MDR-TB IN TANZANIA: PREVALENCE, RISK FACTORS AND PATTERN OF ANTI TB DRUG RESISTANCE

By

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A dissertation submitted to the University of Zambia, in partial fulfilments of the requirements for the award of the Degree of Master's of Science in One Health Analytical Epidemiology

UNIVERSITY OF ZAMBIA

DECLARATION

I, ROBERT MAKORI HONGO does hereby declare that the contents of this dissertation are my own work. The dissertation has not previously been submitted for the award of degree to any University.

Signature.. Date...

DEDICATION

I dedicate this work to my lovely wife Vailet Hance, my children Eunice and John my mother Eunice K. Hongo. Brother Juma John Hongo and all other Hongo family for their full support during the preparation of this dissertation and when I was far from them for school, may Almighty God bless them abundantly.

Finally, I am dedicating this work to those who are suffering from TB in Tanzania and I am also presenting my work to my grandmother Bhunuri Nyamwiharo who has passed away but she was tirelessly pray for me, who her prayer is now a success.

CERTIFICATE OF APPROVAL

This dissertation submitted by **Robert Makori Hongo** is approved as fulfilling part of the requirements for the award of the degree of Master of Science in One Health Analytical Epidemiology (OHAE) at the University of Zambia.

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ABSTRACT

The emergence of drug resistance tuberculosis (DR-TB) is a significant challenge for TB control and prevention programmes, and the major problem is multidrug resistant tuberculosis (MDR-TB) which can develop to extensively drug resistant tuberculosis (XDR-TB). This epidemiological scenario of TB is worsened by its worldwide distribution with a growing trend displaying new and highly antibiotic resistant strains. Thus the present study was carried out to determine the emerging epidemiological trends of TB in the presence of MDR-TB and XDR- TB in Tanzania as well as to ascertain the likely risk factors linked to the occurrence and distribution patterns of anti TB drugs resistance and to determine XDR-TB prevalence among MDR-TB Isolates. We used survey proportion estimates of positive cases whilst possible risk factors were investigated using the multiple logistic regression model. Consequently, 278 MDR-TB isolates from the Central Tuberculosis Reference Laboratory (CTRL) which were diagnosed as new and re-treated tuberculosis cases collected between January 2011 and December 2014 were included in the study after subjecting them to a series of selection criteria for inclusion and exclusion. Isolates were tested for susceptibility to first line and second line drugs.

From the MDR-TB 278 isolates, 136 (48.9%) were resistant only to H, R, S, E, 20 (7.2%) were resistant only to H,R,E, 61,(21.9%) were resistant only to H, R ,S, 60 (21.6%) isolates were resistant only to H and R alone and Only 1 isolate (0.4%) were resistant to Km and Oflx. The general tuberculosis prevalence was seen to vary markedly among the 20 regions P = 0.0001, with Dar-es-Salaam contributing over 55.4% of the cases screened at CTRL, followed by Kilimanjaro 23.4%. The prevalence of XDR-TB was calculated to be at 0.36. Key factors linked to the MDR/XDR-TB included sex, with males being twice likely to have TB than females with P = 0.041. Age was another factor with high prevalence in the reproductive active group between 21 to 40 years P= 0.028. Others included the regions of origin of the isolates P = 0.004, the patients with history of re-treatment P = 0.004.

This study provides preliminary information about the potential risk factors associated with MDR-TB and XDR-TB status in Tanzania. Further, although the prevalence of XDR-TB seems low, its potential risk to public health is important based on traditional methods of diagnosis coupled with poor sanitary measures. Taking into consideration that rifampicin is being used throughout the six months of TB treatment; and the treatment is observed at home by a non-medical professional person, drug resistance surveillance in the country should be maintained among both new and re-treatment cases.

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LIST OF ABBREVIATIONS

AOR-	Adjusted Odd Ratio
cOR-	Clude Odd Ratio
CTRL-	Central Tuberculosis Reference Laboratory
CI-	Confidence Interval
CDC-	Centres for Infectious Disease Control and Prevention
DOTS-	Directly Observed Treatment Short Course
DNA-	Deoxyribonucleic Acids
DST-	Drug Sensitivity Testing
DRS-	Drug Resistant Survey
E-	Ethambutol
FDC-	Fixed Dose Combination
G+C-	Guanine and Cytosine
H-	Isoniazid
HIV-	Human Immunaldeficient Viruses
IQR-	Interquartile Range
IUATLD-	International Union against Tuberculosis and Lung Disease
L-J-	Lowenstein Jensen
MTB-	Mycobacterium Tuberculosis
MGIT-	Mycobacteria Growth Indicator Tube
mNRA-	Massenger Ribonucleic Acid

MCF- McFarland

MIC- Minimal Inhibitory Concentration

MDR-TB- Multidrug-resistance Tuberculosis

MTC- Mycobacterium Tuberculosis Complex

MUHAS- Muhimbili University of Health and Allied Sciences

NTLP- National Tuberculosis and Leprosy Program

P-Value- Level of significance at alpha equal to 0.05

PPD - Purified Protein Derivative

QA- Quality Assurance

QC- Quality Control

R- Rifampicin

RNA- Ribonucleic Acid

RNase - Ribonuclease

rRNA - Ribosomal Ribonucleic Acid

S- Streptomycin

SACIDS- Southern Africa Centre for Infectious Disease Surveillance

TB- Tuberculosis

TB/HIV- The intersecting epidemics of TB and HIV Co-infection

TDR-TB - Totally Drug Resistant Tuberculosis

TSB- Trypticase Soy Broth

WHO- World Health Organization

XDR-TB-	Extensively Drug Resistant Tuberculosis
XXDR-TB-	Extremely Drug Resistant Tuberculosis
Z-	Pyrazinamide

GLOSSARY

Amplification of drug resistance: A phenomenon where additional drug resistance is acquired by an already drug resistant isolate

Any type drug resistance: Single, double, triple, quadruple or resistance to five drugs

Combined resistance: Resistance to more than one drug (synonymous with multiple drug resistance)

Case of tuberculosis: A patient in whom tuberculosis has been confirmed by bacteriology or diagnosed by a clinician

Category I TB patients: New smear positive TB patients and severely ill smear negative and extra-pulmonary TB

Category II TB patients: Return after default, treatment failure and relapse after treatment completed or cure

Category III TB patients: Smear negative and extra-pulmonary TB patients who are not seriously ill

Chronic cases: A patient who is still smear positive at the completion of retreatment regimen

Cohort: *A* group of patients in whom TB has been diagnosed and who were registered for treatment during a specified time period

Cured: An initially smear-positive patient who was smear-negative in the last month of treatment and on at least one previous occasion

Defaulter: A patient who has been on treatment for at least 4 weeks and whose treatment was interrupted for more than 8 consecutive weeks

DOTS-plus: TB treatment programme where DOTS is supported by the use of second line anti-TB drugs

Drug resistance without previous treatment for TB: Drug resistance detected in individuals not previously treated with anti-TB drugs or treated for less than a month

Drug resistance with previous treatment: Drug resistance detected in individuals previously treated with anti-TB drugs for more than a month

Mono-resistance: Resistance to a single drug

Multidrug-resistance (MDR): Resistance to at least Rifampicin and Isoniazid.

Multiple drug resistance*:* Resistance to more than one drug including Multidrug resistance

New case: A patient who has never had treatment for tuberculosis or who has taken anti-TB drugs for less than 1 month

Relapse: A patient who has been declared cured or treatment completed of any form of TB in the past, but who reports back to the health service and is found to be smear positive or culture positive

Re-treatment case: A patient previously treated for tuberculosis, undergoing treatment for a new episode of bacteriologically-positive tuberculosis (expressed as a percentage of the number registered in the cohort)

Smear-positive pulmonary: A patient with at least 2 initial sputum smear examinations AFB+; or one sputum examination AFB+ and radiographic abnormalities consistent with active Pulmonary tuberculosis as determined by a clinician; or one sputum specimen AFB+ and culture positive for *M. tuberculosis*

Treatment failure: A patient who, while on treatment, remained smear-positive or became smear-positive at the end of five months or later, after commencing treatment

TB case definition: An individual starting a full course of anti-TB treatment based on clinical and/or radiological evidence

CHAPTER ONE

1.0 INTRODUCTION

Tuberculosis (TB) is an airborne communicable disease caused by *Mycobacterium tuberculosis* complex (MTC). The common source of the infection is an infectious individual who, by sharing common breathing space, transmits the infection to other individuals (CDC, 2000). Regardless of the long existence of standard anti-TB treatment, low success rates continue to hamper TB control strategies and resulting in increased anti-TB drug resistance, plus Multidrug resistance TB (MDR-TB) and recently extensively drug resistance TB (XDR-TB). MDR-TB is defined as resistance to isoniazid and rifampicin which are the best antituberculosis drugs (WHO, 2008). In September, 2006 the World Health Organization (WHO) expressed worries over the emergence of XDR-TB which is defined as resistance to any fluoroquinolone antibiotic plus resistance to at least one of three injectable second line drugs; capreomycin, kanamycin and amikacin, in addition to multidrug resistance. These types of MDR-TB and XDR-TB do not respond to the six month treatment pattern of first-line anti-TB drugs and can take two years or more to treat with drugs that are less effective, more toxic and much more costly (WHO, 2013). The results from the findings of a survey carried out by WHO and the U.S. Centers for Disease Control and Prevention (CDC) found that XDR-TB has been identified in all regions of the world. The report by Bhargava *et al.*, 2012, showed that the former States of the Soviet Union, India, and China have the greatest burden of XDR-TB globally. Extensively drug resistance TB infections are very complicated to treat and are characterized by high death (Bhargava *et al.*, 2012). In 2014, WHO reported that 9.0% of all MDR-TB cases are XDR-TB. In the African region, 9 countries which include: South Africa, Zimbabwe, DR Congo, Tanzania, Ethiopia, Kenya, Nigeria, Uganda, and Mozambique are on the 22 high TB burden list with South Africa having the uppermost TB burden in the region in 2011 (WHO, 2012). Tanzania is positioned number 15 among the 22 World's Top TB incidence countries having an incidence rate of 165 among smear- positive cases per 100,000 inhabitants in 2012. From WHO report it has been estimated that the prevalence of all forms of TB in Tanzania is 295/100,000 (WHO, 2012). In Tanzania, according to the national anti-TB drug resistance surveillance done in 2007, the prevalence of MDR-TB was 1.1% and 3.9% among new and re-treatment cases, respectively (Chonde *et al.,* 2010). This study was therefore conducted to determine the risk factors, pattern, and geographical distribution of MDR-TB patients and the prevalence of XDR-TB cases among the MDR-TB patients in Tanzania.

