The Landscape of Drug Resistant Tuberculosis in South-East Asia (SEA) Region: A Burden of Disease and Challenges in Detection

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of Bachelor of Science in Microbiology

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis/project titled "The Landscape of Drug Resistant Tuberculosis in South-East Asia (SEA) Region: A Burden of Disease and Challenges in Detection" submitted by Shehreen Ferdous (17326007) of Summer 2017, has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Science in Microbiology on 9th October, 2021.

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Abstract

Tuberculosis, mainly caused by Mycobacterium tuberculosis, is a serious bacterial infection that primarily infects the respiratory system but can spread to other organs of the body. Approximately one-third of the world's population is infected with MTB, affecting people from all age groups. Out of the 30 high Tb burden countries, 6 of them are in South-East Asia (SEA) region accounting for 44% of the global Tb burden. This review looks at the distribution of Tb cases within the SEA region. It further explores some of the underlying causes of drug resistant Tb throughout history and how human activities has led to its proliferation. Different phenotypic and molecular methods in diagnosing drug-resistant Tb are also discussed in this review. Comparative analysis between the different methods have also been made in this review. In order to eradicate Tb, emphasis must be put on efficient diagnostic techniques, preventative measures and adequate treatment.

Keywords: tuberculosis; South-East Asia; drug-resistant Tb; diagnostic techniques

Dedication

Dedicated to my loving parents and

my brother

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List of Acronyms

MTC: Mycobacterium Tuberculosis Complex

MTB: Mycobacterium Tuberculosis

TB: Tuberculosis

HIV: Human Immunodeficiency Virus

AIDS: Acquired Immune Deficiency Syndrome

SEA region: South-East Asia region

WHO: World Health Organization

PAS: Para-amino salicylic acid

INH: Isoniazid

RIF: Rifampicin

EMB: Ethambutol

PZA: Pyrazinamide

SM: Streptomycin

MDR: Multidrug-resistant

XDR: Extensively Drug Resistant

DOTS: Directly Observed Treatment Short course

DST: Drug Susceptibility Testing

OFX: Ofloxacin

GFX: Gatifloxacin

SES: Socioeconomic Status

LJ medium: Lowenstein-Jensen medium

MODS: Microscopic-Observation Drug-Susceptibility

PNB: P-nitrobenzoic Acid

MGIT: Mycobacteria Growth Indicator Tube

CFU: Colony-forming unit

OADC: Oleic acid, Albumin, Dextrose and Catalase

PANTA: Polymyxin B, Amphotericin B, Nalidixic Acid, Trimethoprim, Azlocillin

MDL: Microbial Diseases Laboratory

CDPH: California Department of Public Health

NTRL: National Tuberculosis Reference Laboratory

ZN staining: Ziehl–Neelsen staining

SIRE: Streptomycin, Isoniazid, Rifampicin and Ethambutol

BAL: Bronchoalveolar lavage fluid

RT-PCR: Reverse Transcriptase- Polymerase Chain Reaction

LPA: Line Probe Assays

MDR/RR-TB: Multi-drug Resistant or Rifampicin-Resistant TB

Chapter 1

INTRODUCTION

Tuberculosis, a deadly yet curable, disease has plagued mankind for years on end. Tuberculosis is caused by several mycobacterial species that belongs to the Mycobacterium tuberculosis complex (MTC) which is a surprisingly homogenous group. Among them *M. bovis* (primary causative agent of tuberculosis in cattle) and *M. tuberculosis* (responsible for most of the tuberculosis cases in humans) are the most popular ones. Mycobacterium tuberculosis (MTB) is an obligate aerobic intracellular bacterium with an unusual waxy lipid layer surrounding the bacteria. This waxy layer, created due to the presence of mycolic acid, resists Gram staining; hence special acid-fast stains such as Ziehl Neelson stains are used instead for identification.

It has been estimated that one third of the world's population is infected with MTB, but only 5-10% of the infected individuals develop active tuberculosis in their lifetime within a span of first 5 years of the initial infection (Druszczyńska et al., 2012). Despite being a curable disease, an estimated 1.2 million people died from TB worldwide in 2019 and an additional 208 000 people who were HIV- positive. In 2019 ranking above HIV/AIDS, TB remains the top cause of death from a single infectious agent. Tuberculosis affects people all over the world, including all genders and age groups but the distribution of cases is not uniform. In 2019 approximately 10 million people were infected with TB, the cases were greater in men (56%) compared to women (32%) and children (below the age of 15 years) (12%). In that same year, the highest number of cases were seen in the South-East Asia (44%) Africa (35%), Western Pacific (18%) along with smaller number of cases in Eastern Mediterranean (8.2%), the Americas (2.9%) and Europe (2.5%). (WHO, 2019)

It is important to note that MTB is carried in airborne particles which can remain suspended into the air for several hours depending on the environment. MTB is considered highly contagious and droplet nuclei transmission is possible when it comes to MTB (Chiang & Starke, 2018). Upon inhalation of the infected droplet nuclei, the pathogen enters the body through the respiratory tract (principal route of infection in case of TB) and if they are able to avoid the defenses there (mucociliary clearance and cough) they reach the alveoli of the lungs where they are ingested by the alveolar macrophages (Ahmad, 2011).

Perhaps one of the greatest unsolved mysteries regarding tuberculosis is how such an aggressive immune response in a healthy individual fails to eradicate the infection completely, instead resulting in latency. Some of the most foundational questions concerning latent tuberculosis remains unanswered due to the lack of animal model following the natural progression of the disease, especially the latent stage.(Basaraba & Hunter, 2017)

A key feature of tuberculosis, used in day-to-day diagnosis, is the formation of caseating granuloma (which is the cheese-like appearance of the granuloma due to necrosis). It has long been hypothesized that caseous granuloma is the characteristic lesion of both primary and secondary TB. This theory has been disproved multiple times by scientists who now claims that "post-primary TB develops as an obstructive lobular pneumonia that spreads asymptomatically via bronchi within individuals with a high degree of M. tuberculosis-specific immunity".(Basaraba & Hunter, 2017). Airflow obstruction has been associated with Tb in other studies as well (Amaral et al., 2015). In almost all post-primary Tb pneumonia cases bronchial obstruction has been observed. Moreover, treatment of such airway obstruction has shown to dramatically ameliorate the disease (Richards et al., 2018).

Chapter 2

EPIDEMIOLOGY

Brief History of TB

Robert Koch a German microbiologist and physician, often regarded as one of the main founders of modern bacteriology, made a crucial discovery that was the first most important step in controlling and eliminating the widespread deadly disease tuberculosis. He concentrated all his efforts on finding the causative agent of TB. After successful staining and isolation of the TB bacilli he announced his findings on 24th March, 1882 at the Berlin Institute for Physiology. Robert's revolutionary discovery made him famous overnight and resulted in him winning the Nobel Prize in 1905. (Keshavjee & Farmer, 2012)

In 1943, the first efficacious drug against Tb, streptomycin was discovered in Selman Waksman's laboratory at Rutgers University. (Woodruff, 2014) The following year, streptomycin was administered to a patient who yielded positive results. Despite multiple reports of successful treatment with streptomycin, resistance against the drug quickly developed. To combat the resistance against streptomycin other anti-tuberculosis drugs such as para-amino salicylic acid (PAS) and isoniazid (INH) soon developed marking the start of combination therapy for tuberculosis. Before long other important anti-tuberculosis drugs (pyrazinamide, ethionamide, rifampin and ethambutol) emerged, which are still used to this day as first line drugs. (Keshavjee & Farmer, 2012). First line antituberculosis drugs, often considered the core of treatment regimens, comprises of Isoniazid (INH), rifampicin (RIF), ethambutol (EMB), pyrazinamide (PZA) and streptomycin (SM). The standard short course therapy also known as "short-course chemotherapy" includes a combination of these first-line drugs mainly isoniazid and rifampicin (most effective first line drugs) for approximately 6-8 months. Sadly, the phenomenon of multidrug-resistant TB has become increasingly common nowadays. Multidrug-resistant TB is referred to Tb bacteria that are resistant to both isoniazid and rifampin, the two most effective TB drugs. According to (Dheda et al., 2017) "Approximately 20% of tuberculosis isolates globally are estimated to be resistant to at least one major drug (first-line or group A or B second-line), with approximately 10% resistant to isoniazid."

Epidemiology of SEA-Region

Even though the incidence of TB has reduced substantially over the past decade, the disease still remains a cause for concern in some parts of the world including Asia and Africa. It has been reported that out of the 30 high Tb burden countries, 6 of them (Bangladesh, Democratic People's Republic of Korea, India, Indonesia, Myanmar and Thailand) are in the SEA (South- East Asia region) accounting for 44% burden of TB incidence. In order to end the global TB epidemic, targets were set as part of the World Health Organization's End TB Strategy (2016–2035) and the Sustainable Development Goals (2016–2030). Unfortunately, it has been reported that the number of cases is not declining quick enough for most high burden countries to reach the 2020 goals of End TB strategy. To reach the 2020 goals, between the years 2015 to 2020 a 20% drop was required whereas only a 9% reduction took place, with a 2.3 % decline from 2018-2020. On the contrary, European region of the WHO seems to be just on point to reach the target with a 33% drop in the number of TB deaths from 2015 to 2020. (WHO,2019)

The continuing rise of drug resistance in MTB strains are threatening the progress in containing the global Tb epidemic. An estimated 10% rise in multidrug- or rifampicin-resistant TB (MDR/RR-TB) cases was reported from 2018 (186 883 cases) to 2019 (206 030 cases) according to WHO. Despite considerable progress against drug-resistant TB in many countries, only 57% of MDR-TB cases are treated efficiently worldwide. Approximately 50% of MDR-TB burden lies in China (14%), India (27%) and Russian Federation (8%). (WHO,2019)

The SEA region is home to a significant number of MDR and XDR TB cases. Bangladesh, one of the top high Tb burden countries, ranked 7th in the top 8 high TB countries, accounting for 4% of TB cases globally. Even though the short- course program introduced in 1993 in Bangladesh showed high cure rates (95% of cases successfully treated in 2016) for tuberculosis, it wasn't enough to completely eradicate the disease from the country since resistance was soon reported. A recent systematic review was done to access the burden of antibiotic resistant tuberculosis in Bangladesh. The study showed cycloserine (44.6%) and isoniazid (35%) at the top of list along with ethambutol (16.2%) and gatifloxacin (0.2%). The study also determined the frequency of , mono, multi, poly, and extensive anti-TB antibiotic-resistances which were as follows : any drug

resistance 45.3% [95% CI: 33.5–57.1], mono-drug resistance 14.3% [95% CI: 11.4–17.2], multi-drug resistance 22.2% [95% CI: 18.8–25.7], poly-drug resistance 7.7% [95% CI: 5.6–9.7], and extensive- drug resistance 0.3% [95% CI: 0.0–1.0]. Upon comparison with neighboring countries, it was seen that India's state of drug resistance TB was much worse. India had a higher prevalence of XDR and mono- resistance to first-line anti-Tb drugs compared to Bangladesh. Prevalence of XDR-TB in Bangladesh (0.3%) was lower than that of India (1.9%) and China (2%). All in all, the frequency of MDR-TB is much higher in Bangladesh (22.2%) compared to China, Pakistan and Nigeria. (Kundu et al., 2020).

