medical School
Korle-Bu.

ETHICAL CLEARANCE

Protocol Identification Number: ***CHS-ET/M.6-P 4.4/2021-2022***

FWA: 000185779 **IORG: 0005170** **IRB: 00006220**

The College of Health Sciences Ethical and Protocol Review Committee (EPRC) on April 19, 2022 reviewed and approved your re-submitted research protocol.

Title of Protocol: **"The Burden of Extensively Drug Resistance and Pre-Extensively Drug Resistance Tuberculosis among Multidrug-Resistant Mycobacterium Tuberculosis Patients in Ghana"**

Principal Investigator: **Mr. Stephen Ofori Yirenkyi**

This approval requires that you submit six-monthly review report(s) of the study to the Committee and a final full review report to the EPRC at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study before, during and after implementation.

Please note that any significant modification(s) to this project/study must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the EPRC within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till April 19, 2023.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
Professor Andrew Anthony Adjei
Chair, Ethical and Protocol Review Committee

cc: Provost, CHS
Dean, UGMS
Head, Medical Microbiology

• P. O. Box KB 52, Korle Bu, Accra, Ghana. Telephone: +233 (0) 302 665103/4 • Fax: +233 (0) 302 660762
• Email: admin.chs@ug.edu.gh /provost.chs@ug.edu.gh • Websi

7.7 Appendix VII: Facility Approval Letter

In case of reply the number and the date of this letter should be quoted

My Ref. No GHS/ ERHK /GF/ 037

Your Ref. No .....

GHANA HEALTH SERVICE
Your Health - Our Concern

EASTERN REGIONAL HOSPITAL
P.O. BOX 201
KOFORIDUA, E/R.
GHANA WEST AFRICA.

TEL. # 03420- 23011
FAX # 03420- 25294
Website:www.erhk.org
Email:info@erhk.org/
eastern.rh@ghs.gov.gh

15TH JUNE, 2022

THE PRINCIPAL SUPERVISOR
DEPARTMENT OF MEDICAL MICROBIOLOGY
UNIVERSITY OF GHANA MEDICAL SCHOOL
TEL: 0244789209
ACCRA

RE: RESEARCH STUDY: THE BURDEN OF PRE-EXTENSIVELY AND EXTENSIVELY DRUG RESISTANCE AMONG MULTI DRUG RESISTANT PATIENTS IN GHANA

Your letter dated 25th April, 2022 with respect to the above subject matter refers.

This is to inform you that approval has been given to Mr. Stephen Ofori Yerenkyi an MPHIL student at the Department of Medical Microbiology, UGMS to conduct a research at the Eastern Regional Hospital, Koforidua on the Topic **"The Burden of Pre-extensively and Extensively Drug Resistance among Multi Drug Resistant Patients in Ghana".**

You are therefore requested to submit copies of the research report to the office of the Medical Director upon completion of the study or research.

Thank you.

DR. FORSTER AMPONSAH-MANU (MD, FGCS)
CONSULTANT, GENERAL SURGEON
CLINICAL DIRECTOR
CONSULTANT GENERAL SURGEON
CLINICAL DIRECTOR
EASTERN REGIONAL HOSPITAL
KOFORIDUA

DR. FORSTER AMPONSAH-MANU (MD, FGCS)
CONSULTANT, GENERAL SURGEON
CLINICAL DIRECTOR
HEAD OF SURGICAL DEPARTMENT

CC: HOD, Medical Microbiology Dept.
Mr. Stephen Ofori Yirenkyi

IN CASE OF REPLY THE REFERENCE NUMBER AND DATE OF THIS LETTER SHOULD BE QUOTED

Our Ref
Your Ref

DEPARTMENT OF MEDICINE
KORLE BU TEACHING HOSPITAL
P.O.BOX KB 77
KORLE BU ACCRA
Tel 233-21-673033-6
Fax 233-21-667759
E-mail Korlebu@ghana.com
Website www.korlebu.com

5th May, 2022.