2.0 STATEMENT OF THE PROBLEM

MDR-TB continues to present a global challenge to TB control. Coverage of Drug Sensitivity Test (DST) for TB patient remains low as a result of a minority of drug-resistant TB patients who are detected and notified. Information remains incomplete but TB cases are allied with high morbidity and mortality with high cost of treatment. More seriously however, has been the emergence of new and drug resistant strains causing drug-resistant tuberculosis (DR-TB), developing into multi-drug resistant tuberculosis (MDR-TB), which can further become extensively drug resistant tuberculosis (XDR-TB) to extremely drug resistant tuberculosis (XXDR-TB) and finally into totally drug resistant tuberculosis (TDR-TB) as has been evidenced from many parts of the world. It is important to identify the true prevalence, factors and patterns of anti TB drug resistance in Tanzania which are associated with MDR-TB and XDR-TB to improve patient management. According to the national anti-TB drug resistance surveillance done in 2007, the prevalence of MDR-TB was 1.1% and 3.9% among new and re-treatment cases, respectively (Chonde *et al*., 2010). This level of drug resistance in Tanzania is said to be one of the lowest among the African countries with an estimated level of drug resistance. However, these reports are not backed by empirical evidence as well as organised and well formulated studies to augment these current assertions.

3.0 RATIONALE

The TB control program in Tanzania faces the challenge of emerging MDR-TB, and in order to control this, there is need to understand factors associated with the occurrence of MDR-TB and the distribution patterns. Understanding risk factors for developing MDR-TB is critical in designing intervention strategies that are best suited for the Tanzanian situation. Understanding these factors will provide information that is crucial in formulating policies that will help in allocating resources, targeting and strengthen the surveillance activities, promoting early diagnoses, treatment and ultimately preventing MDR-TB and its progression to XDR-TB.

4.0 RESEARCH QUESTIONS

1. What is the true prevalence of XDR-TB cases among MDR-TB Patient in Tanzania?
2. What is the geographical distribution of MDR-TB in Tanzania?
3. What are the patterns of anti- TB drug resistance in Tanzania?
4. What are the risk factors associated with MDR-TB in Tanzania?

5.0 OBJECTIVE

5.1 GENERAL OBJECTIVE

To identify risk factors, pattern, and geographical distribution of MDR-TB patients and the prevalence of XDR-TB cases among the MDR-TB patients in Tanzania.

5.2 SPECIFIC OBJECTIVES

1. To determine the prevalence of XDR-TB cases among MDR-TB Patient in Tanzania
2. To assess geographical distribution of MDR-TB drug resistance in Tanzania
3. To determine the pattern of anti-TB drug resistance among *M. tuberculosis* strains isolated from MDR-TB patients in Tanzania
4. To identify the risk factors for MDR-TB in Tanzania

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 BACKGROUND

Tuberculosis (TB) is a disease that is caused by members of the M*ycobacterium tuberculosis complex (*MTC) (van Soolingen *et al.,* 1997). There are eight closely related mycobacteria responsible for TB, These include; *Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium africanum, Mycobacterium microti, Mycobacterium caprae, Mycobacterium pinnipedii, Mycobacterium Canetti* and *Mycobacterium Mungi* together comprise what is known as the *Mycobacterium tuberculosis* complex (MTC) (van Soolingen *et al.,*1997). *Mycobacterium tuberculosis* is the commonly transmitted between humans through the airborne route. Tuberculosis usually manifests as a respiratory illness, but can present in other areas of the body such as joints, kidneys, and the brain, and if not treated properly, it can be fatal. Tuberculosis is a highly contagious disease that is spread through the air. One must inhale the infectious droplets to become infected. However, TB is not spread through person to person by physical contact, sharing bodily fluids, or touching infected surfaces. There are no known animal reservoirs of *Mycobacterium tuberculosis*, while that of *Mycobacterium microtti* are rodents and *Mycobacterium bovis* are a wide range of wild, domestic animals and occasionally humans. *Mycobacterium bovis* may penetrate the gastrointestinal mucosa or invade the lymphatic tissue of the oropharynx when ingested in milk from diseased cows (NTMG, 2014). Repetitive DNA elements such as insertion sequence (*IS6110)* and direct repeat have been found to be restricted to the MTC (van Soolingen *et al*., 1997). The complete genome of the *Mycobacteria,* strain H37Rv has been sequenced and is known to contain 4,411,529 bps consisting of about 4,000 genes with Guanine and Cytosine (G+C) content of 65.6% (van Soolingen *et al.*, 1997). Global efforts to control TB pandemic have been undermined by the emergence and spread of strains that are resistant to the commonly used first line anti-TB drugs isoniazid (H), rifampicin (R), ethambutol (E), and streptomycin (S) (WHO,2014). Strains resistant to at least H and R, the two most efficacious TB drugs are termed MDR-TB (CDC, 2006). MDR-TB treatment is rather complicated as it requires second line drugs some of which are only injectable, are less efficacious, more toxic and more expensive than the first line agents (WHO, 2006). Treatment lasts for 18-24 months but only around 50% – 60% of MDR-TB patients will be cured compared with 95%–97% cure rate for patients with drug-susceptible strains treated with first line agents (Goble, 1993).

2.2 EPIDEMIOLOGY OF TB

2.2.1 GLOBAL EPIDEMIOLOGY OF TB

The TB burden remains a major global health problem. The number of TB deaths is unacceptably large given that most are preventable. According to WHO 2014, 9 million people fell ill with TB in 2013, including 1.1 million cases among people living with HIV .In 2013, 1.5 million people died from TB, including 360 000 among people who were HIV-positive. About 510 000 women died from TB in 2013, including 180 000 among women who were HIV-positive (WHO, 2014). Of the overall TB deaths among HIV-positive people, 50% were among women (WHO, 2014). Tuberculosis is one of the top killers of women of reproductive age (WHO, 2014). An estimated 550 000 children became ill with TB and 80 000 children who were HIV-negative died of TB in 2013 (WHO, 2014). The TB mortality rate has decreased 45% since 1990. On average, an estimated 9% of people with MDR-TB have XDR-TB. The majority of cases worldwide in 2012 were in the South-East Asia (29%), African (27%) and Western Pacific (19%) regions. India and China alone accounted for 26% and 12% of total cases, respectively (WHO, 2014).

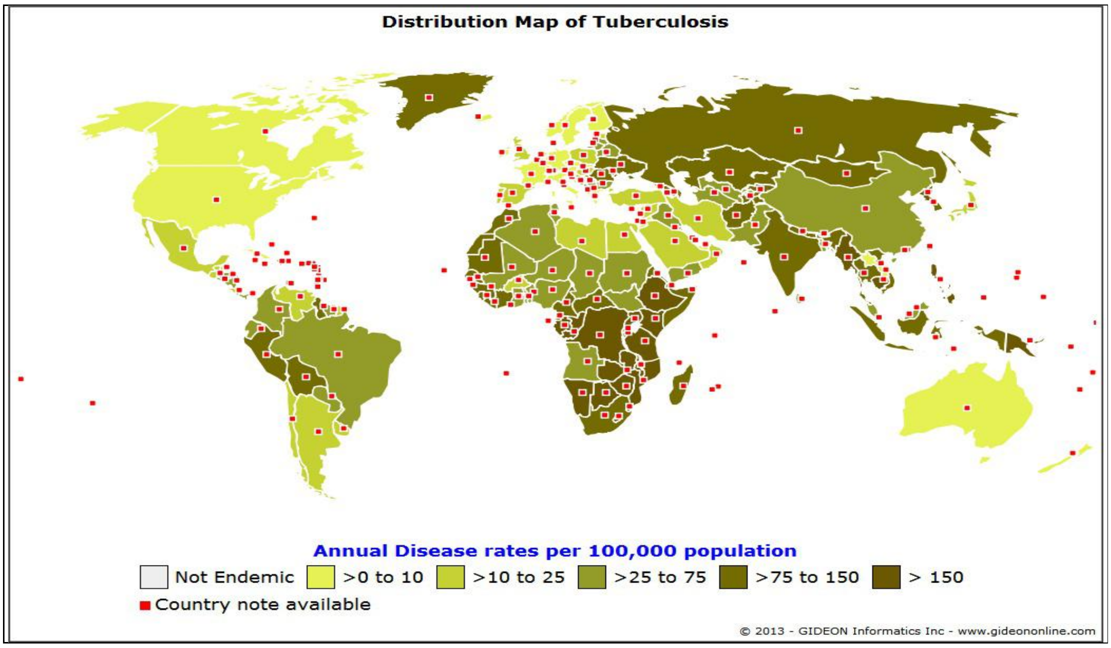

Figure 2.1 Global map showing distribution of Tuberculosis. (WHO, 2013).

The TB incidence rate at country level ranges substantially, with around 1000 or more cases per 100 000 people in South Africa and Swaziland, and fewer than 10 per 100 000 population in parts of the Americas, several countries in Western Europe, Japan, Australia and New Zealand (WHO, 2014).

2.2.2 GLOBAL MDR-TB SITUATION

Globally, in 2013, an estimated 480 000 people developed MDR-TB and there were an estimated 210 000 deaths from MDR-TB. The number of people diagnosed with MDR-TB tripled between 2009 and 2013, and reached 136 000 worldwide. This was equivalent to 45% of the estimated MDR-TB cases among notified TB patients (WHO, 2014). Progress in the detection of drug-resistant TB has been facilitated by the use of new rapid diagnostics. A total of 97 000 patients were started on MDR-TB treatment in 2013, a three-fold increase compared with 2009.