Almost half a million deaths in India are due to tuberculosis. India is considered one of the highest MDR-Tb burden countries globally, responsible for 25% of the global MDR-Tb burden alone. (Chatterjee et al., 2018). A systematic review was done recently to access the prevalence of TB in India. The study was divided into 2 decades, decade 1 was from the year 1995 to 2005; and decade 2 was from 2006 to 2015 where drug resistance Tb was defined as "resistance to one first-line anti-tubercular drug only" and MDR-Tb was defined as "TB with resistance to at least both isoniazid and rifampicin". The study showed out of 45,076 people tested 40% of patients showed resistance to any first-line anti-TB drugs. The overall frequency of both MDR-TB and drug resistance TB has increased over the years. During decade 1 drug resistance Tb was 37.7% (95% CI = 29.0; 46.4, n = 25) vs decade 2, (46.1% (95% CI = 39.0; 53.2, n = 36)). Similarly, during decade 1 MDR-Tb rates were (14.9% [95% CI = 11.0; 18.7, n = 24]) vs decade 2, (27.9% [95% CI = 23.8; 32.1, n = 49]). In 2015 a mere 46% of MDR-TB patients were treated effectively. Evidently, statistics show that India still has a long way to go in regaining control of this Tb- epidemic and requires an intervention to prevent transmission, improve drug susceptibility testing and provide personalized treatment for TB.(Goyal et al., 2017)

Tuberculosis continues to be a huge public health concern for Indonesia which remained in the 3rd position for Tb incidences worldwide. In 2018, roughly 845 000 people were infected with TB, killing approximately 98 300 people (WHO,2018). A study was done in Tangerang, one of the biggest cities in Indonesia to access the burden of drug resistance TB. 127 samples were tested; out of 22% of samples which displayed resistance to first line drugs, 0.8% showed MDR resistance and 20.5% showed resistance to at least one of the first line drugs. 55.1 % of the patients were male and 44.9% female living in cities.(Cucunawangsih et al., 2015). Worrisome rates have

emerged in Thailand as well. A national survey to access the burden of drug resistant Tb are done in Thailand every 5 years. The survey done from 2017 to 2018 showed MDR rates for new and previously treated cases were 0.8% (95% CI: 0.5–1.4) and 13.0% (95% CI:6.5–24.4) respectively. Out of 1501 new TB cases 14.0% [95% confidence interval (CI): 12.1–16.1] tested positive for resistance to ant anti-Tb drug and among 69 previously treated TB cases, 33.4% (95% CI: 23.6–44.8), showed resistance to any anti-TB drug. (Kamolwat et al., 2021)

Myanmar secured a place among the top 30 high TB burden countries with a prevalence rate of 5% MDR-TB cases among new patients and 27%MDR-TB cases among previously treated patients. Concerning rates of XDR-TB among MDR-Tb cases (13.5%) were also found in Myanmar (Ei et al., 2018) (Oo et al., 2019). Drug resistant TB continues to pose a major public health threat in Democratic People's Republic of Korea. In South Korea a study done between January 2015 and December 2018 showed 4.1% MDR-TB cases with rifampicin (1.2%) and isoniazid mono-resistant TB (7.2%) cases present. (Lee et al., 2020).

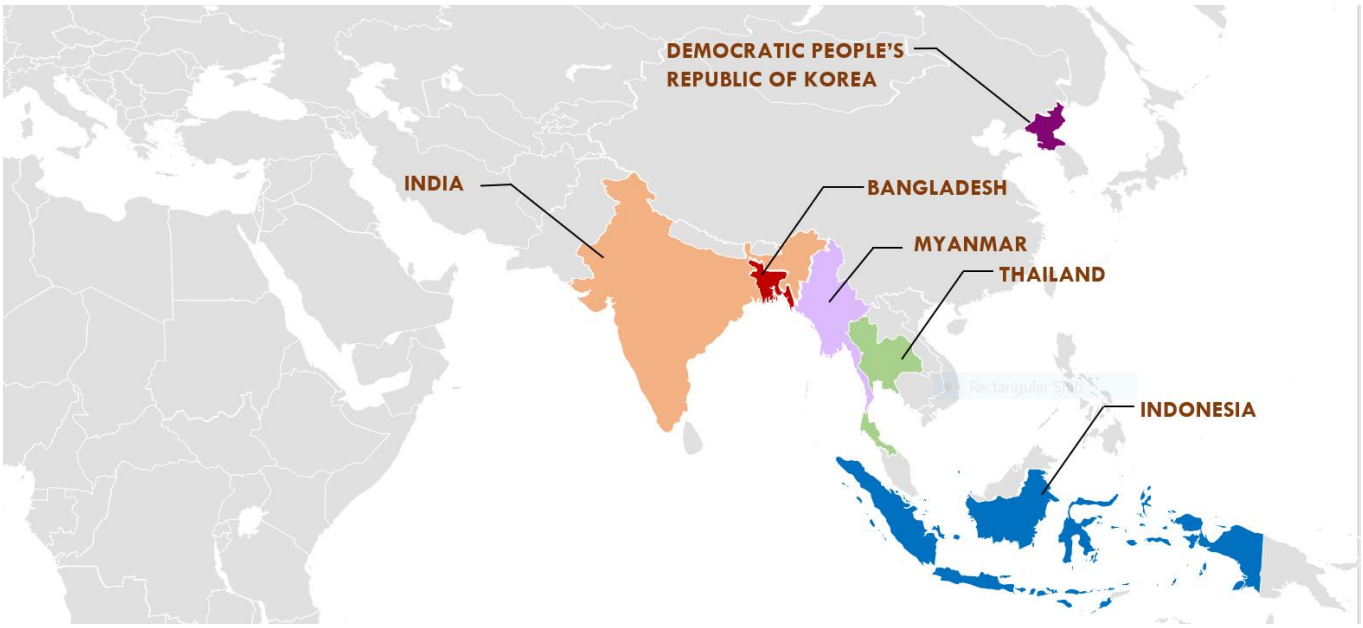

Figure 1- The six high-burden TB countries in South-East Asia Region

Table 1- Frequency distribution of drug resistant TB among SEA region countries

SEA Region	Prevalence of Drug-Resistant TB	References
Bangladesh	Bangladesh ranked 7th in the top 8 high TB countries accounting for 4% of TB cases globally. Prevalence of multi-drug resistance 22.2% in Bangladesh proved to be much higher compared to neighboring countries like Pakistan. On the other hand, frequency of extensive-drug resistance 0.3% was lower than that of India (1.9%) and China (2%).	(Kundu et al., 2020).
India	India is considered one of the highest MDR-Tb burden countries globally, responsible for 25% of the global MDR-Tb burden alone. A systematic study was done to evaluate the severity of Tb burden in India. The study was divided into two decades. Decade 1 (1995 to 2005) VS Decade 2 (2006 to 2015). The study showed Tb burden has continued to deteriorate over the decades with MDR rates showing a substantial increase. MDR-Tb rates during decade 1 (14.9%) vs decade 2 (27.9%)	(Chatterjee et al., 2018) (Goyal et al., 2017)
Indonesia (Tangerang)	A study was done in Tangerang, one of the biggest cities in Indonesia to access the burden of drug resistance TB. 127 samples were tested; out of 22% of samples which displayed resistance to first line drugs, 0.8% showed MDR resistance and 20.5% showed resistance to at least one of the first line drugs.	(Cucunawangsih et al., 2015).
Thailand	The national survey done from 2017 to 2018 showed MDR rates for new and previously treated cases were 0.8% and 13.0% respectively. Out of 1501 new TB cases 14.0% tested positive for resistance to ant anti-Tb drug and among 69 previously treated TB cases, 33.4% showed resistance to any anti-TB drug.	(Kamolwat et al., 2021)

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Democratic People's Republic of Korea	Drug resistant TB continues to pose a major public health threat in Democratic People's Republic of Korea. In South Korea a study done between January 2015 and December 2018 showed 4.1% MDR-TB cases with rifampicin (1.2%) and isoniazid mono-resistant TB (7.2%) cases present.	(Lee et al., 2020)

Chapter 3

CAUSES OF DRUG RESISTANT TB

As efforts to improve drug-resistant Tb surveillance and drug susceptibility testing procedures grew all over the world, it became evident that drug-resistant TB is a worldwide phenomenon and a serious public health issue. A person can be affected by drug-resistant Tb in one of two ways. The term primary resistance or transmitted resistance is used when a person is directly infected with a drug-resistant strain. On the other hand, secondary or acquired resistance takes place when a person who initially had drug sensitive TB but the strain has now become drug-resistant due to incorrect treatment protocols, non-compliance etc. To begin with, a patient carrying drug-sensitive TB acquires resistance and then later transmits this drug resistant strains to other vulnerable members of the population.(Müller et al., 2011)

Multi-drug resistant (MDR)Tb is defined as drug resistant Tb that are resistant to the two most powerful first-line anti-Tb drugs isoniazid and rifampicin, whereas extensively drug resistant (XDR) Tb is defined as MDR but with additional resistance to any of the fluoroquinolones (such as levofloxacin or moxifloxacin) and to at least one of the three injectable second-line drugs (amikacin, capreomycin or kanamycin). Treatment of MDR usually requires use of second-line drugs which are considered more expensive and toxic in nature. (Chen et al., 2016). According to WHO Tb treatment consists of two phases. The first intensive phase consists of a 2-month regimen including isoniazid, rifampicin, pyrazinamide and ethambutol followed by the continuation phase for 4 months continuing rifampicin and isoniazid. The duration of therapy may be extended depending on radiological and bacteriological data and the clinical judgment of the treating physician. It is recommended that treatment protocols should be guided by DST testing. Unfortunately, in many high Tb burden countries first-line drugs are used to treat MDR without proper DST. This usually happens in resource-limited countries where second-line drugs are unavailable, proper DST laboratory facilities are absent, lab personnel don’t have sufficient training, poor management systems etc.(Müller et al., 2011)

Acquired and Transmitted Resistance

Short course chemotherapy

The DOTS (Directly Observed Treatment Short course) strategy was implemented in 1994 by WHO as a means to control the global spread of Tb. This short-standardized course treatment was recommended for use in any countries where the patients sputum smear tested positive for Tb. DOTS involved treatment with a four-drug regimen consisting of isoniazid (INH), Rifampicin (Rif), Pyrazinamide (PZA) and Ethambutol (EMB) for 6-9 months. Although DOTS strategy proved to be an overall success for many countries, it was not the "magic bullet" that many had hoped would eradicate the disease.(Obermeyer et al., 2008) What DOTS didn't take into account is the emergence of drug resistant Tb. Treatment of MDR using DOTS strategy not only failed to cure the disease, when used in significant doses promoted resistance. Furthermore, using DOTS strategy to treat drug-resistant strains have been known to cause amplification of resistance (adding more resistance to an already resistant strain) that is worsening the overall situation.(Seung et al., 2004). Delay in diagnosis has also been cited as a reason for amplification of resistance. Diagnostic delay treating new undetected MDR cases will result in treatment using first-line drugs. The drugs pyrazinamide and ethambutol are not very effective in preventing resistance to other drugs. Hence treatment of MDR using these drugs during the first 2-month intensive phase will likely result in MDR acquiring further resistance to at least one other drug. To make matters worse, the undiagnosed infectious MDR patient will likely transmit the drug-resistant strains to other members of the community.(Müller et al., 2011)

Inability of this short course chemotherapy to treat MRD-Tb proved to be a massive blow since many countries had already adopted the strategy. A prime example of this can be seen in Peru during the year 1995 in the north region of Lima. There patients were identified with strains of Tb that showed broad spectrum resistance to first-line drugs. Instead of a more personalized treatment approach guided by DST results, WHO recommended the Peruvian government to use short course chemotherapy to treat the mutant bacteria. This resulted in massive devastation where hundreds of Peruvian people lost their lives.(Keshavjee & Farmer, 2012)