THE PRICIPAL SUPERVISOR
DEPARTMENT OF MEDICAL MICROBIOLOGY
UNIVERSITY OF GHANA MEDICAL SCHOOL

Dear Sir,

RE: RESEARCH STUDY: THE BURDEN OF PRE-EXTENSIVELY AND EXTENSIVELY DRUG RESISTANCE AMONG MULTI DRUG RESISTANT PATIENTS IN GHANA

I refer to your letter dated April 25, 2022 on the above subject and wish to inform you that approval has been granted Mr. Stephen Ofori Yirenkyi, an MPhil candidate at the Department of Medical Microbiology of the University of Ghana Medical School to use archival samples of Mycobacterium tuberculosis complex from the Chest Clinic Laboratory, Korle Bu Teaching Hospital for the study.

You are requested to share the research findings with the laboratory unit upon completion in a form of a hard copy of the final write up.

Thank you.

Yours faithfully

Honesty M. Ganu (MSc, BSc)
Prin. Med Lab Scientist
Dept. of Chest Diseases
Korle Bu Teaching Hospital

Honesty Mensah Ganu (FWAPCMLS, Medical Bacteriology)
Head of Unit, Chest Clinic Laboratory

Cc: HOD, Medical Microbiology Dept.
Mr. Stephen Ofori Yirenkyi

7.8 Appendix VIII: Table of Results of Isolates

Date	Path No.	Culture Results	RIF. Mutations	INH Mutations	FQs Mutations	AMGs Mutations	Genotypic Results - First Line	Genotypic Results - Second line
24/02/2016	38-16	MDR-TB	S531L	S315T1			MDR-TB	
17/10/2016	207-16	MDR-TB	D516V	at codon 315			MDR-TB	
11/11/2016	211-16	MDR-TB	at Codon 530-533				Rif Resistance Inferred.	
3/3/2017	78-17	MDR-TB	S531L	S315T1			MDR-TB	
21/3/2017	103-17	MDR-TB	F505L	S315T1			MDR-TB	
12/4/2017	135-17	MDR-TB	D516V	S315T1			MDR-TB	
12/5/2017	155-17	MDR-TB	S531L	S315T1			MDR-TB	
7/7/2017	234-17	MDR-TB	H526D	at codon 315			MDR-TB	
17/7/2017	244-17	MDR-TB	S531L		D94A		RR-MTB	Pre-XDR-TB
18/7/2017	247-17	MDR-TB	at codons 526-529				Rif Resistance Inferred.	
26/7/2017	252-17	MDR-TB	S531L				RR-MTB	
5/9/2017	303-17	MDR-TB	S531L	S315T1	D94G		MDR-TB	Pre-XDR-TB
13/9/2017	325-17	MDR-TB	S531L	S315T2			MDR-TB	
25/9/2017	338-17	MDR-TB	S531L	S315T1		C1402T	MDR-TB	Pre-XDR-TB
3/10/2017	352-17	MDR-TB	at codon 530-533			at 1484 region	Rif Resistance Inferred.	AMG resistance inferred

9/10/2017	367-17	MDR-TB	at codon 530-533		D94A		Rif Resistance Inferred.	Pre-XDR-TB
14/11/2017	416-17	MDR-TB						
22/11/17	423-17	MDR-TB						
30/11/2017	432-17	MDR-TB	S531L			C1402T	RR-MTB	Pre-XDR-TB
30/11/2017	437-17	MDR-TB						
5/12/2017	457-17	MDR-TB	S531L	C-15t			MDR-TB	
9/1/2018	Nov-18	MDR-TB	at codons 510-517				Rif Resistance Inferred	
21/2/2018	86-18	MDR-TB	S531L	S315T1			MDR-TB	
28/2/18	97-18	MDR-TB						
5/3/2018	109-18	MDR-TB	at codon 530-533				Rif Resistance Inferred.	
5/3/2018	110-18	MDR-TB	S531L	S315T1			MDR-TB	
14/3/2018	115-18	MDR-TB	S531L	S315T1, t-8c			MDR-TB	
20/3/2018	121-18	MDR-TB	S531L	S315T1			MDR-TB	
5/4/2018	134-18	MDR-TB						
10/4/2018	147-18	MDR-TB						
26/4/2018	163-18	MDR-TB	S531L			G1484T	RR-MTB	Pre-XDR-TB