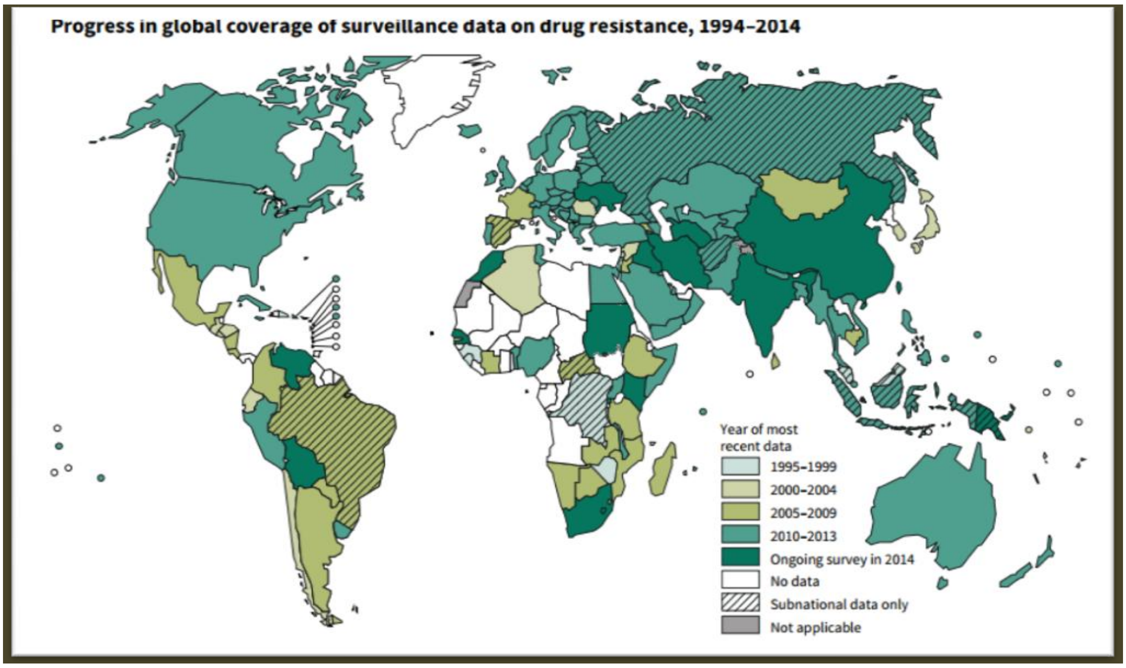

Figure 2.2 Map of Surveillance data on drug resistance 1994-2014. (WHO.2014)

However, the gap between diagnosis and treatment widened between 2012 and 2013 in several countries. XDR-TB has been reported by 100 countries in 2013. On average, an estimated 9% of people with MDR-TB have XDR-TB (WHO, 2014).

2.2.3 MDR-TB IN AFRICA

In Africa, there is a low proportion of MDR-TB reported among newly identified TB cases in contrast to that in regions such as Eastern Europe and Central Asia, due in part to the inadequate laboratory ability to conduct drug resistance surveys (WHO, 2014). Most recent estimates of WHO put the number of MDR-TB cases emerging in 2008 in Africa at 69, 000. Earlier reports found high levels of death among people living with HIV and infected with MDR-TB and XDR TB. In 2006 XDR-TB outbreak in KwaZulu-Natal, South Africa, 52 of 53 people who contracted the disease died within months. It is estimated that 70% of XDR-TB patients die within a month of diagnosis. The most recent drug-resistance surveillance data issued by the WHO estimates that an average of roughly 5 per cent of MDR-TB cases are XDR-TB. Estimating the incidence of XDR-TB is extremely difficult because most laboratories are ill equipped to detect and diagnose it; it is thought that the majority of XDR-TB cases go undocumented (WHO, 2012).

2.2.4 DRUG SENSITIVE TB IN TANZANIA

In 2013, the National TB and Leprosy Control report Program in Tanzania (NTLP), showed that a total of 65,732 cases of all forms of TB were notified in 2013, which showed an increase of 1,840 cases or 2.9% compared to the year 2012 (NTLP,2014). Among all the cases notified, new cases were 62,952 (95.8%) and the retreatment cases were 2,780 (4.2%) which is almost same proportions for the past three years (NTLP, 2014).

2.2.5 MDR-TB IN TANZANIA

According to the WHO 2013 global report, it is estimated that Tanzania has 1.1 % prevalence of MDR-TB (WHO, 2014). In 2011 there were an estimated 500 MDR-TB cases among notified pulmonary TB cases. The national drug resistant survey (DRS) that was conducted in 2006/2007 showed MDR- TB prevalence of 1.1% among new cases and 3.9% among re-treatment cases (Chonde *et al.*, 2010).

2.3 TRANSMISSION OF TB

Mycobacterium tuberculosis is an airborne particle, called droplet nuclei, of 1– 5 microns in diameter (CDC, 2003). Infectious droplet nuclei are generated when persons who have pulmonary or laryngeal TB disease cough, sneeze, shout, or sing

(CDC, 2003). In 1995, the study done by Well, it was observed that depending on the environment, these tiny particles can remain suspended in the air for several hours (Well, 1995). *Mycobacterium tuberculosis* is transmitted through the air, not by surface contact (Well, 1995). Transmission occurs when a person inhales droplet nuclei containing *Mycobacterium tuberculosis* (Canetti, 1955) and the droplet nuclei pass through the mouth or nasal passages, upper respiratory tract, and bronchi to reach the alveoli of the lungs Figure 2.3.

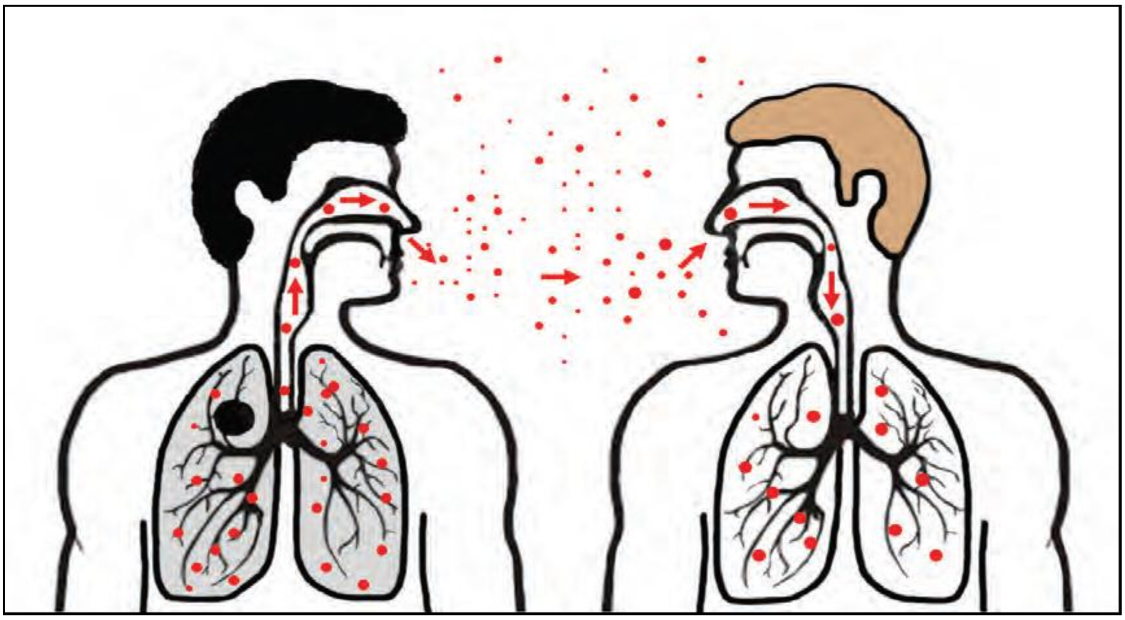

Figure 2. 3 TB is spread from person to person through the air. The red dots in the air represent droplet nuclei containing tubercle bacilli (Source from CDC).

2.4 RISK FACTORS ASSOCIATED WITH DRUG RESISTANT TB

The risk of progression to infection and disease is two different aspects and proper understanding of these factors is essential for planning TB control strategies (Romieu and Trenga, 2001). Different factors are suggested to be associated with drug resistant TB; there is no consensus for many of the implicated risk factors. Previous treatment, age, sex and region variability are factors usually considered with drug resistant TB.

2.4.1 PREVIOUS TREATMENT

A TB strain previously exposed to anti-TB drugs is likely to undergo selection and to have a greater proportion of drug resistant bacilli (Castello *et al.*, 1980). Hence previously treated patients are more likely to harbour drug resistant strains. Patients who failed on treatment, defaulters and relapses cases after cure fit this category. Previous treatment is a factor associated with development of drug resistance more consistently (de Melo *et al.,* 2003). A greater proportion of treatment failures, in particular, have drug resistant TB (Quy *et al.*, 2003).

2.4.2 AGE AND SEX

Even if many studies were not conclusive to whether MDR/XDR-TB have special treatment with certain age group or sex, In Argentina, young people were considerably found to have MDR-TB and it was more among women than men (Palmero *et al.,* 2003).This on the other hand may differ from country to country and from place to place within the same country. Children are at risk of being infected by their infected parents or caretakers with either drug susceptible or MDR/XDR-TB (Schaaf *et al*., 2007). This chance is significantly higher if a child is HIV positive (Schaaf *et al.,* 2007). In the studies done in South Africa, it was observed 78% of children who suffered from TB were exposed to infected parents or close relatives (Schaaf *et al.*, 2007). In the study done in Namibia it was observes most age group that affected by TB is 15-54years and more men than women are affected (MoHSS, 2008).This is the reproductive age group which is also worse affected by HIV epidemic (MoHSS, 2008). A systematic review done by Storla *et al.*, 2008 on delay to accessing treatment highlighted elderly people as being at risk of developing TB due to their poor immunity, and they are also more likely to delay in accessing treatment because of their dependency to other people (Storla *et al.,* 2008). People, who take certain medications like older adults, such as corticosteroids and transplant suppression drugs, also accelerate the progression to active disease (Pratt *et al.,* 2011).

2.4.3 REGION VARIABILITY

The occurrences and spread of MDR /XDR-TB may differ from place to place depending on the factors that favour the spread. In a study conducted in South Africa, it was found that some provinces have higher rate of MDR-TB than others (Andrews *et al.,* 2010). Congregate settings with poor ventilation favours, the spread of pulmonary TB. In a study done by Habeenzu *et al.*, 2001, it was observed that spread of MDR-TB in the Zambian prisons is 10 folds that of the general population. Tanzania has one MDR-TB hospital which is situated at Hai District in Kilimanjaro region in northern Tanzania this makes access to medical treatment and care for MDR/XDR-TB patients very limited in the country (NTLP, 2014). As reported by Holtz *et al.,* 2007, hospital wards, overcrowded waiting rooms in hospitals and clinics are regarded as potential places for the spread of MDR/XDR-TB. MDR/XDR-TB does not know state border as infectious patient continue to travel across borders and by public transport (Donnelly *et al.,* 2009). In a study conducted in Argentina, it was found that new MDR-TB was introduced by migrants from neighbouring countries (Palmero *et al.,* 2003). In another study conducted in Lesotho, a country that is surrounded by South Africa, the cases of MDR-TB were observed to have increased and this was blamed on daily mobility between the two countries (Setti *et al.,* 2008). Dar es Salaam is the largest commercial city of Tanzania having an important port and an international airport. The City has the highest proportion (22%) of all TB cases (61,000) notified in Tanzania in 2011

(MoHSW, 2012). Therefore, the city is a key area for TB control efforts in Tanzania (MoHSW, 2012).