Similar incidences were also reported in Russia during 1996-2000. In 1994 Russia, like many others, also adapted DOTS strategy in hopes of controlling the Tb epidemic so they had plenty of first-line anti-tb drugs in store with a limited reserve of second-line anti-tb drugs. In Tomsk, Siberia despite DST results patients with previously reported drug-resistant Tb received standardized short course treatment. Therefore, a retrospective study done in Tomsk presented the perfect occasion to analyze the effect of standardized short course chemotherapy on patients with drug resistant strains. Data for 1681 patients were available for investigation. Acquired resistance in this study was defined as "new drug resistance (during or at the end of treatment) that was not present at the beginning of treatment". The results found strong association between treatment outcome and initial drug resistance. Among patients who received previous treatment and showed resistance to isoniazid or rifampicin but not both, 70.8% (17 out of 24) of them acquired MDR resistance. At the same time among pretreatment patients with strains which exhibited resistance to only streptomycin or strains which were susceptible to all first-line drugs, 41.9% (13 out of 31) of them involving treatment failures acquired new multidrug resistance. The study demonstrates the use of short course chemotherapy to treat drug-resistant strains can lead to amplification of resistance. For countries where DST is not readily available, physicians should be well aware that failure of treatment using short course chemotherapy could possibly lead to MDR and should act accordingly.(Seung et al., 2004)

Inadequate treatment and poor adherence

Incorrect treatment and poor adherence are some of the underlying causes for the spread of MDR-TB. Any sort of interruption in treatment could lead to poor treatment outcome and hence encourage the transmission of drug-resistance. It is important to understand the full scale of the problem regarding drug-resistant TB to organize interventions, reassess protocols to prevent the spread of drug-resistance.

Africa being a developing nation has always struggled with control of Tb. In Africa magnitude of the problem regarding MDR-Tb remains unknown due to poor surveillance and limited DST capacity. In August 2017 a sudden upsurge in MDR-TB cases in Arua District, Uganda attracted a lot of attention. Hence an investigation was done to access the burden of MDR-Tb in the district.

Compared to 2013-2016 MDR-Tb cases had more than doubled in 2017. Poor adherence and delay of initial treatment were thought to be the main reasons behind the progression to MDR in patients. Further research revealed possible reasons behind poor adherence which were as follows-health facilities running out of Tb drugs, insufficient training of laboratory personnel, lack of financial and social support, adverse side effect of drugs etc. Delay in starting treatment prolongs the time that patients carry the TB bacteria and allows the disease to progress without proper intervention and the reason behind delay was due to prolonged time period for diagnostic results to come back.(Okethwangu et al., 2019)

Poor adherence was even observed in developed countries such as the UK, where successful outcome for MDR-Tb treat was high as 74%. A study done from 2008 to 2014 consisting of 100 cases showed 14% (14/100) negative outcome, the primary reason for which was poor adherence 62% (9/14). Treatment protocols for MDR-Tb in England is considered very rigorous (18-24) months with long hospital visits. Furthermore, treatment for MDR is also very expensive ranging as high as ten times compared to fully sensitive Tb treatment.(Arnold et al., 2017)

China, not only accounts for 10% of the global Tb burden, it also ranked second highest country to carry MDR-Tb burden worldwide(Li et al., 2020). To identify the factors associated with MDR-Tb a study was performed in Heilongjiang, China consisting of 1995 patients. Out of the patients tested 12% tested positive for MDR-Tb. MDR-Tb in retreatment cases were more likely (5.48 times (95% CI 4.04 to 7.44)) compared to newly diagnosed cases indicating an association with inadequate treatment. Specifically, patients who received treatment for more than 180 days were 4.82 times (95% CI 2.97 to 7.81) times more likely to develop MDR-Tb compared to those who received less. As for reasons behind interruption in treatment, financial challenges, limited knowledge and adverse side effects of the drugs were noted. Treatment in Tb dispensaries were free unlike hospitals and clinics where greater percentage (50.7% compared to 29.9% in Tb dispensaries) of incomplete treatment was reported. Limited knowledge regarding the disease also lead to poor adherence where patients were likely to stop receiving treatment when the symptoms disappeared.(Liang et al., 2012) Similar reasons behind treatment interruption were found in another cross-sectional study done in Guizhou, China. Adverse side effects of drugs and financial burden was cited as the top 2 reasons behind interruption. Most frequently interrupted drugs included amikacin (18.3%) due to its adverse side effects, and cycloserine (10.2%) due to its cost.

Of 202 patients, short treatment interruption was observed in 37.6% of the cases where as, serious treatment interruption found in 28.7% of the cases. (Y. Wang et al., 2019)

Community and Facility based Transmission

Back in the days, the hypothesis that claimed drug resistant Tb was mostly acquired and rarely transmitted was flawed. This idea stemmed from the fact that a mutation in katG gene reduced the virulence of the drug resistant bacteria. Hence it was believed that rate of transmission would be less due to the fitness cost the drug- resistant strain was bearing. This was further supported by the fact that greater resistance was observed in retreatment cases rather than newly diagnosed cases. Before long the hypothesis was challenged by the frequent transmission of MDR-Tb.(Müller et al., 2011)

Regarding drug-resistance, it is often assumed that if a patient is newly diagnosed with MDR-Tb then it is primary resistance, whereas MDR-Tb detected in retreatment cases is considered acquired resistance. It has been estimated that more than half of MDR-Tb and XDR-Tb cases were newly diagnosed and not treated beforehand indicating the significance of transmission. It is also possible for patients who had previously been treated for Tb, to get re-infected with drug resistant Tb. Transmission of MDR and XDR-Tb can be tracked using genotypic method. For examples if through genotyping methods it is seen two individuals in the same hospital have the exact same MDR-Tb strain then transmission is considered to be the case.(Olson et al., 2011)

Very recently there has been a notable rise in XDR-Tb cases in South Africa, the reasons behind which still remains somewhat unclear. A prospective study including 404 patients was performed to decipher whether acquired resistance or transmitted resistance played a bigger role in gaining drug resistance. Both genotypic and clinical approach was taken to access the burden. This is absolutely crucial since findings can reveal whether targeted interventions should be focused on preventing transmission or revision of treatment protocols. XDR-Tb is prevalent in South Africa, to the point where the number of XDR-Tb cases had escalated by a factor of 10 compared to the last decade. It was estimated that in 31% of the cases (124 participants) XDR-Tb was acquired

where as in the remaining 69% of the cases (280 participants) XDR-Tb was obtained through transmission. Hence transmission resistance was found to be the main factor driving XDR-Tb in KwaZulu-Natal Province of South Africa. Further research tracing back social networks revealed both community and nosocomial transmission could have played a role in transmission.(Shah et al., 2017)

A study done in Charghat Bangladesh, a high-Tb burden country, showed that transmission is possible even in rural sub-districts which are not overpopulated. Secondary Tb cases were detected 10 years later after initial outbreak. Out of the 765 cases detected 2.1% (16 cases) developed MDR among which 2 XDR-Tb cases were detected as well. The reason behind the development of XDR-Tb was revealed as private treatment, which lead to acquisition of ofloxacin (OFX) resistance further amplifying resistance resulting in his death. On the contrary, the second case of XDR-Tb was successfully treated with gatifloxacin (GFX) based regimen. (Gumusboga et al., 2012) GFX regimen for treatment of drug resistance Tb has shown considerable success in Bangladesh. (Van Deun et al., 2010). Development of MDR-Tb resulted from inadequate treatment due to the unnecessary strict guidelines followed by hospitals and mandatory hospitalizations (which patients were unwilling to follow). Strict treatment protocols resulted in the death of MDR-Tb patients who (according to National Tuberculosis Control Program criteria) failed to qualify for second-line treatment. Even known XDR-Tb patients were deemed non-eligible unless they went through first-line treatment first. (Gumusboga et al., 2012)

The role of nosocomial transmission in the fight against Tb epidemic cannot be underestimated. An investigation including four hospitals in the Republic of Moldova, a high-Tb burden country, showed how nosocomial transmission especially in hospitals without proper control measures can contribute to the spread of drug resistant Tb. The study was done to inspect whether non MDR-Tb patients with treatment failure developed MDR-Tb during inpatient treatment signifying nosocomial transmission. Molecular techniques such as genotyping were used to identify and track specific MDR and non-MDR strains. It was shown that about 5.1% of inpatients developed MDR-Tb during their stay. According to the results “In 75% of the cases the MDR-TB strain was genetically distinct from the non-MDR-Tb strain at baseline, suggesting a high rate of nosocomial transmission of MDR-Tb bacilli.” Establishing strict control measures in hospitals to isolate MDR-

Tb infectious patients in separate wards and discharging non-infectious Tb patients could be way to reduce nosocomial transmission.(Olmsted et al., 1995)

Other studies found similar result where hospitalization increased the chances of developing MDR-Tb. A study done in Tomsk, Siberia revealed an association between poor adherence and treatment outcome. At the same time data also showed a significantly higher risk for patients who were hospitalized or received in patient treatment later on compared to those who did not(Gelmanova et al., 2007)

Tuberculosis has also been termed as "the disease of the poor" since Tb disproportionately affects the poor. It is no coincidence that some of the highest Tb burden countries just happen to be poor developing nations like Bangladesh India and Africa. Particularly in places where people have limited access to healthcare, nutrition, overcrowded household conditions etc Tb is more commonly seen. Yet few studies focus on the socio-economic factors and its association with Tb. A study done in Lima, Peru attempted to investigate the link between socioeconomic status (SES) and acquired and transmitted Tb. The results showed an interesting finding, that people with higher SES had a greater chance (3-fold increase risk) of transmitted rather than acquired resistance. Conversely, people with lower SES were at a greater risk for acquiring resistance. Some plausible explanations could be people with higher SES have better access to healthcare, therefore proper treatment protocols and regular monitoring compared to people with lower SES, so they have lower chances of acquiring drug resistant Tb through inadequate treatment. Also, people with higher SES tend to work less, stay at home more, have bigger social gatherings leading to transmission of drug resistant strain to other members of the community. (Odone et al., 2016)

Chapter 4

DIAGNOSIS OF TB

Tuberculosis has re-emerged in the past decade throughout the world. In industrialized countries, it has been associated with population growth and immigration whereas in developing countries such as Africa it has been associated with HIV epidemic, poverty, poorly implemented control programs etc. To make matters worse available data suggests that drug resistant TB, namely MDR and XDR TB, are on the rise as well. Hence early diagnosis and treatment remain absolutely critical, now more than ever, to limit the spread of TB. With early diagnosis and proper treatment, there is 70-80 chances in a 100 of curing or hindering the progression of the disease. (Frank, 2015)

Diagnosis of drug resistant TB remains a challenge in developing countries due to the absence of affordable, reliable and quick techniques to detect drug susceptibility. Having proper DST facilities is a top priority for most developing countries since it allows definite diagnosis of drug resistant Tb. Early detection and adequate treatment of drug resistant Tb could significantly reduce morbidity and mortality rates, leading to a favorable outcome. With recent advances in TB control many different phenotypic and genotypic methods have been explored and evaluated to detect drug resistance in both developing and industrialized countries.(Migliori et al., 2008).