7/5/2018	179-18	MDR-TB	H526Y	S315T1			MDR-TB	
24/5/2018	217-18	MDR-TB	H526Y	S315T1			MDR-TB	
11/6/2018	227-18	MDR-TB	at codons 526-529				Rif Resistance Inferred	
15/6/2018	230-18	MDR-TB	at codon 530-533			at position 1484	Rif Resistance Inferred.	AMG Resistance inferred
19/6/2018	234-18	MDR-TB	at codons 526-529				Rif Resistance Inferred	
22/6/2018	241-18	MDR-TB	D516V	S315T2			MDR-TB	
25/6/2018	243-18	MDR-TB	D516Y at codons 513-519		S91P		Rif Resistance Inferred.	Pre-XDR-TB
29/6/2018	250-18	MDR-TB	H526D	S315T1			MDR-TB	
30/6/2018	251-18	MDR-TB	S531L	S315T1			MDR-TB	
19/7/2018	266-18	MDR-TB	S531L	C–15T			MDR-TB	
20/7/2018	267-18	MDR-TB	H526D				RR-MTB	
1/8/2018	284-18	MDR-TB	D516V	S315T1			MDR-TB	
18/8/2018	285-18	MDR-TB	D516Y at codons 513-519	S315T1			MDR-TB	
18/8/2018	288-18	MDR-TB	D516V	S315T1	D94A		MDR-TB	Pre-XDR-TB
28/8/2018	314-18	MDR-TB	S531L	S315T1			MDR-TB	
1/9/2018	331-18	MDR-TB	H526D			C1402T	RR-MTB	Pre-XDR-TB

1/9/2018	332-18	MDR-TB	S531L				RR-MTB	
1/9/2018	333-18	MDR-TB	D516V	S315T1			MDR-TB	
20/9/2018	336-18	MDR-TB	S531L	at codon 315			MDR-TB	
12/10/2018	401-18	MDR-TB	H526D	S315T1			MDR-TB	
9/1/2019	15-19	MDR-TB						
10/4/2019	72-19	MDR-TB						
12/6/2019	109-19	MDR-TB	D516V	S315T1			MDR-TB	
9/7/2019	136-19	MDR-TB	S531L	in the -15 region			MDR-TB	
7/8/2019	155-19	MDR-TB	S531L	S315T1	A90V		MDR-TB	Pre-XDR-TB
10/9/2019	186-19	MDR-TB						
30/10/2019	227-19	MDR-TB	S531L				RR-MTB	
13/11/2019	233-19	MDR-TB	H526D	-15 region			MDR-TB	
30/11/2019	244-19	MDR-TB	H526D	at codon 315			MDR-TB	
17/12/2019	254-19	MDR-TB						
18/1/2019	CL-181-19	MDR-TB	S531L				RR-MTB	
1/1/2019	CL-061-19	MDR-TB	S531L				RR-MTB	

8/1/2020	Mar-20	MDR-TB	D516V	S315T1			MDR-TB	
8/1/2020	Apr-20	MDR-TB	at codons 505-509	S315T1			MDR-TB	
27/3/2020	67-20	MDR-TB						
8/4/2020	79-20	MDR-TB	at codon 530-533	S315T1			MDR-TB	
18/9/2020	170-20	MDR-TB	S531L	S315T1			MDR-TB	
1/12/2020	202-20	MDR-TB	S531L				RR-MTB	
5/9/2020	C4-95-20	MDR-TB	at codon 530-533				RR-MTB	
10-Oct-2020	C-100-20	MDR-TB	H52Y				RR-MTB	
25/9/2020	CL-259-20	MDR-TB	H52D	S315T1			MDR-TB	
5-Nov-2020	CL-115-20	MDR-TB	at codon 530-533				Rif Resistance Inferred	
2-Mar-2020	C-032-20	MDR-TB	at codon 526-529	S315T1			MDR-TB	
4/5/2020	C-045-20	MDR-TB	D516V	S315T1			MDR-TB	
9/2/2020	CL-092-20	MDR-TB	H526Y				RR-MTB	
27/5/2020	C-275-20	MDR-TB	at codon 530-533				RR-MTB	
5/7/2020	C-057-20	MDR-TB	H526D	S315T1			MDR-TB	
19/9/2020	C-199-20	MDR-TB	S531L				RR-MTB	

1/2/2020	C-012-20	MDR-TB	S531L	S315T1			MDR-TB	
14/6/2020	CL-146-20	MDR-TB	H526Y	S315T1			MDR-TB