2.5 PATHOGENESIS OF TB

When a person is infected with *Mycobacterium tuberculosis*, and the droplet nuclei are in the alveoli, local infection establish followed by dissemination to draining lymphatics and haematogenous spread throughout the body (WHO, 2011). Principally a primary complex builds up in the lungs, which commonly heals and forms calcifications (Krauss *et al*., 2003). But in 90-95% of the cases, the host immune response generated against the microbes is capable of preventing its development and reproduction, leading to a latent infection with no clinical symptoms (Delogu *et al.*, 2009). They perhaps become progressive in 5-10% of the cases (Delogu *et al.*, 2009). If the infection becomes progressive, patients present with cough, weight loss, night sweats, low-grade fever, dyspnea, lymphadenopathy, chest pain, and even pneumonia or phthisis (Pfyffer, 2007). Next after the development of the cavernous lesions, primary tuberculosis may also have an exudative course (Krauss *et al.*, 2003). This exudative course involves pleural effusion with high fever and chest pain (Krauss *et al.*, 2003). Tuberculosis can also be extra pulmonary which has an effect on any organ system and may cause inflammations of the organ it affects like cervical lymphadenitis, pleuritis, pericarditis, synovitis, meningitis, and infections of the skin, joint or bones (Pfyffer, 2007). Disseminated tuberculosis (Milliary TB) is characterized by high and sustained fever, night sweats, dry cough, malaise, splenomegaly, and skin lesions (Krauss *et al.*, 2003). In Meningitis clinical features like high fever, cranial nerve deficits, and psychic changes build up in 50% of the TB cases with a high mortality rate, if left untreated (Krauss *et al*., 2003). In addition, these patients may also develop tuberculous peritonitis with fever, ascites and increase in abdominal girth (Krauss *et al.,* 2003). Even if *Mycobacterium bovis* infection in humans causes a disease very similar to *Mycobacterium tuberculosis*, non-pulmonary lesions are more common than pulmonary lesions, given that *Mycobacterium bovis* infection is mainly acquired by consuming soiled milk or meat with the microbes (Gilchrist *et al.*, 1995).

2.6 DIAGNOSIS OF TB

Like many diseases, tuberculosis is diagnosed through a combination of clinical examinations and laboratory tests. Clinical examination involves collecting information on the patient's history which includes whether the person has been in a country with a high TB incidence rate (Pratt *et al*., 2005). In addition to clinical examination, there are other tests that have to be done such as bacteriological diagnosis, radiological examination, tuberculin test, and others. However, only the most common screening test is discussed.

2.6.1 TUBERCULIN TEST (MANTOUX TEST)

This test is given by injecting preparation of PPD (purified protein derivative) usually 0.1 ml or 0.05 in infants into the dermal layer of the skin, usually on the forearm. The test is read after 72 hours by measuring the transverse diameter of the induration which may be felt and not the redness (Pratt *et al.*, 2005). A patient with a positive test result has an induration of six mm or more; positive result means that the person is sensitive to tuberculin test, but it doesn't mean that he or she may transmit the disease. The tuberculin test does not differentiate between TB bacteria and BCG reactions which is one of the test's disadvantages (CDC, 2000). The tuberculin test must be evaluated individually to identify the risk for developing the disease. Therefore, one has to consider if the person has stayed in an area with a high TB incidence rate or if he or she has been in contact with other TB patients. One also has to check also if the person has been vaccinated with BCG. At the same time, the results may lead to a kind of suspicion. However, if the swelling is six mm and the patient has come from a high TB incidence rate country while another patient has indurations of 10 or 15 mm but has come from a low TB incidence rate country (FHI, 2007).

2.6.2 RADIOLOGICAL EXAMINATION

The radiology examination, particularly of the chest, is widely used for the diagnosis of tuberculosis. It could involve the use of full-size chest radiographs, miniature chest radiographs, and fluoroscopy. The WHO has particularly recommended the last test (Schaefer-Prokop *et al.,* 2008). Miniature X- ray has been used in mass screening, but it is not cost effective and is now rarely used (WHO, 2011). Full-size X-rays are more sensitive because they show even the smallest lesions in the lungs; however, it is still difficult to differentiate between TB lesions and others (Graham *et al.,* 2002). It is important to know that radiology may be of great help, but other investigations are required to confirm the diagnosis (Pratt *et al.,* 2005).

2.6.3 SMEAR MICROSCOPY

Smear microscopy of sputum is often the first TB test to be used in countries with a high rate of TB infection. Sputum is a thick fluid that is produced in the lungs and the airways. A sample of sputum is usually collected by the person coughing. Sputum smear microscopy is inexpensive and simple, and people can be trained to do it relatively quickly and easily. In addition, the results are available within hours. The sensitivity though is only about 50-60 % (Siddiqi, 2003). In countries with a high prevalence of both pulmonary TB and HIV infection, the detection rate can be even lower, as many people with HIV and TB co-infection have very low levels of TB bacteria in their sputum, and are therefore recorded as sputum negative (Kent *et al.,* 1985).

2.6.4 CULTURING METHOD

Culturing is a method of studying bacteria by growing them on media containing nutrients (Linda *et al.,* 2011). Media can be either solid media on culture plates or bottles of liquid media (culture broths). Different media are used to make it as easy as possible for the suspected microorganisms to grow (Linda *et al.,* 2011). Culturing and identification of *M. tuberculosis* provides a definitive diagnosis of TB and can significantly increase the number of cases found (Moore *et al*., 2006). Culture can also provide drug susceptibility testing, showing which TB drugs a person's bacteria are resistant to, and if the person has got MDR or XDR- TB (De Beenhouwer *et al.,* 1995). However, culture is much more complex and expensive than microscopy to perform as it requires specific equipment and enhanced laboratory facilities. Diagnosing TB using culture can also take weeks because of the slow growth of TB bacilli (WHO, 2009). It takes on averages 4 weeks to get a conclusive test result using the most common methods of solid media, with another 4-6 weeks to produce drug susceptibility results.

2.6.5 DRUG SUSCEPTIBILITY TESTING

Drug susceptibility testing (DST) means testing to find out which drugs the TB bacteria in a patient are susceptible to, and can therefore determine whether the person has got drug resistant TB. Some drug susceptibility tests, such as the Xpert TB test can be used to diagnose TB, as well as testing for some types of TB drug resistance (Weyer *et al.,* 2012). Different molecular techniques which rely on the detection of mutation in the regions of gene (s) associated with drug action could also be useful for rapid diagnosis of DR-TB (Heifets and Cangelosi, 1999).

2.7 TREATMENT OF TB

2.7.1 FIRST LINE ANTI-TB DRUGS

Any drug used in the anti-TB regiment is supposed to have an effective sterilizing activity that is capable of shortening the duration of treatment. Currently, a five-drug regiment is used consisting of H, R, PZA,S and E. Resistance to first line anti-TB drugs has been linked to mutations in at least 10 genes; katG, inhA, ahpC, kasA and ndh for H-resistance; rpoB for R-resistance, embB for EMB resistance, pncA for PZA resistance and rpsL and rrs for S- resistance.

Isoniazid is a pro-drug that is activated to different oxygen free radicals and organic radicals which then attack different targets. The primary target of inhibition is the cell wall mycolic acid synthesis pathway, where enoyl-acyl carrier protein reductase (ACP) (*Inh*A) was identified as the target of Isoniazid inhibition (Zhang *et al.*, 1992). This is an important enzyme for the synthesis of the cell wall (Heiym *et al.,* 1993). The active species for *Inh*A inhibition has been found to be isonicotinic acyl radical, which reacts with nicotinamide adenine dinucleotide (NAD) to form H-NAD adduct and then inhibits the *Inh*A enzyme (Rozwarski *et al*., 1998). The reactive species produced during H activation could also cause damage to DNA, carbohydrates, and lipids (Zhang, 2005) and inhibit NAD metabolism (Winder and Collins, 1968). Isoniazid and Rifampicin have high early bactericidal activity on rapidly proliferating bacilli (Mitchison, 2000). These drugs account for killing of approximately 95% of bacilli in the first two days of treatment. These drugs, therefore, effectively prevent transmission of tuberculosis during early weeks of treatment. **Rifampicin** is an efficacious drug that binds to the -subunit of RNA polymerase and inhibits the process of transcription (Zhang, 2005).

Pyrazinamide (Z) on the other hand, is a pro-drug that is activated to pyrazinoic acid by Pyrazinamidase (Konoko *et al.,* 1967). The mechanism of action for PZA is not fully understood but one possible mechanism is believed to be inhibition of fatty acid synthase I (FASI), an enzyme involved in fatty acid biosynthesis (Zimhony *et al.,* 2000). Another mechanism suggested is cytoplasmic acidification and has effect on membrane energy metabolism (Zhang, 2005). Rifampicin and Pyrazinamide are drugs that are particularly effective in eliminating special subpopulations of bacilli such as those in semi-dormant states and in acidic environment respectively. These drugs have the highest sterilizing effect and often kill the last few bacilli during the course of treatment. Therefore, they effectively shorten duration of treatment (Mitchison, 2000). **Streptomycin,** an aminoglycoside antibiotic, primarily interferes with protein synthesis by inhibiting initiation of mRNA translation, facilitating misreading of the genetic code and damaging the cell membrane (Zhang, 2005). The site of action is in the small 30S subunit of the ribosome, specifically at ribosomal protein S12 (*rps*L) and 16S rRNA (*rrs*) in protein synthesis (Garvin *et al.,* 1974).

Ethambutol is one of the first-line anti-TB drugs which inhibit cell wall synthesis. Ethambutol inhibits arabinosyl transferase, an enzyme important for cell wall synthesis (Telenti *et al.,* 1997). It acts on rapidly proliferating organisms and less effective drug compared to other drugs. All first-line anti-TB drugs are said to be bactericidal except Ethambutol which is bacteriostatic (Zhang, 2005). The treatment regimen that is given for smear positive category I patients is two months of treatment with R, H, Z with S or E, followed by six months of E and H. In the continuation phase the semi-dormant bacilli are more common than in the rapidly proliferating bacilli (Mitchison, 2000). There are also recommendations to replace E with R in the continuation phase for enhanced efficacy. This is expected to shorten the duration of treatment by two months (Mitchison, 2000).