Phenotypic Methods

Conventional culture-based method – LJ Medium

Traditional culture-based methods, such as Lowenstein-Jensen medium, are regarded as the gold standard for detection of drug-resistant tuberculosis. LJ medium is a solid selective medium used for the cultivation and isolation of Mycobacterial species, specifically Mycobacterium tuberculosis. The green color of the media is due to the presence of malachite green, which is responsible for preventing the growth of any contaminants that may have survived decontamination at the same time favoring the growth of mycobacterial species. Glycerol acts as

the carbon source in the medium encouraging the growth of human type bacilli. For growth of M.bovis species sodium pyruvate is added instead of glycerol. M.tuberculosis appear as brown granular colonies in LJ medium. (Hi Media, 2019). LJ media can also be used for DST testing using antibiotics to distinguish between drug susceptible and resistance strains. Despite being comparatively inexpensive the process is time-consuming which makes it a challenge for use in middle and low- income country. Owing to the slow growth of MTB it takes anywhere from 2–3 weeks to several months to yield colonies of M. tuberculosis. Due to the long turnaround time LJ medium is not a preferred choice of culture method in resource limited countries since inappropriate treatment can lead to death in just weeks (especially in case of XDR with HIV-coinfection cases). Delay in diagnosis paired with inappropriate treatment can further amplify resistance and promote further spread of drug resistant strains in communities.(Migliori et al., 2008)

TK Medium

TK medium is a colorimetric method where the presence of mycobacteria can be detected by a change in color from red to yellow. The color change occurs due to the metabolic activity of mycobacteria (caused by changes in pH) which can be detected even before growth of mycobacterial colonies. A big advantage of TK medium is that contamination such as other Gram-negative bacteria or fungi causes the media to change its color from red to green instead of yellow. Tk medium is a biphasic medium since it displays the growth of mycobacterial colonies in the solid phase whereas the liquid phase is used to visualize features such as the cord factor. As for nutritional requirements for mycobacteria it contains egg, iron, glutamic acid etc. TK medium can also be used for drug susceptibility testing and even allows differentiation between mycobacterial and non-mycobacterial species. (Kocagöz et al., 2012). Compared to LJ medium TK medium provides results much faster (average time for detection is around 2 weeks). Unlike other media which needs to be rehydrated and assembled requiring time and effort, TK medias are ready to use which significantly reduces the chances of contamination caused by handling media. Specifically, TK selective media contains antimicrobials that further reduces contamination rate. Studies done in turkey revealed that sensitivity of TK medium was similar to that of LJ medium.(Pai et al., 2006)

Microscopic-Observation Drug-Susceptibility (MODS)

MODS is a high-performance assay that allows both early detection of MTB (uses a liquid culture where growth of MTB is faster than solid media) and DST testing simultaneously, eliminating the need to perform drug susceptibility tests separately thereby reducing occupational risk. The process utilizes an inverted light microscope to detect early growth of Mycobacterium tuberculosis as strings and tangles. (Brady et al., 2008). MODS consists of a total of 24 plates where 4 plates are reserved for a single patient. Two of these plates are drug free whereas the other two consists of isoniazid and rifampicin where sputum samples are directly inoculated for detection of MDR-TB. (L. Wang et al., 2015)Detection in MODS is much quicker (average 8 days) compared to conventional LJ medium. Previous concerns regarding the ability to microscopically distinguish between mycobacterium Tb and other non-tuberculosis mycobacterium. were met with the introduction p-nitrobenzoic acid (PNB) in microtiter wells. MTB does not grow in the presence of PNB. Hence visualization of morphological characteristics pertaining to MTB in non-PNB wells suggests the presence of MTB growth. According to WHO MODS showed high sensitivity (pooled estimate 98%; 95CI 95% - 99%) and specificity for rifampicin and isoniazid resistance were (pooled estimate 99%; 95CI 96% - 100%) and (pooled sensitivity 91%; 95CI 87% - 95%) respectively. MODS has been recommended by WHO as an affordable, rapid and highly sensitive assay compared to gold standard liquid culture techniques used for MTB detection. (World Health Organisation, 2010). Regardless due to safety concerns, MODS are still not widely used in developing countries. Handling liquid cultures carries a significant occupational risk of aerosolization and spillage. This technique also requires trained technicians to take manual reading which can be particularly challenging in high TB burden countries where huge number of samples need to be tested.

The auto-MODS technique was developed which follows similar principles to MODS but with some key adjustments to address the previous concerns. Modifications included the use of screw caps instead of wells (to reduce risk of exposure), use of PNB wells for differentiation of MTB and non-Tb mycobacterium, use of computer assisted digital camera to take reading and use of

low-speed centrifuge to remove any large particles in the sample. Studies were done in Thailand to evaluate the auto-MODS assay which found it to be a highly sensitive, specific and cost-effective, recommending it for use in limited resource settings.(L. Wang et al., 2015).

Mycobacteria Growth Indicator Tube (MGIT)

Mycobacteria Growth Indicator Tube (MGIT) is a non-radiometric method that uses liquid culture to detect the presence of mycobacteria. Most pulmonary and extra-pulmonary clinical sample (except blood and urine) can be incorporated into the system to detect mycobacteria growth. The tubes contain a fluorescent compound present at the bottom which is sensitive to the oxygen dissolved in the broth. Actively growing and respiring microorganisms uses up oxygen and increases the fluorescence. The fluorescence can be read manually using a longwave UV light (Wood's lamp) or it can be completely automated (Growth & Tube, 2012). MGIT uses modified Middlebrook 7H9 broth base which improves recovery and enhances growth of mycobacteria. In automated systems, the instrument scans the MGIT every 60 minutes for increased fluorescence. A positive tube contains approximately 105 to 106 colony-forming units per milliliter (CFU/mL). Absence for any sort of growth for 42 days (up to 56 days) is considered negative. Growth can also be detected visually by the presence of turbidity and small grains or flakes in the culture media. For MGIT the average turnaround time for smear positive samples are typically 17 days. At the same time, it consists of OADC (Oleic acid, Albumin, Dextrose and Catalase) enrichment. Oleic acid is an important constituent of mycobacterial metabolism whereas albumin binds free fatty acids that could be toxic to mycobacterium species. Dextrose is used by the bacteria as an energy supply and catalase destroys any toxic peroxides that may be present. Chances of contamination in liquid culture is higher than solid media hence PANTA (Polymyxin B, Amphotericin B, Nalidixic Acid, Trimethoprim, Azlocillin) antimicrobial mixture are added to reduce the contamination rate. All these supplements are essential for growth of many mycobacteria, especially those belonging to M. tuberculosis complex.(Salman & Rüsch-Gerdes, 2006) (Foundation for Innovative New Diagnostics)

Most commonly BD BACTEC MGIT 960 and BD BACTEC MGIT 320 systems are used. They are both fully automated mycobacterial systems where both detection and susceptibility testing

can be done. They both use the same technology, the only difference being BD BACTEC MGIT 320 (holds 320 MGIT tubes) is designed for smaller-capacity laboratories with limited space and workload whereas BD BACTEC MGIT 960 (holds 960 MGIT tubes) is designed to meet the needs of medium- and high-volume laboratories (Duque et al., 2013).Typically, in MGIT drug susceptibility testing uses Streptomycin (STR), isoniazid (INH), rifampin (RIF), ethambutol (EMB) (collectively known as SIRE), and pyrazinamide (PZA) antibiotics incorporated into tubes in the system. Growth in the presence of antibiotics displays resistance. The principle behind drug susceptible testing is similar to the previous one. Same sample is inoculated into two tubes with one tube without antibiotic (control tube) and another tube with a known concentration of the antibiotic. If growth appears in the presence of antibiotic fluorescence will increase and if the antibiotic inhibits the growth of mycobacteria, then fluorescence will be suppressed. The results will be automatically interpreted by the system.(Salman & Rüsch-Gerdes, 2006) (Foundation for Innovative New Diagnostics)

From April 2010 to February 2011, two laboratories, the Microbial Diseases Laboratory (MDL) of the California Department of Public Health (CDPH), USA and the National Tuberculosis Reference Laboratory (NTRL) of Bangkok, Thailand conducted a study to evaluate the growth and detection of mycobacteria and drug susceptibility testing of Mycobacterium tuberculosis using BD Bactec MGIT 320 where BD Bactec MGIT 960 was used as a reference. For detection of mycobacteria, 359 processed sputum samples were tested, and the result showed 99.7% agreement between the MGIT 320 and MGIT 960. As for drug susceptibility SIRE and pyrazinamide (PZA) antibiotics were used on 89 clinical strains, prepared from both liquid and solid inocula. The experiment yielded positive results which showed 100% reproducibility between the two instruments tested at both laboratories. (Duque et al., 2013)

When compared with two standard methods, proportion and resistance ratio methods, the MGIT system presented an overall agreement of 96% proclaiming it as a rapid, sensitive and efficient method for early detection of multidrug-resistant M. tuberculosis.(Telles et al., 2002)

Molecular methods for TB diagnosis

In the past two decades, better understanding of drug resistance at the molecular level led to the development of rapid and novel molecular techniques which aimed to identify specific mutations responsible for drug resistance. Initially these techniques were developed to swiftly identify drug resistance, but later were upgraded for detection of MTB isolates from clinical samples. Molecular techniques hold several advantages over conventional DST testing. They have significantly faster turnaround times (days rather than weeks), can directly be tested from clinical samples without the need to isolate and grow the bacteria, and even on non-viable bacteria (eg that have been killed or inactivated by chemicals or heat). Hence these novel rapid molecular techniques for detection of MTB isolates and drug resistance patterns have become a priority for TB research and development. (Elisa Tagiliani, 2018)(Migliori et al., 2008)(Elisa Tagiliani, 2018)

DNA Line Probe Assays

Line probe assays also known as Genotype MTBDRplus, is a molecular assay that relies on reverse hybridization and polymerase chain reaction (PCR) to identify and determine drug resistance profile. At first the DNA is extracted (this can be done from MTB or directly from clinical specimens). Next through PCR, using biotinylated probes, amplification of specific resistance-determining region of the gene takes place. After that, these PCR amplicons are hybridized onto specific oligonucleotides probes immobilized onto strips. After washing the plates to remove any non-specific binding, the captured probe hybrids can be visualized as colored lines on the strips. The total turnaround time for this method is around 5-7 hours.(Nguyen et al., 2019) To detect MDR resistance, probes are used to detect mutations in rpoB gene for rifampicin resistance, katG gene for high-level isonizaid resistance, and inhA gene (promoter region) for low-level isoniazid resistance. Commercially available kits recommended by WHO includes GenoType MTBDRplus VER 1 and 2 (Hain Lifescience, Germany) and Nipro NTM+MDRTB detection kit 2 (Nipro, Japan). The Hain version 1 and 2 assays include probes to identify Mycobacterium. tuberculosis complex (MTBC), and detect mutations in the rpoB gene, katG gene and in the inhA promotor region. On the other hand Nipro assay allows differentiation between four important Mycobacterium species (M. avium, M. intracellulare and M. kansasii from MTBC and from other

non-tuberculous mycobacteria). It also allows detection of MTBC resistance to rifampicin and isoniazid. Mutations are detected by the binding of amplicons to probes targeting the most commonly occurring mutations (MUT probes) or indicated by the lack of hybridization or binding of the amplicons to the corresponding WT probes.(WHO, 2008)

It has been advised that DNA line probe assays should not be carried out on smear negative samples. Since before DNA extraction, digestion, decontamination and concentration of clinical specimens need to be carried out, performing this assay holds a risk for contamination and infection. The processing of specimens for line probe assays should be performed in a laboratory with adequate and appropriate biosafety level precautions. Appropriate laboratory staff also need to be trained to conduct LPA procedures.(WHO, 2008)