2.7.2 SECOND LINE DRUGS USED IN TB TREATMENT

According to the WHO second line drugs can be classified as: Aminoglycosides (kanamycin and amikacin). Polypeptides (capreomycin, viomycin and enviomycin). Fluoroquinolone (ofloxacin, ciprofloxacin, and gatifloxacin), D-cycloserine and ethionamide (ethionamide and prothionamide) (WHO, 2001). Unfortunately, second-line drugs are more toxic and less efficient than first-line drugs (WHO, 2001). As stated, second line drugs are mostly used in the treatment of MDR-TB and as a result prolong the total treatment time from 6 to 9 months (Cheng *et al.*, 2004). In different studies showed that quinolones target and inactivate DNA gyrase, a type II DNA topoisomerase (Cynamon and Sklaney, 2003; Ginsburg *et al.*, 2003; Rattan *et al.*, 1998). DNA gyrase is encoded by gyrA and gyrB (Rattan *et al.*, 1998; Takiff *et al.*, 1994) and introduces negative supercoils in closed circular DNA molecules (Rattan *et al.*, 1998; Ramaswamy and Musser, 1998). The quinolone resistance-determining region (QRDR) is a conserved region in the gyrA (320bp) and gyrB (375bp) genes (Ginsburg *et al.*, 2003) which is the point of interaction of FQ and gyrase (Ginsburg *et al.*, 2003). Mutations in codon 90, 91, and 94 of gyrA are related with resistance to FQs (Takiff *et al.*, 1994; Xu *et al.*, 1996). Aminoglycosides Kanamycin (KAN) and Amikacin (AMI) are aminoglycosides which inhibit protein synthesis and thus cannot be used against dormant *M. Tuberculosis*. Aminoglycosides bind to bacterial ribosomes and agitate the elongation of the peptide chain in the bacteria. Mutations in the rrs gene encoding for 16s rRNA are associated with resistance to KAN and

AMI. In the study done by Suzuki *et al.,* 1998, it showed that nucleotide changes at positions 1400, 1401 and 1483 of the rrs gene is specifically associated with KAN resistance. A change of A→G at codon 1400 in the rrs gene showed resistance to KAN of MICs more that 200 μg/ml (Taniguchi *et al.,* 1997; Suzuki *et al.,* 1998).

2.7.3 ANTI-TB DRUG RESISTANCE

Drug resistance is defined as a decrease in susceptibility of an isolate to a sufficient degree to be reasonably certain that the strain concerned is different from a wild strain that has never come in contact with the drugs (WHO, 1997). Generally, when one percent or more of an isolates are resistant to an anti-TB drug, therapeutic success is less likely to occur. The isolate is then considered resistant to the drug (Canetti *et al.,* 1963).

2.7.4 MECHANISM OF ANTI-TB DRUG RESISTANCE

Resistance of *M. tuberculosis* to anti-TB drugs is a man-made amplification of a natural phenomenon. Wild strains of *M. tuberculosis* that have never been exposed to anti-TB drugs are almost never resistant, though natural resistance to specific drugs has been documented for *M. bovis* and other atypical mycobacteria (WHO/IUATLD, 2000). During bacterial multiplication, resistant bacilli evolve through spontaneous mutation and with defined frequency. Mutation that results in H resistance to *M. tuberculosis,* for example, occurs at a rate of 10^{-7} to 10^{-9} per cell division and leads to an estimated resistance of 1 in 10^{6} bacilli in drug-free environment (Lambregts-van Weezenbeek *et al.,* 1998). Thus, resistant organisms (mutants) evolve in the absence of antimicrobial exposure. However, these mutants are diluted within the majority of drug-susceptible mycobacteria since bacillary populations greater than 10^{7} are common in the lung of TB patients (Canetti, 1965). The presence of a single antimicrobial drug, to which mutation has been developed, provides the selective pressure that helps resistant organisms to predominate. This process could especially be common in situations where there is a large load of bacilli such as in cavities (Howard *et al.*, 1949). Exposure to a single anti-TB drug due to irregular intake, poor drug quality, inappropriate prescription and/or poor adherence to treatment could result in functional monotherapy (Howlett *et al.*, 1949). This will suppress the growth of bacilli susceptible to that drug but permits the multiplication of drug resistant organisms (Crofton and Mitchison, 1948; Mitchison, 1950). This phenomenon is called *Acquired Drug Resistance* (secondary drug resistance). Subsequent transmission of such bacilli to other persons may lead to disease which is drug-resistant from the outset, a phenomenon known as *Initial Resistance* (Figure 2.4).

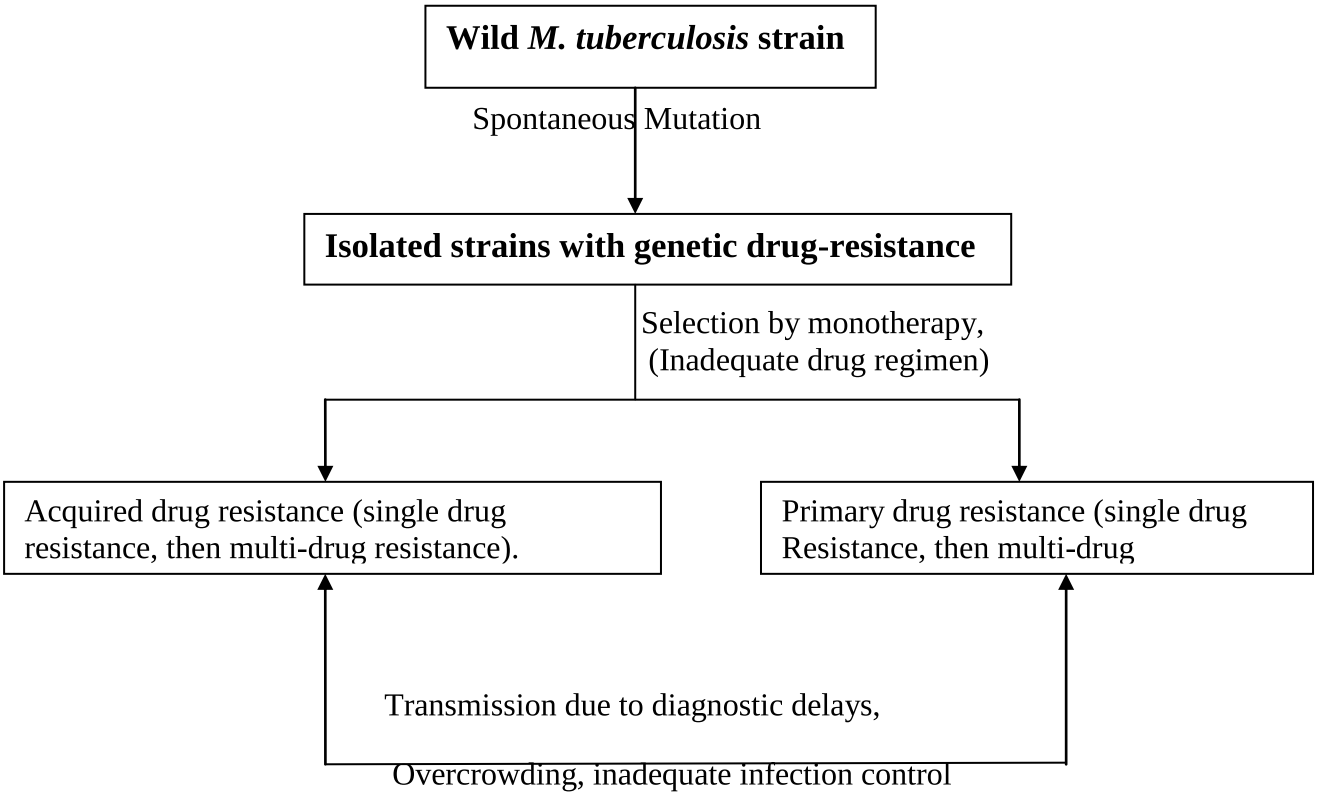

Figure 2. 4 Development and spread of drug and multi- drug resistance tuberculosis (Global project on anti -TB drug resistance Surveillance 2000)

When this process is repeated through the same process, multiple drug or multidrug-resistance will be developed. Every drug effective against TB is bound to select for resistant bacilli (Canetti, 1965).

Treatment of MDR-TB is a difficult and an expensive process. It can take up to two years to treat with drugs that are less potent and more toxic than regular TB drugs (Jenkins et *al.*, 2011). The drug regimen for treating drug-resistant TB depends on the exact drugs the patient is resistant to. The drugs that can be used as part of the treatment regimen are isoniazid (H); rifampicin (R); pyrazinamide (Z); ethambutol (E); streptomycin (S); ciprofloxacin or ofloxacin or moxifloxacin or gatifloxacin (FQ); injectable agents like Streptomycin or kanamycin or amikacin or capreomycin or viomycin; and second-line drugs such as rifabutin, ethionamide, prothionamide, *para*-amino salicylic acid, D-cycloserine and thiacetazone (Zignol *et al.,* 2011). Treatment regimens can include anywhere from three to many of these drugs at a time and last anywhere from six months to over two years (Ahmad *et al.,* 2010). Patients must be closely monitored to ensure they follow the treatment regimen exactly. The outcome of the treatment depends on the type of drug-resistance, the availability of the previously listed drugs, and the patient's adherence to the therapy (Migliori *et al.*, 2009). In extreme cases, surgical resection of the infected lung tissue has been used as treatment. This treatment method is only used in cases with severe drug resistance with a high probability of medication therapy failing, localized disease with good postoperative expectations for lung function, and ample activity of TB drugs to ensure proper healing of the surgery stump. Surgical measures should only be offered after medication treatment has been tried for at least three months (Migliori *et al.*, 2009).

2.8 PROBLEMS ASSOCIATED WITH DRUG RESISTANT TB

Due attention should be given to anti-TB drug resistance since the situation causes different problems such as failing on treatment, relapse after cure and increased likelihood of transmission as some of the risks that affect the effectiveness of TB control programs (Noeske and Nguenko, 2002).

2.8.1 TREATMENT FAILURE AND DEATH

In a study done by Noeske and Nguenko, 2002, patients with drug resistant TB are observed frequently to fail on treatment. Noeske and Nguenko, 2002 state that, patients with MDR-TB specifically were 15 times more likely to fail on treatment than patients with drug sensitive TB. Death was also significantly higher in patients harbouring MDR-TB. Particularly, life span of HIV patients with drug resistant TB is short (Fischl *et al.*, 1992). Treatment failure was also more common in other types of resistance than MDR-TB. An approximately linear increase in the likelihood of treatment failure was observed as the number of drugs to which the strains were resistant increased (Espinal *et al.*, 2000).There are also reports indicating that around 90% of re-treatment cases failing on the standard re-treatment regimen have MDR-TB (Espinal *et al.*, 2000). Moreover, around 65% of patients with no initial resistance or other types of resistance than MDR-TB will acquire MDR-TB at failure. In general failure cases on 2SRHZ/6HE had a high prevalence of MDR-TB (80%), half of which was primary drug resistance and the remaining half was acquired (Quy *et al.*, 2003).