A systemic meta-analysis study was done to assess 3 DNA line probe assays- Hain Genotype MTBDRplusV1, MTBDRplusV2 and Nipro NTM+MDRTB. After much deliberation, 74 studies were included. Reportedly, out of 21225 samples included, for rifampicin resistance pooled sensitivity and specificity (with 95% confidence intervals) were 96.7% (95.6–97.5%) and 98.8% (98.2–99.2%) respectively. Out of 20954 samples included, for isoniazid resistance, pooled sensitivity and specificity were 90.2% (88.2–91.9%) and 99.2% (98.7–99.5%) respectively. For M. tuberculosis detection (3451 samples), pooled sensitivity was 94% (89.4–99.4%) for smear-positive specimens and 44% (20.2–71.7%) for smear-negative specimens.(Nathavitharana et al., 2017)

Gene Xpert MTB/RIF

In 2008, WHO started to recommend rapid nucleic acid amplification tests such as LPA to effectively detect and treat drug resistant TB. (WHO, 2008) However, the problem with such techniques were that they were expensive, technically challenging, required trained personnel to operate, and were not recommended for sputum negative samples due to their low sensitivity and cross contamination risk. Around 2010 WHO endorsed Xpert MTB/RIF test using sputum samples for adults to diagnose pulmonary Tb. The test simultaneously detects Mycobacterium tuberculosis complex (MTC) and resistance to rifampin (RIF) in less than 2 hours using RT-PCR and molecular beacon technology.(World Health Organization (WHO), 2011) This test was able to overcome some of the limitations of LPA. Xpert MTB/RIF is a fully automated system requiring a single

disposable cartridge that reduces biohazard risk and contamination. It is relatively simple test to perform and can be done using clinical specimens, regardless of their smear status. Around 2013 WHO recommended the use of Xpert MTB/RIF test for children and certain extrapulmonary Tb.(Lawn & Nicol, 2012)

Xpert MTB/RIF assay uses Real Time-PCR to amplify 81 bp region of the bacterial RNA polymerase rpoB gene since 96% of the time rifampicin resistance strain consists of mutations within this region, whereas drug susceptible strain is usually wild type in this segment. Rifampicin resistance is indicative of MDR-Tb since resistance to rifampicin is naturally accompanied by resistance to isoniazid (unlike rifampicin, sole resistance to isoniazid is caused by mutations in a number of genes hence resistance to isoniazid is not considered a good indicator for MDR-Tb). Moreover, rpoB core region is flanked regions that are representative to MTB making it possible to simultaneously detect rifampicin resistance along with MTB using a simple amplicon.(Lawn & Nicol, 2012).(Lawn & Nicol, 2012)

Molecular beacon technology relies on its ability to bind to target sequences that are perfectly complementary to the probe. Five probes are used in this assay that bind to different target regions, overlapping one another, within this 81bp region of rpoB gene. Each probe is labelled with a different fluorophore. Molecular beacons are single stranded nucleic acid molecules that form a stem- loop structure. The two arms are designed to be complementary to one another so that they form a loop and the probe sequence is present within this loop. Regarding the two ends of the arms, one contains a fluorophore molecule and the other a quencher molecule that suppresses the fluorescence of the fluorophore when they are in close proximity. Whenever the beacon comes across its target sequence, the probe is strongly attracted to the target sequence forcing the arms to separate. This in turn causes the quencher molecule to separate from the fluorophore causing it to emit fluorescence. The probes are designed to hybridize to rifampicin susceptible or wild-type rpoB sequences. Hence any mutation in this region will cause partial inhibition or complete suppression of fluorescence (Piatek et al., 1998)

Xpert MTB/RIF assay uses a single cartridge to perform the entire experiment thereby reducing cross contamination. Before being loaded onto the cartridge the sample is treated with sodium hydroxide and isopropanol-containing sample reagent for about 15 minutes. This is done to reduce the viability of the existing MTB bacteria to minimize biohazard risk. The sample is then loaded

into specific compartment inside the cartridge. The MTB bacilli are trapped inside a filter and the rest of the sample is kept in a waste chamber. Through sonication the MTB bacilli are broken apart and the genetic material inside released. The liberated genetic material is then transferred to the reaction chamber which contains reaction beads. The module is heated and cooled in cycles and it is exposed to illumination by LED.(Lawn & Nicol, 2012) For smear positive samples sensitivity and specificity were reported to be 100 and 99%, respectively whereas for smear negative samples sensitivity and specificity reached upto 67 and 99%, respectively. However, single use cartridge makes the assay expensive, also rifampicin resistance cannot confirm MDR-Tb so usually other DST tests need to paired up with this technique for confirmation. The machine also happens to be sensitive to dust and heat and requires constant electrical supply.(Nguyen et al., 2019)

Chapter 5

DISCUSSION

Even in the 21st century Tb remain lethal as ever, ranking above AIDS/HIV as the principal cause of death from a single infectious agent. Furthermore, it is in the top 10 list for cause of death worldwide. Globally approximately 10 million people were infected with Tb in 2019, out of which 5.6 million were men, 3.2 million were women and 1.2 million children. The 30 high Tb burden countries represent 87% of Tb cases whereas the SEA region accounts for 44% of this burden. Although the global incidence of Tb is gradually declining, there had been a 10% increase (from 2018) in drug resistance TB, in particular (MDR/RR-TB) in 2019. An estimated 206 030 people with MDR/RR-TB were detected in 2019.

Evaluation of different phenotypic techniques

MGIT VS LJ

According to WHO Ethiopia remains one of the 30 high burden Tb and MDR-burden countries globally. In 2019 approximately 140 per 100,000 population were affected with Tb and the mortality rate was about 21 per 100,000 population. MGIT is limited in use in high burden resource limited countries. Ethiopia, where MGIT is well established method, did a cross-sectional study to evaluate its effectiveness against conventional LJ medium. The study was conducted from 2013 to 2014 using 908 clinical sputum samples which were processed using standard protocols and then inoculated into MGIT tubes and LJ slants. For confirmation ZN staining and SD Bioline test (Specific detection immune chromatographic test which can discriminate MTBC and non-Mycobacterium tuberculosis complex) was used. According to the results MGIT showed a better recovery rate compared to LJ medium for both sputum positive and negative samples. For smear positive samples the recovery rate for LJ and MGIT were 66.7% (74/111) and 87.4% (97/ 111) respectively. The overall recovery rates were 26% (236/908) and 20% (182/908), for MGIT and LJ respectively. The average turnaround time was also much faster for MGIT compared to LJ. For smear positive samples the turnaround times were 16 and 31 days for MGIT and LJ respectively. Total time for smear negative results to come back was 20 days for MGIT and 36 days for LJ. However, the contamination rates were higher for MGIT (15%) compared to LJ (9.3%). All in all, MGIT automated liquid culture system had a much faster turnaround time and better recovery rates for MTB compared to conventional solid LJ media method.(Diriba et al., 2017)

A similar study was done in Nigeria which compared BACTEC-MGIT-960 and LJ method for its ability to isolate MTB and drug susceptibility testing. 527 samples were cultured in the study,81% (428 samples) were culture positive with BACTEC-MGIT-960 whereas 78% (411 samples) were culture positive with LJ media. The contamination rate was higher for MGIT 960 (7%) than LJ (4%). The average detection time for MGIT and LJ were 11 (6) and 30 (11) days respectively. As for drug susceptibility testing results for Rifampicin and isoniazid were similar for both methods. Ethambutol resistance was detected more commonly in MGIT compared to LJ whereas streptomycin resistance was more often in LJ compared to MGIT. However, these differences were not statistically relevant. LJ method successfully managed to detected 27 MDR-TB cases while MGIT detected 25 .(Lawson et al., 2013)

A study in Pakistan showed higher recovery rates. 260 different clinical specimens were cultured, out of which recovery rate of M. tuberculosis complex was 97.6% on BACTEC MGIT 960 system and 83.7% on LJ medium. Similar to other studies the contamination rates were much higher for BACTEC MGIT 960 (9.6%) than it was for LJ method (3.4%). (Satti, L. et al., 2010)

MGIT VS MODS

China is recognized as a “hot-spot” for Tb ranking as one of the top MDR-Tb burden countries in the world. According to WHO in 2019, 833,000 people were affected by Tb in China. A study was performed including 5 different laboratories in China, to assess the performance of MGIT against MODS assay. 532 patient samples were tested out of which MODS assay detected 200 (37.6%) as positive culture (MTB) where as MGIT, which was used as a reference method, was able to detect 213 (40.0%) as positive cultures. For MODS assay the overall sensitivity for M. tuberculosis detection was 87.8%–94.3% and specificity was 96.8%–100%. MODS assay took a shorter time to detect positive cultures compared to MGIT (8 days for MODS versus 11 days for MGIT, P,0.001). Overall MODS proved to be more sensitive and rapid technique compared to MGIT for drug-resistant TB detection and showed tremendous potential for use in high burden resource limited settings.(Huang et al., 2015)

Another observational cohort study was done from January 2010 to October 2015 to assess MODS technique to detect MTB and its susceptibility to isoniazid and rifampicin to identify MDR-TB. In

the study MODS was compared with a validated method Mycobacteria Growth Indicator Tube/Antimicrobial Susceptibility Testing/Streptomycin, Isoniazid, Rifampicin and Ethambutol (MGIT/AST/SIRE. A total of 98 patients were selected as per the criteria for the study. Participants were above 12 years with suspected TB and naive to anti-TB drugs or had failed treatment. According to the results MODS had a sensitivity and specificity of around 94.12% and 85.71% respectively for detecting TB. The average time for culture positivity were 8 days (IQR 5-11) for MODS and 6 days (IQR 5-6) for MGIT. The sensitivity and specificity of MODS assay were respectively 100% and 95.92% in detecting MDR-TB patients. The positive predictive value was 66.7% whereas negative predictive value was 100%. For MODS assay 8 days were required for drug susceptibility test results to come back whereas 35 days were required or MGIT/AST/SIRE method.(Sanogo et al., 2017) MODS assay can be used in any biosafety level 2 laboratory with an inverted optical microscope, a centrifuge and an incubator (Caviedes et al., 2000). Hence this affordable technique with good sensitivity and specificity is feasible for use in resource limited areas.(Sanogo et al., 2017)

Detection and drug susceptibility test using automated rapid techniques such as MGIT and MODS in adults have been well researched and explored unlike cases in children. Diagnosis of Tb, in children is particularly challenging due to paucibacillary samples, cultures and smears often appearing negative, inadequate amount of samples for testing (due to difficulty in extracting samples from children). Hence in Vietnam, MODS and MGIT were evaluated in the diagnosis of Tb in children. 96 children (under the age of 16) participated in the study, and from them 217 samples were taken (sputum (n = 132), gastric fluid (n = 50), CSF (n = 32) and pleural fluid (n = 3)). The average time for detection was 8 days for MODS and 13 days for MGIT. Since the samples are paucibacillary for children, it is important to determine which types of samples shows the highest sensitivity. According to this study, sputum and gastric fluid samples gave the best results for pediatric patients. Sensitivity for MODS and MGIT was comparable when it came to per patient sample. However, when it came to per sample analysis, MODS showed greater sensitivity than smear (P,0.001) but less than MGIT (P = 0.027). Overall, MODS was able to diagnose 88% of TB cases. MGIT was able to detect slightly more TB patients compared to MODS (33/35 patients for MGIT vs 31/33 patients for MODS).(Ha et al., 2009)