2.8.2 RELAPSE

Quy et al., (2003) state that, relapse after cure was observed more frequently in patients with drug resistant TB compared to patients with drug sensitive TB. An estimated 77% of patients with at least one anti-TB drug resistance will develop relapse (Quy *et al;* 2003). Among treatment relapse cases, MDR-TB was much less common (8%, all of them acquired) compared to the treatment failures. Combined primary Isoniazid and Streptomycin resistance was a strong risk factor for relapse and for acquired MDR-TB among relapse cases (Quy *et al;* 2003).

2.8.3 INCREASED LIKELIHOOD OF TRANSMISSION

Smear positive TB patients are more likely to transmit TB to others than smear negative patients (Grzybowski *et al.,* 1975). Under a successful treatment, patients convert to negative smear and stop transmission within an average of two weeks of treatment initiation (Sacks *et al.,* 2001). Factors such as the presence or absence of cavitary disease and drug resistance dictate the rate of conversion to negative smear. TB patients with drug resistance will be slower to smear conversion since sterilization by first line anti-TB drugs is rendered difficult (Sacks *et al;* 2001). Therefore, these patients will transmit their drug resistant TB to individuals in their vicinity resulting in higher incidence of TB, and specifically of drug resistant ones.

2.9 CONTROL OF TB

2.9.1 THE GLOBAL TB CONTROL STRATEGY

In 2006, the WHO started a new strategy to stop the spread of tuberculosis. This strategy was based on the, Directly Observed treatment System (DOTS), and a well-known TB approach launched by WHO in 1995. Since its inception, more than 22 million patients have been treated under DOTS-based service (WHO, 1995). The new strategy consists of six points which are built on the success of the DOTS program (WHO, 2007). In addition to the six points, the program also takes into consideration certain challenges like TB/HIV, equity and quality constraints, and adopts evidence. It is also founded on innovations from engaging with private-care providers, empowering affected people, communities and helping to strengthen health systems, and promote research.

2.9.2 COMPONENTS OF THE STOP TB STRATEGY (WHO 2007)

1. Pursuing high-quality DOTS expansion and enhancement.

Making high-quality services widely available and accessible to all those who need them. The poorest and most vulnerable requires DOTS expansion to even the remotest areas. In 2004, 183 countries (including all 22 of the high-burden countries which account for 80% of the world's TB cases) were implementing DOTS in at least each part of the country.

2. Addressing TB/HIV, MDR-TB and other challenges.

Addressing TB/HIV, MDR-TB and other challenges requires much greater action and input than DOTS implementation and is essential to achieving the targets set for 2015, including the United Nations Millennium Development Goal relating to TB (Goal 6; Target 8).

3. Contributing to health system strengthening.

National TB control programmes must contribute to overall strategies to advance financing, planning, management, information and supply systems and innovative service delivery scale-up.

4. Engaging all care providers.

TB patients seek care from a wide array of public, private, corporate and voluntary health-care providers. To be able to reach all patients and ensure that they receive high-quality care, all types of health-care providers are to be engaged.

5. Empowering people with TB and communities.

Community TB care projects have shown how people and communities can undertake some essential TB control tasks. These networks can mobilize civil societies and also ensure political support and long-term sustainability for TB control programmes.

6. Enabling and promoting research.

While current tools can control TB, improved practices and elimination will depend on new diagnostics, drugs and vaccines.

2.9.3 BASIC PRINCIPLES OF TB CONTROL IN TANZANIA

Early case finding and adequate treatment of tuberculosis patients is the corner stone of tuberculosis control (NTLP, 2006). The aim of treatment is to cure TB patients, to prevent death from active TB or its late effects and to prevent further transmission of tuberculosis to the community. The DOTS strategy is the gold standard to achieve these aims and to prevent the development of anti-TB drug resistance. In order to achieve effective treatment of tuberculosis, adequate chemotherapy should be prescribed in appropriate combination of at least three anti tuberculosis drugs (Mono-therapy must be avoided). Every confirmed tuberculosis patient should take the drugs regularly for a sufficient period of time (NTLP, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 STUDY DESIGN

This was a case study utilized retrospective data of previously diagnosed cases of MDR-TB where all available archived MDR-TB isolates were retrieved from the Central Tuberculosis Reference Laboratory at Muhimbili National Hospital for further analysis. The characteristic to be investigated was proportion of drug resistance among newly and re-treated MDR-TB Banked isolates from TB patients with and without HIV infection.

3.2 STUDY AREA

The United Republic of Tanzania is a union between Tanganyika and Zanzibar, which was formed in April 1964 after attaining independence in 1961. It is the largest country in East Africa, occupying an area of about 945,087 sq. km, and has common boarders with eight neighbouring countries; Kenya and Uganda to the north; Rwanda, Burundi and Democratic Republic of Congo to the west, Zambia, Malawi and Mozambique to the South. The country's eastern border is formed by the Indian Ocean. The country is divided into 30 administrative regions, five on the semi-autonomous islands of Zanzibar and 25 on the mainland in the former Tanganyika. It lies between the latitudes 1°S and 12°S and longitudes 30°E and 40°E. The results of this study is helpful in designing polices in order to reach patients across the country particularly when dealing with diseases of public health importance such as MDR-TB and XDR-TB.

3.3 STUDY PERIOD

The study was carried out between November 2014 and July 2015. The study and the recruitment process started after obtaining ethical clearance.

Figure 3.1 Map of Tanzania showing regions and bordering countries (Source: www.google.com)

3.4 STUDY POPULATION

The study targeted all isolates confirmed MDR-TB patients who were diagnosed between January 2011 and December 2014.

3.5 CASE DEFINITIONS

A new case is defined as a patient who has never had treatment for TB before or has been on treatment for not more than four weeks.

Relapse case is defined as a patient declared cured or treatment completed but who reports back to the health facility and is found to be acid-fast bacilli (AFB) positive.
A resistant case is defined as any individual who was resistant to any one of the drug tested either as single drug or in a combination of two or more drugs.

Primary drug resistance is the presence of resistant strains of M. TB in a newly diagnosed patient who has never received TB drugs or has received them for less than one month of treatment.

Acquired or secondary drug resistance is the resistance found in a patient who has previously received at least one month of TB therapy.

Multi-drug resistant TB (MDR-TB) is defined as disease due to M.TB that is resistance to at least two first-line drugs; rifampicin and isoniazid (WHO, 2011).

3.6 SELECTION CRITERIA

3.6.1 INCLUSION CRITERIA

All MDR-TB isolates available at CTRL archives that grow on subculture in a plane LJ media were included for the study.

3.6.2 EXCLUSION CRITERIA

All non MDR-TB isolates that were found in the CTRL archives

All MDR-TB isolates that did not grow on subculture of LJ

3.6.3 SAMPLE SIZE

All MDR-TB Isolates which met the selection criteria were selected a total of 278 MDR-TB Isolates were included in the study.

3.7 DATA COLLECTION

3.7.1 SOURCE OF ISOLATES

A record review of laboratory Drug Sensitivity Test (DST) records was done to find MDR-TB isolates identified at the CTRL TB Laboratory between January 2011 and December 2014. The list of MDR-TB isolates generated from the record review was used to locate and retrieve the archived isolates from the laboratory repository. Only the first MDR-TB isolate for each patient were included, excluding multiple isolates from the same patient. Another record review of "request for examination" forms submitted to the laboratory along with the original patient specimen was used to collect patient demographics. Data on age, gender, TB treatment history, and region of origin were collected using a coded form.

3.7.2 PREPARATION OF LÖWENSTEIN–JENSEN MEDIUM

Löwenstein-Jensen (LJ) medium is most widely used for mycobacterial culture and is recommended by WHO. Different ingredients can favour different growth: LJ containing glycerol (Stonebrink) favours the growth of *M. tuberculosis*; LJ without glycerol but with pyruvate encourages the growth of *M. bovis.* Both should be used in countries or regions where patients may be infected with either organism. We used the commercially available mineral salt solution and the 2% malachite green solution. The mineralized solution was sterilized by autoclaving at 121 °C for 30 minutes and then cooled to room temperature. This solution was stored in the refrigerator. Fresh hen's eggs of not more than 7 days old were used where by firstly, they were cleaned by scrubbing thoroughly in warm water with a plain alkaline soap, then rinsed with water and soaked in 70% ethanol for 15 minutes. To avoid contamination, the eggs were cracked with a sterile knife into a sterile flask and beaten with a sterile blender for 5 minutes. For preparing 600 slants, 75 eggs were used. All the prepared solutions were well mixed in a large sterile flask; eggs were added by filtering them through a sterile fabric. Then 6ml of the solution was distributed in the McCartney bottles and coagulated in the inspissator for not more than 45 minutes to avoid sedimentation of the heavier ingredients. Before inspissations of the prepared medium, the inspissator was heated to 80 °C, then McCartney bottles with 6ml of the solutions were placed in a slanted position (5-10° to the horizontal) in the inspissator. The medium was coagulated for 45minutes at 85^{o}C. Since the medium had been prepared using sterile procedures this heating was to solidify the medium and not to sterilize it. A relative humidity of 80% is necessary for good heat transmission. To check sterility, we incubated each new batch of media at 35–37 °C for 48 hours. Growth of any organism indicates poor procedure; Results of the sterility were recorded on the appropriate form.

3.7.3 PREPARATION OF DRUG TO BE USED IN MEDIUM

Susceptibility testing for rifampicin, Ethambutol, Kanamycin, Ofloxacin, Streptomycin and Isoniazid were performed. Working drug dilutions were prepared on the day of use; 1 ml of working solutions was added to 500 ml of L-J medium to yield final concentrations of isoniazid 0.2μg/ml, rifampicin 40μg/m, Ethambutol 2μg/ml, Kanamycin 30.0 μg/ml, Ofloxacin 2.0 μg/ml and Streptomycin 4 μg/ml. (WHO,2008;Sangaré,2011). The control medium without drugs was prepared at the same time as the drug-containing media. The period of validity of media stored at 4^0C was one month. The slopes were Incubate at 37^0C for 6 weeks. After incubation at 37^0C for 6 weeks, the slopes were read and the isolate was considered to be sensitive if there was no growth or less than 1% of colonies grew compared to the drug free controls, and resistant if 1% or more of the colonies are growing compared to the drug free controls. DST was repeated for strains with less than 5 of more than 100 colonies on the most diluted control slopes.