MODS VS LJ

Similar findings were seen in another study in Peru involving pediatric patients (under the age of 12). The study was conducted to assess the speed and sensitivity of MODS against conventional LJ methods in children. A total of 165 patients were evaluated, two specimens of each type (gastric aspirate, nasopharyngeal aspirate, and stool specimens) were collected from each patient and consecutively examined by auramine stain, and cultured by Microscopic Observation Drug Susceptibility and Lowenstein Jensen technique. MODS was able to detect 87% of pediatric Tb cases (33/38 cases) whereas LJ was able to detect 55% (21/38). This significant difference was due to the fact that MODS was able to recover MTB from most auramine-negative specimens unlike LJ (19 of 23 by MODS vs 9 of 23 by LJ). This is particularly important since most pediatric specimens are paucibacillary and may appear auramine negative. MODS also outcompeted LJ when it came to time for bacterial isolation. MODS proved be much faster compared to LJ.(Oberhelman et al., 2006)

Another study was done in Peru, to evaluate MODS against conventional LJ technique (used as reference standard) in terms of both detection of MTB and drug susceptibility testing to determine drug resistance. 10.7% (401) samples gave positive results out of 3760 sputum samples tested. MODS proved to be superior to LJ method when it came to sensitivity for detection and time for culture positivity. Sensitivity for MODS was reported to be 97.8% whereas for LJ culture it was 84% ($P<0.001$). The overall specificity of detection was 99.6% for MODS culture and 100.0% for Löwenstein–Jensen culture. Time for culture to become positive for MODS and LJ were 7 days and 26 days respectively ($P<0.001$). As for detection of drug resistance the following antibiotics were used - isoniazid, 0.1 and 0.4 μg per milliliter; rifampin, 1 and 2 μg per milliliter; ethambutol, 2.5 and 5.0 μg per milliliter; and streptomycin, 2 and 6 μg per milliliter. For isolated from Löwenstein–Jensen culture, indirect drug-susceptibility testing was performed with the use of the proportion method21 (by an external laboratory). For MDR detection a strong agreement was observed between MODS and the reference standard for susceptibility ,100% for rifampin, 97% for isoniazid, 99% for rifampin and isoniazid. For other drugs the following agreement was found ,95% for ethambutol, and 92% for streptomycin. The average time from sample processing to receiving drug susceptibility test results was also significantly less for MODS (7 days) compared to LJ (68 days). Overall, MODS showed great potential for use in resource limited settings for

both MTB detection and detection of multi- drug resistant TB compared to LJ technique.(Moore et al., 2007)

TK VS MGIT

A total of 146 clinical sputum samples were tested in an experiment in Turkey to evaluate TK SLC-L system (that uses a liquid medium) and MGIT system. The samples were examined by ZN staining prior to inoculation and was decontaminated using the same procedures. Growth detected by the systems were further re-confirmed using smear and microcopy. Culture positivity of both systems were very close to one other (TK SLC-L 24.0% and MGIT 23.3%). Average time to growth detection was significantly less for MGIT (13.1 days) compared to TK SLC-L (18.3 days). The sensitivity and specificity for TK SLC-L and MGIT were 76.1% ,100% and 73.9% ,100% respectively. However, the contamination rates were much less for TK SLC-L (1.3%) than MGIT (13.7%). This could be due to the fact that TK SLC-L systems are always ready to use whereas MGIT systems require handling (addition of OADC nutrients and selective antimicrobials before inoculation) thereby increasing the chances of contamination. Further practical use of such systems in resource limited areas could provide insight as to which system may be better for use in high TB burden countries.(Feyzioglu et al., 2014)

MGIT VS TK VS LJ

A study was conducted between May and August 2012 to compare 3 different methods- TK Rapid Mycobacterial Culture System (which uses a liquid broth), BACTEC MGIT 960 method and conventional solid LJ medium. 192 patients were included in the study, and 200 clinical specimens were collected from them (152 sputum, 41 Bronchoalveolar lavage fluid (BAL), 4 gastric aspirations, 2 urine and 1 wound). Results revealed the contamination rates were as follows- TK 1.5%, LJ 6.5%, and BACTEC MGIT 960 systems 9%. This was interesting since all samples were decontaminated using the same protocols. Tk system doesn't contain any selective antimicrobials,

hence this suggests that perhaps contamination was by the different tubes and caps or the selectivity of the content of the medium. Commination in TK is very easy to detect due to the color change from red to green. Contamination rates were highest in MGIT, this could be due to the preparatory work required before the inoculation of samples. Growth rate for MTB were similar for the three methods 7.5%, 7% and 6.5% by TK culture system, MGIT and LJ, respectively. The average detection times for LJ, TK, and MGIT method were 20.1, 17.1, and 8.3 days respectively. TK system showed a significantly lower contamination rate compared to MGIT system but the turnaround time for TK is disadvantageous. TK could be promising in that regard since accurate and reliable diagnosis of TB is vital. MGIT showing higher contamination rates took the least amount of time to deliver results. MGIT could be very useful in situations where rapid results are needed to detect then later prescribe anti-TB drugs. Both MGIT and TK has its pros and cons is promising in the diagnosis of tuberculosis in the future.(Çiftci & Karakeçe, 2014)

Table 2- Comparison among the different phenotypic methods in diagnosing drug resistant Tb

Phenotypic Methods	Evaluation of Phenotypic Methods	References
MGIT VS LJ	**MGIT automated liquid culture system had a much faster turnaround time and better recovery rates for MTB compared to conventional solid LJ media method** A study in Ethiopia showed overall recovery rates were 26% and 20% for MGIT and LJ respectively. However, the contamination rates were higher for MGIT (15%) than LJ (9.3%).	(Diriba et al., 2017)
	MGIT provided results much faster but showed higher contamination rates compared to LJ. 81% (428 samples) were culture positive with BACTEC-MGIT-960 whereas 78% (411 samples) were culture positive with LJ media in a study in Nigeria. The contamination rate was higher for MGIT 960 (7%) than LJ (4%). The average detection time for MGIT and LJ were 11 (6) and 30 (11) days respectively. As for drug susceptibility testing results for Rifampicin and isoniazid were similar for both methods. Ethambutol resistance was detected more commonly in MGIT compared to LJ whereas streptomycin resistance was more often in LJ compared to MGIT. However, these differences were not statistically relevant. LJ method successfully managed to detected 27 MDR-TB cases while MGIT detected 25.	(Lawson et al., 2013)
	A study in Pakistan produced similar results as above. recovery rate of M. tuberculosis complex was 97.6% on BACTEC MGIT 960 system and 83.7% on LJ medium. Similar to other studies the contamination rates were much higher for BACTEC MGIT 960 (9.6%) than it was for LJ method (3.4%).	(Satti, L. et al., 2010)
	A study in China, showed MODS to be more sensitive and rapid technique compared to MGIT for drug-resistant TB detection. For MODS assay the overall sensitivity for M.	

MGIT VS MODS	tuberculosis detection was 87.8%–94.3% and specificity was 96.8%–100%. MODS assay took a shorter time to detect positive cultures compared to MGIT (8 days for MODS versus 11 days for MGIT, P,0.001).	(Huang et al., 2015)
	According to the results done in an observational cohort study, MODS had a sensitivity and specificity of around 94.12% and 85.71% respectively for detecting TB. The average time for culture positivity were 8 days (IQR 5-11) for MODS and 6 days (IQR 5-6) for MGIT. For MODS assay 8 days were required for drug susceptibility test results to come back whereas 35 days were required or MGIT method.	(Sanogo et al., 2017)
	When it came to diagnosing Tb in children in Vietnam, MODS and MGIT both showed potential. The average time for detection was 8 days for MODS and 13 days for MGIT. Sensitivity for MODS and MGIT was comparable when it came to per patient sample. However, when it came to per sample analysis, MODS showed lower sensitivity (P,0.001) than MGIT (P = 0.027). MGIT was able to detect slightly more TB patients compared to MODS (33/35 patients for MGIT vs 31/33 patients for MODS).	(Ha et al., 2009)
MODS VS LJ	**In a study done in Peru MODS also outcompeted LJ when it came to time for bacterial isolation. MODS proved be much faster compared to LJ.** MODS was able to detect 87% of pediatric Tb cases whereas LJ was able to detect 55%. This significant difference was due to the fact that MODS was able to recover MTB from most auramine-negative specimens unlike LJ (19 of 23 by MODS vs 9 of 23 by LJ). This is particularly important since most pediatric specimens are paucibacillary and may appear auramine negative.	(Oberhelman et al., 2006)
	In a study done in Peru MODS proved to be superior than LJ in terms of detection. Sensitivity for MODS was reported to be 97.8% whereas for LJ culture it was 84%. The overall specificity of detection was 99.6% for MODS culture and	(Moore et al., 2007)

	100.0% for LJ culture. Time for culture to become positive for MODS and LJ were 7 days and 26 days respectively. **As for MDR detection a strong agreement was observed between MODS and LJ for susceptibility**. The agreements were as follows-100% for rifampin, 97% for isoniazid, 99% for rifampin and isoniazid. For other drugs the following agreement was found ,95% for ethambutol, and 92% for streptomycin. The average time from sample processing to receiving drug susceptibility test results was also significantly less for MODS (7 days) compared to LJ (68 days). **Overall, MODS showed great potential for use in resource limited settings for both MTB detection and detection of multi-drug resistant TB compared to LJ technique.**	
TK VS MGIT	**A study done in Turkey showed MGIT to have a much faster turnaround when compared to TK, whereas TK medium showed significantly lower contamination rates**. Culture positivity of both systems were very close to one other (TK SLC-L 24.0% and MGIT 23.3%). Average time to growth detection was significantly less for MGIT (13.1 days) compared to TK SLC-L (18.3 days). However, the contamination rates were much less for TK SLC-L (1.3%) than MGIT (13.7%).	(Feyzioglu et al., 2014)
MGIT VS TK VS LJ	**A study done in 2012 proved both MGIT and TK has its pros and cons and showed promise in the diagnosis of tuberculosis.** The contamination rates were as follows- TK 1.5%, LJ 6.5%, and BACTEC MGIT 960 systems 9%. Growth rate for MTB were similar for the three methods 7.5%, 7% and 6.5% by TK culture system, MGIT and LJ, respectively. The average detection times for LJ, TK, and MGIT method were 20.1, 17.1, and 8.3 days respectively. TK system showed a significantly lower contamination rate compared to MGIT system but the turnaround time for TK is disadvantageous.	(Çiftci & Karakeçe, 2014)

Evaluation of Molecular Methods

Gene Xpert MTB/RIF VS DNA Line Probe Assays

A study consisting of 300 patients from May 2012 to April 2013 was done in Bangladesh to assess molecular techniques gene Xpert MTB/RIF against DNA Line Probe Assays for detection of MDT-Tb. Conventional DST testing using LJ medium was also done as reference alongside the molecular techniques. Out of 300 sputum samples tested, 277 isolates were detected to be Mycobacterium tuberculosis. LPA was successfully able to detect 191 (63.7%) and GeneXpert method detected 193 (64.3%) of rifampicin resistant cases whereas 189 (63%) cases of rifampicin resistance were detected by conventional DST methods. Conversely, when it came to detecting isoniazid resistant cases LPA was able to detect 196 (65.3%) and DST method detected 191 (63.7%). When it came to detecting MDR-Tb cases LPA and DST methods were able to detect 189 (95.6%) and 187 (96.9%) respectively. Due to their high sensitivity and specificity the study was in favor of using gene Xpert MTB/RIF and DNA Line Probe Assays for detection of MDT-Tb.(Aurin et al., 2014)

In 2013 a similar study was done in South India to evaluate DNA line probe assays against Xpert MTB/Rif assay and culture-based method. 91 suspected MDT-Tb patients were included in the study. Line probe assays showed sensitivity and specificity of 81.5% (95%CI 67.4–91.1%) and 87.5% (95%CI 71–96.5%) for the detection of tuberculosis against culture-based method. For rifampicin resistance, line probe assay showed sensitivity and specificity of 100% (95%CI 85.2–100%) and 93.8% (95%CI 69.8–99.8%), respectively, compared to culture-based method., whereas for isoniazid resistance it was, sensitivity- 89.3% (95%CI 71.8–97.7%) and specificity- 100% (95%CI 71.5–100%), respectively. Against gene Xpert MTB/Rif assay, the LPA showed a sensitivity of 80% (95%CI 68.2–88.9%) and specificity of 100% (95%CI 85.8–100%) for the detection of tuberculosis and a sensitivity of 94.3% (95%CI 80.8–99.3%) and specificity of 94.1% (95%CI 71.3–99.9%) for rifampicin resistance. According to the study LPA showed good detection for smear positive samples unlike smear negative samples. Therefore, the study recommended the use of LPA to detect MDR-Tb and isoniazid resistance in particular since thar cannot be detected by gene Xpert MTB/Rif assay.(Ninan et al., 2016).

Chapter 6

CONCLUSION

Well into the 21st century, Tb remains a global threat. The SEA region alone accounts for a huge burden of Tb. According to reports MDR-Tb cases, in high burden Tb cases within the SEA region, are rising at an alarming rate. Some of the main causes behind drug resistant Tb has been identified as: the use of short course treatment strategies, inadequate treatment of patients, poor adherence to treatment plans and through community and facility-based transmission. In fact, it can be reasoned that drug resistant Tb is a man-made problem. Modern technology has allowed the discovery and implementation of various new phenotypic and molecular methods to diagnose Tb. Both methods have their unique advantages and limitations. While phenotypic methods are considered the gold standard in Tb detection some of them tend to be very time consuming and end up delaying treatment. On the other hand, most molecular methods despite providing fast and reliable results, are considered too expensive for resource limited countries. Effective Tb- control regimens, including early detection and diagnosis, proper treatment protocols to treat and prevent transmission, are urgently required and should become a priority in Tb burden countries. For countries to adopt such Tb control guidelines strong commitment and collaborations between healthcare facilities and government are needed. Only together, we can hope to win this battle against Tb and completely eradicate this deadly disease.

Chapter 7

REFERENCES

Ahmad, S. (2011). Pathogenesis, immunology, and diagnosis of latent mycobacterium tuberculosis infection. *Clinical and Developmental Immunology*, *2011*. https://doi.org/10.1155/2011/814943

Amaral, A. F. S., Coton, S., Kato, B., Tan, W. C., Studnicka, M., Janson, C., Gislason, T., Mannino, D., Bateman, E. D., Buist, S., Burney, P. G. J., Zhong, N., Liu, S., Lu, J., Ran, P., Wang, D., Zheng, J., Zhou, Y., Kocabaş, A., … Islam, M. (2015). Tuberculosis associates with both airflow obstruction and low lung function: BOLD results. *European Respiratory Journal*, *46*(4), 1104–1112. https://doi.org/10.1183/13993003.02325-2014

Arnold, A., Cooke, G. S., Kon, O. M., Dedicoat, M., Lipman, M., Loyse, A., Butcher, P. D., Ster, I. C., & Harrison, T. S. (2017). Drug resistant TB: UK multicentre study (DRUMS): Treatment, management and outcomes in London and West Midlands 2008–2014. *Journal of Infection*, *74*(3), 260–271. https://doi.org/10.1016/j.jinf.2016.12.005

Aurin, T. H., Munshi, S. K., Kamal, S. M. M., Rahman, M. M., Hossain, M. S., Marma, T., Rahman, F., & Noor, R. (2014). Molecular approaches for detection of the Multi-Drug Resistant Tuberculosis (MDR-TB) in Bangladesh. *PLoS ONE*, *9*(6). https://doi.org/10.1371/journal.pone.0099810

Basaraba, R. J., & Hunter, R. L. (2017). Pathology of Tuberculosis: How the Pathology of Human Tuberculosis Informs and Directs Animal Models. *Microbiology Spectrum*, *5*(3), 1–13. https://doi.org/10.1128/microbiolspec.tbtb2-0029-2016

Brady, M. F., Coronel, J., Gilman, R. H., & Moore, D. A. J. (2008). The MODS method for diagnosis of tuberculosis and multidrug resistant tuberculosis. *Journal of Visualized Experiments*, *18*, 2–5. https://doi.org/10.3791/845

Caviedes, L., Tien-Shun, L., Gilman, R. H., Sheen, P., Spellman, E., Lee, E. H., Berg, D. E., Montenegro-James, S., Fuentes, P., Valencia, T., Chumpitaz, R., Salas, I., Recavarren, S., Cama, R., Cabrera, L., Franchi, L. M., Ticona, E., Ticona, L., Chavez, V., … Evans, C. (2000). Rapid, efficient detection and drug susceptibility testing of Mycobacterium tuberculosis in sputum by microscopic observation of broth cultures. *Journal of Clinical Microbiology*, *38*(3), 1203–1208. https://doi.org/10.1128/jcm.38.3.1203-1208.2000

Chen, Y., Yuan, Z., Shen, X., Wu, J., Wu, Z., & Xu, B. (2016). Resistance to second-line antituberculosis drugs and delay in drug susceptibility testing among multidrug-resistant tuberculosis patients in Shanghai. *BioMed Research International*, *2016*. https://doi.org/10.1155/2016/2628913

Çiftci, I. H., & Karakeçe, E. (2014). Comparative evaluation of TK SLC-L, a rapid liquid

mycobacterial culture medium, with the MGIT system. *BMC Infectious Diseases*, *14*(1), 2–5. https://doi.org/10.1186/1471-2334-14-130

Diriba, G., Kebede, A., Yaregal, Z., Getahun, M., Tadesse, M., Meaza, A., Dagne, Z., Moga, S., Dilebo, J., Gudena, K., Hassen, M., & Desta, K. (2017). Performance of Mycobacterium Growth Indicator Tube BACTEC 960 with Lowenstein-Jensen method for diagnosis of Mycobacterium tuberculosis at Ethiopian National Tuberculosis Reference Laboratory, Addis Ababa, Ethiopia. *BMC Research Notes*, *10*(1), 1–6. https://doi.org/10.1186/s13104-017-2497-9

Druszczyńska, M., Kowalewicz-Kulbat, M., Fol, M., WŁodarczyk, M., & Rudnicka, W. (2012). Latent M. tuberculosis infection - Pathogenesis, diagnosis, treatment and prevention strategies. *Polish Journal of Microbiology*, *61*(1), 3–10. https://doi.org/10.33073/pjm-2012-001

Duque, A., Grace Lin, S. Y., Desmond, E., Rienthong, S., Rienthong, D., & Boonin, C. (2013). Evaluation of the BD bactec MGIT 320 system for detection of mycobacteria and drug susceptibility testing of mycobacterium tuberculosis. *Journal of Clinical Microbiology*, *51*(10), 3403–3405. https://doi.org/10.1128/JCM.01357-13

Ei, P. W., Aung, W. W., Nyunt, W. W., Swe, T. L., Htwe, M. M., Win, S. M., Aung, S. T., Chang, C. L., Lee, H. Y., & Lee, J. S. (2018). Extensively drug-resistant tuberculosis in Myanmar: Burden and mutations causing second-line drug resistance. *International Journal of Tuberculosis and Lung Disease*, *22*(1), 47–53. https://doi.org/10.5588/ijtld.17.0321

Elisa Tagiliani, D. C. (2018). *Line probe assays for drug- resistant tuberculosis detection*. 34.

Feyzioglu, B., Dogan, M., Sanli, O. O., Ozdemir, M., & Baykan, M. (2014). Comparison of the performance of TK system with LJ and MGIT methods in the diagnosis of tuberculosis. *International Journal of Clinical and Experimental Medicine*, *7*(4), 1084–1088.

Frank, R. (2015). *depends largely*. 3–6.

Gelmanova, I. Y., Keshavjee, S., Golubchikova, V. T., Berezina, V. I., Strelis, A. K., Yanova, G. V., Atwood, S., & Murray, M. (2007). Barriers to successful tuberculosis treatment in Tomsk, Russian Federation: Non-adherence, default and the acquisition of multidrug resistance. *Bulletin of the World Health Organization*, *85*(9), 703–711. https://doi.org/10.2471/BLT.06.038331

Growth, M., & Tube, I. (2012). *Bbl ™ mgit ™*. 1–12.

Gumusboga, A., Aung, K. J. M., Rigouts, L., & Van Deun, A. (2012). Transmission of multidrug-resistant and extensively drug-resistant tuberculosis in rural Bangladesh: lessons learnt. *Public Health Action*, *2*(3), 76–78. https://doi.org/10.5588/pha.12.0015

Ha, D. T. M., Lan, N. T. N., Wolbers, M., Duong, T. N., Quang, N. D., van Thinh, T. T., Ngoc, L. T. H., Anh, N. T. N., van Quyet, T., Tuyen, N. T. B., Ha, V. T., Day, J., Hoang, T. T. H., Kiet, V. S., Nho, N. T., Hoa, D. V., Dung, N. H., Lan, N. H., Farrar, J., & Caws, M. (2009). Microscopic observation drug susceptibility assay (MODS) for early diagnosis of

tuberculosis in children. *PLoS ONE*, *4*(12). https://doi.org/10.1371/journal.pone.0008341

Hi Media, L. (2019). *Lowenstein Jensen Medium Base HiMedia*. 10–12.