Figure 3. 2 McCartney bottle contain L-J media during inspissations

3.7.4 MDR-TB ISOLATE RECOVERY FROM STORAGE MEDIA

Isolates were retrieved from the freezer and allowed to thaw to room temperature. Slopes were incubated for a maximum of 4 weeks and checked weekly for growth. Pure colonies of positive isolates were harvested by scraping the colonies of MTB from LJ slopes for use as inoculum for 2nd line DST.

Figure 3.3 McCartney bottles contain L-J media with resistant strain of TB.

3.8 DATA STORAGE AND ANALYSIS

Social demographic and clinical data of all MDR-TB isolates received at CTRL from January 2011 to December 2014 were extracted from the standardized program registers together with clinical data. The data was double-entered into a Microsoft excel database, validated and subsequently imported into STATA version 12.0 for statistical analysis. The chi-square (χ^2) was used to test for association between variables. Adjusted analysis of proportional was made by logistic regression. The odds ratio was used to measure the degree of association. Hypothesized risk factors with variables $p < 0.05$ were considered as having significant association with MDR-TB. A probability of <0.05 was considered significant. The map of distribution of MDR-TB isolate was generated by using Arch GIS version 9.1.

3.9 ETHICAL CONSIDERATION

Approval and clearance was sought from Muhimbili University of Health and Allied Sciences (MUHAS). Information obtained was treated with confidentiality. Confidentiality was assured through the use of codes in records. The results that were obtained were sent to Muhimbili national hospital and Ministry of health and social welfare.

CHAPTER FOUR

4.0 RESULTS

4.1 DESCRIPTIVE RESULTS

4.1.1 TREATMENT HISTORY

A total of 278 MDR-TB isolates were identified and retrieved from the CTRL archive. These originated from across 20 regions of Tanzania. The general tuberculosis prevalence was seen to vary markedly among these 20 regions (P = 0.0001), with Dar-es-Salaam contributing over 55.4% of the cases screened at the CTRL, followed by Kilimanjaro at 23.4%. Of these 21.6% (95% CI = 16.8 - 26.5%) were isolated from TB new cases and 78.4% (95% CI = 73.5 - 83.2%) were isolated from TB retreated cases sent in from different regions all over the country (Table 4.1).

Table 4.1 Proportion of MDR-TB according to treatment history

Treatment history	*n*	Percent%	95%CI
TB new case	60	21.58	16.78 -26.54
TB Retreated cases	218	78.42	73.46 - 83.22

4.2 DEMOGRAPHICS OF MDR-TB ISOLATES

4.2.1 SEX

Laboratory records of all 278 identified isolates were investigated for demographic data. The study population consisted of 70.8% (95% CI: 65.4-76.2) males = 196 and 29.2 % (95% CI: 23.9-34.6) females = 81, with gender data not available for 0.4 %

(95% CI: 0.0-1.1) of the isolates (Table 4.2). All 278 isolates grow after subculture onto fresh L-J media, and these were included in second line DST tests.

Table 4. 2 Proportion by Sex of MDR-TB *n* = 278

Sex	*n*	Percent%	95% CI
Male	196	70.76	65.37-76.15
Female	81	29.24	23.85-34.63
Unknown	1	0.36	0.0-1.10

4.2.2 AGE

The median age and interquartile range (IQR) was 33 (27 to 45) years. The isolates were categorized into four groups with the following populations in each group: age 0-20 years: 38 cases 13.8 %(95% CI: 09.6 - 17.7) age 21-40 years: 149 cases 53.6 % 95% CI: 47.7 - 59.5), age 41-6 years: 76 cases 27.3% (95% CI: 22.1 - 32.6) and 61-100 years: 15cases 5.4% (95% CI: 2.7 - 08.1) (Table 4.3)**.**

Table 4. 3 Proportion of MDR-TB by age categories

Age Categories	*n*	Percentage (%)	95% CI
0-20	38	13.67	(09.6-17.7)
21-40	149	53.6	(47.7-59.5)
41-60	76	27.34	(22.1-32.6)
61-100	15	5.40	(2.7-08.1%)

MDR-TB was seen to mostly affect young adults, in their most reproductive **age,** as reflected in Figure 4.1.

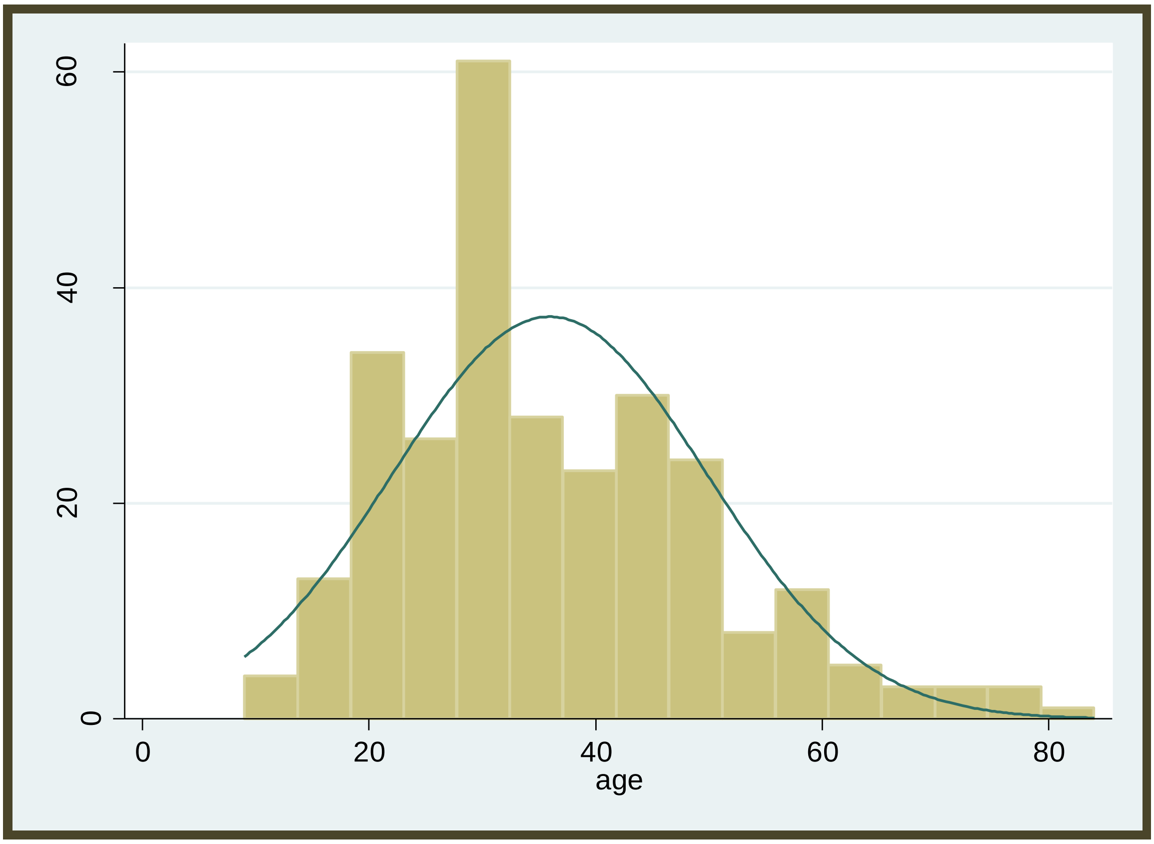

Figure 4. 1 Show distribution of MDR-TB by Age Category.

4.3 GEOGRAPHICAL DISTRIBUTION OF MDR-TB ISOLATE

It was observed that there were more MDR-TB cases from region of Dar-es-salaam and Kilimanjaro as shows on figure 4.2. Almost all the regions had at least reported one or more case of MDR-TB whilst the latter two regions had the highest frequency of cases (Figure 4.2).

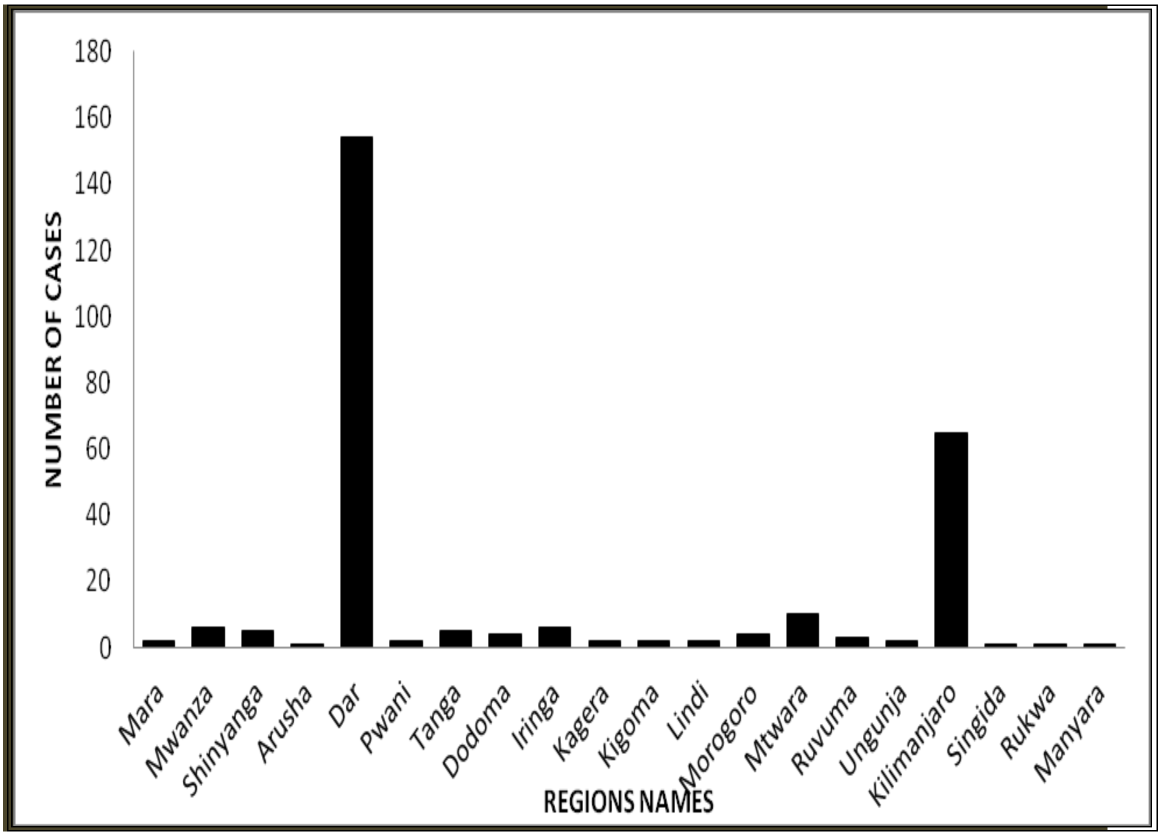

Figure 4. 2 The geographical regions showing distribution of MDR-TB isolates

4.4 EFFECT OF AGE ON THE DISTRIBUTION OF MDR-TB ISOLATES

The proportion of MDR-TB differed across regions depending on the factors that favoured its occurrence. In our findings it shows that there was an association that existed between region of origin of the isolates, age and MDR-TB prevalence. This is further confirmed using a Lowess- smoother scatter plot incorporating a trend line (Figure 4.3). Both charts (Figures 4.2 and 4.3 Scatter plot) show that increase in MDR-TB positivity is correspondingly associated with an increase in the region of origin and the most important indicator is the mean age of the patients as shown along the trend line (Figure 4.3). The effect of MDR-TB prevalence was markedly noted in the isolates that were found to come from Kilimanjaro. Where the likelihood of getting at least one positive isolate correspondingly increased with the increase in the region with MDR-TB referral services.