Huang, Z., Qin, C., Du, J., Luo, Q., Wang, Y., Zhang, W., Zhang, X., Xiong, G., Chen, J., Xu, X., Li, W., & Li, J. (2015). Evaluation of the microscopic observation drug susceptibility assay for the rapid detection of mdr-tb and xdr-tb in china: A prospective multicentre study. *Journal of Antimicrobial Chemotherapy*, *70*(2), 456–462. https://doi.org/10.1093/jac/dku384

Keshavjee, S., & Farmer, P. E. (2012). Tuberculosis, Drug Resistance, and the History of Modern Medicine. *New England Journal of Medicine*, *367*(10), 931–936. https://doi.org/10.1056/nejmra1205429

Kocagöz, T., Altin, S., Türkyilmaz, Ö., Taş, I., Karaduman, P., Bolaban, D., Yeşilyurt, E., Öktem, S., Aytekin, N., Şinik, G., Mozioğlu, E., & Silier, T. (2012). Efficiency of the TK Culture System in the diagnosis of tuberculosis. *Diagnostic Microbiology and Infectious Disease*, *72*(4), 350–357. https://doi.org/10.1016/j.diagmicrobio.2011.12.004

Lawn, S. D., & Nicol, M. P. (2012). Erratum: Xpert® MTB/RIF assay: Development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance (Future Microbiology (2011) 6:9 (1067-1082)). *Future Microbiology*, *7*(8), 1024. https://doi.org/10.2217/fmb.12.77

Lawson, L., Emenyonu, N., Abdurrahman, S. T., Lawson, J. O., Uzoewulu, G. N., Sogaolu, O. M., Ebisike, J. N., Parry, C. M., Yassin, M. A., & Cuevas, L. E. (2013). Comparison of Mycobacterium tuberculosis drug susceptibility using solid and liquid culture in Nigeria. *BMC Research Notes*, *6*(1), 1. https://doi.org/10.1186/1756-0500-6-215

Lee, E. G., Min, J., Kang, J. Y., Kim, S. K., Kim, J. W., Kim, Y. H., Yoon, H. K., Lee, S. H., Kim, H. W., & Kim, J. S. (2020). Age-stratified anti-tuberculosis drug resistance profiles in South Korea: A multicenter retrospective study. *BMC Infectious Diseases*, *20*(1), 1–10. https://doi.org/10.1186/s12879-020-05157-6

Li, B. Y., Shi, W. P., Zhou, C. M., Zhao, Q., Diwan, V. K., Zheng, X. Bin, Li, Y., Hoffner, S., & Xu, B. (2020). Rising challenge of multidrug-resistant tuberculosis in China: A predictive study using Markov modeling. *Infectious Diseases of Poverty*, *9*(1), 1–8. https://doi.org/10.1186/s40249-020-00682-7

Liang, L., Wu, Q., Gao, L., Hao, Y., Liu, C., Xie, Y., Sun, H., Yan, X., Li, F., Li, H., Fang, H., Ning, N., Cui, Y., & Han, L. (2012). Factors contributing to the high prevalence of multidrug-resistant tuberculosis: A study from China. *Thorax*, *67*(7), 632–638. https://doi.org/10.1136/thoraxjnl-2011-200018

Migliori, G. B., Matteelli, A., Cirillo, D., & Pai, M. (2008). Diagnosis of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis: Current standards and challenges. *Canadian Journal of Infectious Diseases and Medical Microbiology*, *19*(2), 169–172. https://doi.org/10.1155/2008/857901

Moore, D. A. J., Evans, C. A. W., D, P., Gilman, R. H., Sc, B., Coronel, J., Vivar, A., Sanchez, E., Saravia, J. C., Salazar, C., Oberhelman, R., Sc, M., Lachira, D., & Escombe, A. R. (2007). UKPMC Funders Group Author Manuscript Microscopic-Observation Drug-Susceptibility Assay for the Diagnosis of TB. *English Journal*, *355*(15), 1539–1550. https://doi.org/10.1056/NEJMoa055524.Microscopic-Observation

Müller, B., Warren, R. M., Williams, M., Böttger, E. C., Van Pittius, N. C. G., & Victor, T. C. (2011). Acquisition, transmission and amplification of drug-resistant tuberculosis. *Antituberculosis Chemotherapy*, *10*, 96–104. https://doi.org/10.1159/000324624

Nathavitharana, R. R., Cudahy, P. G. T., Schumacher, S. G., Steingart, K. R., Pai, M., & Denkinger, C. M. (2017). Accuracy of line probe assays for the diagnosis of pulmonary and multidrug-resistant tuberculosis: A systematic review and meta-analysis. *European Respiratory Journal*, *49*(1). https://doi.org/10.1183/13993003.01075-2016

Nguyen, T. N. A., Berre, V. A. Le, Bañuls, A. L., & Nguyen, T. V. A. (2019). Molecular diagnosis of drug-resistant tuberculosis; A literature review. *Frontiers in Microbiology*, *10*(MAR), 1–12. https://doi.org/10.3389/fmicb.2019.00794

Ninan, M. M., Gowri, M., Christopher, D. J., Rupali, P., & Michael, J. S. (2016). The diagnostic utility of line probe assays for multidrug-resistant tuberculosis. *Pathogens and Global Health*, *110*(4–5), 194–199. https://doi.org/10.1080/20477724.2016.1214350

Oberhelman, R. A., Soto-Castellares, G., Caviedes, L., Castillo, M. E., Kissinger, P., Moore, D. A. J., Evans, C., & Gilman, R. H. (2006). Improved recovery of Mycobacterium tuberculosis from children using the microscopic observation drug susceptibility method. *Pediatrics*, *118*(1). https://doi.org/10.1542/peds.2005-2623

Obermeyer, Z., Abbott-Klafter, J., & Murray, C. J. L. (2008). Has the DOTS strategy improved case finding or treatment success? An empirical assessment. *PLoS ONE*, *3*(3). https://doi.org/10.1371/journal.pone.0001721

Odone, A., Calderon, R., Becerra, M. C., Zhang, Z., Contreras, C. C., Yataco, R., Galea, J., Lecca, L., Bonds, M. H., Mitnick, C. D., & Murray, M. B. (2016). Acquired and transmitted multidrug resistant tuberculosis: The role of social determinants. *PLoS ONE*, *11*(1), 1–7. https://doi.org/10.1371/journal.pone.0146642

Okethwangu, D., Birungi, D., Biribawa, C., Kwesiga, B., Turyahabwe, S., Ario, A. R., & Zhu, B. P. (2019). Multidrug-resistant tuberculosis outbreak associated with poor treatment adherence and delayed treatment: Arua District, Uganda, 2013-2017. *BMC Infectious Diseases*, *19*(1), 1–10. https://doi.org/10.1186/s12879-019-4014-3

Olmsted, R. N., Craig, C. P., & Treston-Aurand, J. (1995). Nosocomial transmission of tuberculosis. *Annals of Internal Medicine*, *123*(7), 1520–1523. https://doi.org/10.7326/0003-4819-123-7-199510010-00015

Olson, S., English, R., & Claiborne, A. (2011). the New Profile of Drug-Resistant Tuberculosis in Russia : a Global and Local Perspective. In *Sciences-New York*.

Oo, T., Kyaw, K. W. Y., Soe, K. T., Saw, S., Satyanarayana, S., & Aung, S. T. (2019). Magnitude and reasons for pre-diagnosis attrition among presumptive multi-drug resistant tuberculosis patients in Bago Region, Myanmar: A mixed methods study. *Scientific Reports*, *9*(1), 1–9. https://doi.org/10.1038/s41598-019-43562-3

Pai, M., Kalantri, S., & Dheda, K. (2006). New tools and emerging technologies for the diagnosis of tuberculosis: Part I. Latent tuberculosis. *Expert Review of Molecular Diagnostics*, *6*(3), 413–422. https://doi.org/10.1586/14737159.6.3.413

Piatek, A. S., Tyagi, S., Pol, A. C., Telenti, A., Miller, L. P., Kramer, F. R., & Alland, D. (1998). Molecular beacon sequence analysis for detecting drug resistance in Mycobacterium tuberculosis. *Nature Biotechnology*, *16*(4), 359–363. https://doi.org/10.1038/nbt0498-359

Richards et al. (2018). 乳鼠心肌提取 HHS Public Access. *Physiology & Behavior*, *176*(5), 139–148. https://doi.org/10.1016/j.tube.2015.11.010.Tuberculosis

Salman, H. S., & Rüsch-Gerdes, S. (2006). MGIT Procedure Manual. *Mycobacteria Growth Indicator Tube (MGIT) Culture and Drug Susceptibility Demonstration Projects*, *July*, pp24-40. http://www.finddiagnostics.org/export/sites/default/resource-centre/find_documentation/pdfs/mgit_manual_nov_2007.pdf

Sanogo, M., Kone, B., Diarra, B., Maiga, M., Baya, B., Somboro, A. M., Sarro, Y. S., Togo, A. C. G., Dembele, B. P. P., Goita, D., Kone, A., M'Baye, O., Coulibaly, N., Diabate, S., Traore, B., Diallo, M. H., Coulibaly, Y. I., Saleeb, P., Belson, M., … Diallo, S. (2017). Performance of microscopic observation drug susceptibility for the rapid diagnosis of tuberculosis and detection of drug resistance in Bamako, Mali. *Clinical Microbiology and Infection*, *23*(6), 408.e1-408.e6. https://doi.org/10.1016/j.cmi.2017.01.004

Satti, L., Ikram, A., Abbasi, S., Butt, T., Malik, N., & Mirza, I. A. (2010). Evaluation of BACTEC MGIT 960 system for recovery of Mycobacterium tuberculosis complex in Pakistan. *Malaysian Journal of Microbiology*, *6*(2), 203–208. https://doi.org/10.21161/mjm.22509

Seung, K. J., Gelmanova, I. E., Peremitin, G. G., Golubchikova, V. T., Pavlova, V. E., Sirotkina, O. B., Yanova, G. V., & Strelis, A. K. (2004). The effect of initial drug resistance on treatment response and acquired drug resistance during standardized short-course chemotherapy for tuberculosis. *Clinical Infectious Diseases*, *39*(9), 1321–1328. https://doi.org/10.1086/425005

Shah, N. S., Auld, S. C., Brust, J. C. M., Mathema, B., Ismail, N., Moodley, P., Mlisana, K., Allana, S., Campbell, A., Mthiyane, T., Morris, N., Mpangase, P., van der Meulen, H., Omar, S. V., Brown, T. S., Narechania, A., Shaskina, E., Kapwata, T., Kreiswirth, B., & Gandhi, N. R. (2017). Transmission of Extensively Drug-Resistant Tuberculosis in South Africa. *New England Journal of Medicine*, *376*(3), 243–253. https://doi.org/10.1056/nejmoa1604544

Telles, M. A. S., Bori, A., Amorim, A. B. R., Cruz, A. F., Pini, M. I. T., & Sato, D. N. (2002). Rapid detection of multidrug-resistant Mycobacterium tuberculosis using the mycobacteria growth indicator tube (MGIT) system. *Brazilian Journal of Medical and Biological*

Research, *35*(10), 1127–1131. https://doi.org/10.1590/S0100-879X2002001000003

Van Deun, A., Maug, A. K. J., Salim, M. A. H., Das, P. K., Sarker, M. R., Daru, P., & Rieder, H. L. (2010). Short, highly effective, and inexpensive standardized treatment of multidrug-resistant tuberculosis. *American Journal of Respiratory and Critical Care Medicine*, *182*(5), 684–692. https://doi.org/10.1164/rccm.201001-0077OC

Wang, L., Mohammad, S. H., Chaiyasirinroje, B., Li, Q., Rienthong, S., Rienthong, D., Nedsuwan, S., Mahasirimongkol, S., & Yasui, Y. (2015). Evaluating the auto-MODS assay, a novel tool for tuberculosis diagnosis for use in resource-limited settings. *Journal of Clinical Microbiology*, *53*(1), 172–178. https://doi.org/10.1128/JCM.01946-14

Wang, Y., Chen, H., Huang, Z., McNeil, E. B., Lu, X., & Chongsuvivatwong, V. (2019). Drug non-adherence and reasons among multidrug-resistant tuberculosis patients in Guizhou, China: A cross-sectional study. *Patient Preference and Adherence*, *13*, 1641–1653. https://doi.org/10.2147/PPA.S219920

WHO. (2008). Molecular Line Probe Assays for Rapid Screening of Patients At Risk of Multidrug-Resistant Tuberculosis Policy Statement Molecular Line Probe Assays for Rapid Screening of. *World Health Organization*, *June*.

World Health Organisation. (2010). *NON-COMMERCIAL CULTURE AND DRUG-SUSCEPTIBILITY TESTING METHODS FOR SCREENING OF PATIENTS AT RISK OF MULTI-DRUG RESISTANT TUBERCULOSIS - POLICY STATEMENT - March 2010*. *March*.

World Health Organization (WHO). (2011). Rapid Implementation of the Xpert MTB / RIF diagnostic test Technical and Operational ‘ How - to .’ *WHO Library Cataloguing-in-Publication Data*.