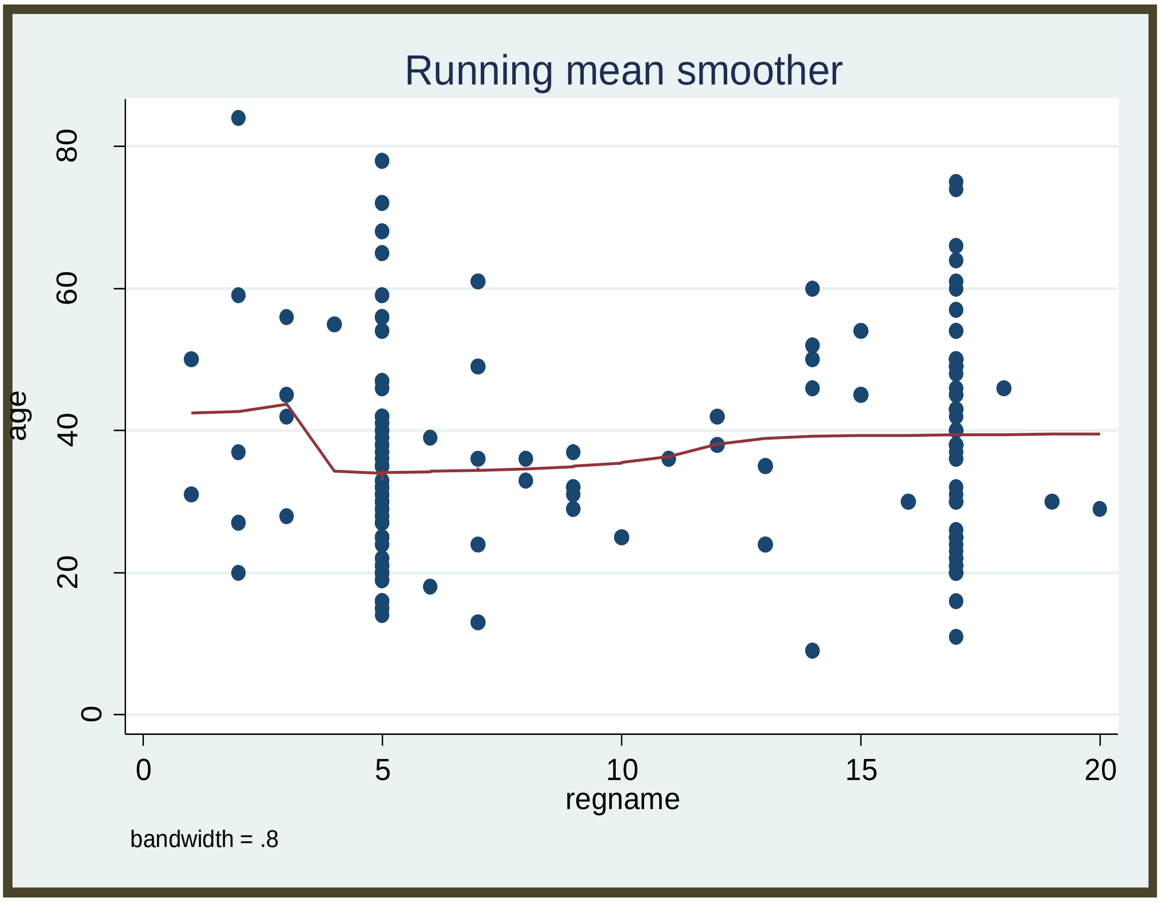

Figure 4. 3 The frequencies of MDR-TB Isolates according to region of origin using a mean Smoother Plot

4.5 SPATIAL DISTRIBUTION OF MDR-TB IN TANZANIA

The number of MDR-TB isolates used in our study are those that had occured in Tanzania from 2011-2014 in both new and re-treated TB cases sent to CTRL from different regions of the country. From our findings five regions of Dar-es-salaam, Kilimanjaro, Mtwara, Mwanza, Tanga and Shinyanga all together account for 86.7% of the overall MDR-TB Cases in Tanzania. The distribution of MDR-TB cases among Tanzanian regions are shown in the map of Tanzania(Figure 4.4).

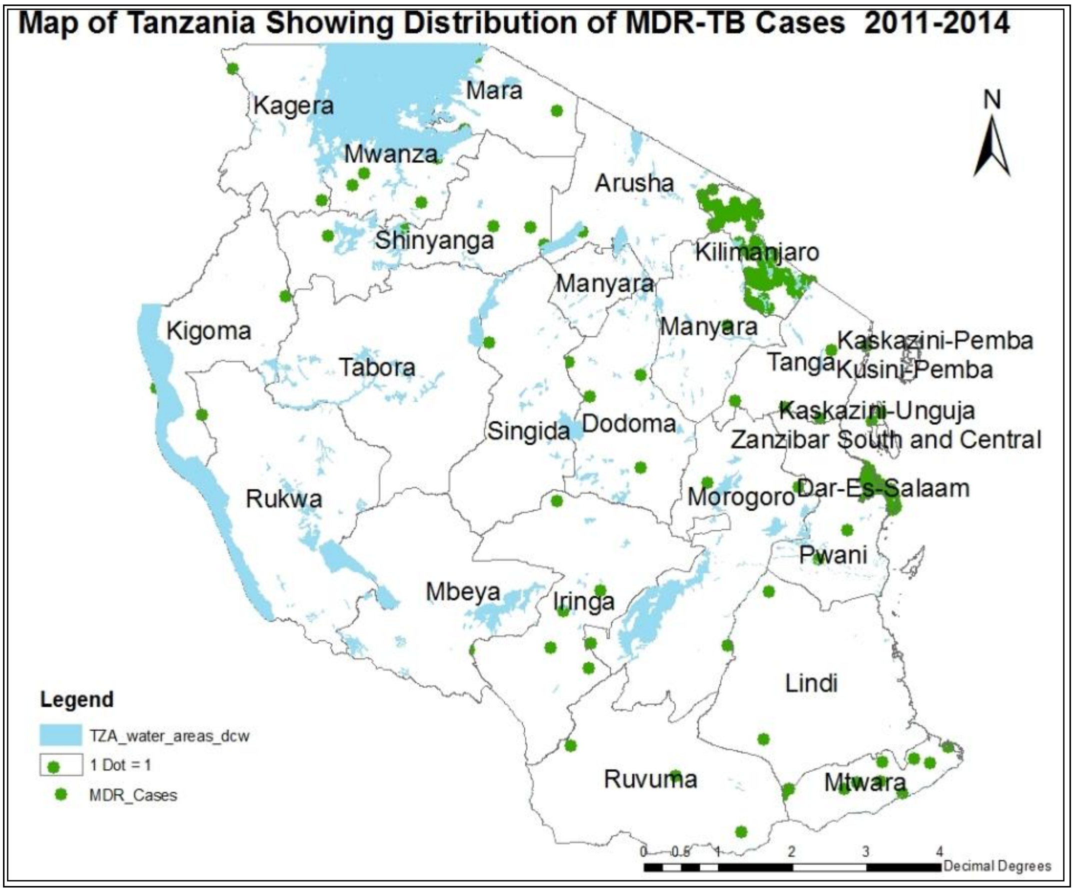

Figure.4.4 Map of Tanzania showing Distribution of MDR-TB case by regions

4.6 PROPORTION OF DST FIRST LINE PROFILE

Approximately twenty percent [21.6% (95% CI = 16.7 -26.4%)] of the isolates were resistant to isoniazid and rifampicin alone, 7.2% (95% CI = 4.1 - 10.3%) were resistant to isoniazid, rifampicin, and ethambutol only, and 21.94% (95% CI = 17.0-26.8%) were resistant to isoniazid, rifampicin, and streptomycin only, with 48.92% (95% CI = 43.0 - 54.8%) being resistant to all four first line TB drug (Table 4.4)

Table 4. 4 Proportion of DST first line profile

DST Profile	Number of Isolates	Percentages (%)	Cumulative	[95 % C I]
Undetermined	1	0.36%	0.40	0.0-1%
1=HR	60	21.58%	21.98	16.7-26.4%
2=HRE	20	7.19%	29.17	4.1-10.3%
3=HRS	61	21.94%	51.11	17.0-26.8%
4=HRES	136	48.92%	100	43.0-54.8%

R= Rifampicin; H = Isoniazid; E = Ethambutol; S =Streptomycin

4.7 LEVEL OF DRUG RESISTANCE PATTERN BY TYPE OF DRUGS AND TB TREATMENT HISTORY

Resistance to all four drugs, rifampicin(R), isoniazid (H), ethambutol (E) and streptomycin(S) (RHES) was observed in 136(48.9%). Resistance to RHS was 61 (21.9%).

Table 4.5 Level of drug resistance pattern

Drug Resistance	All cases combined		New cases		Retreated cases	
	No.	%	New	%	Retreated	%
Undetermined Drug	1	0.36	0	0	1	0.46
Resistance to HR	60	21.58	14	23.33	46	21.10
Resistance to HRE	20	7.19	11	18.33	9	4.13
Resistance to HRS	61	21.94	14	23.33	47	21.56
Resistance to HRES	136	48.92	21	35.00	115	52.75
Total	278	100	60	100	218	100

R = Rifampicin; H = Isoniazid; E = Ethambutol; S = Streptomycin

Resistance to RHE was 20 (7.2%) and to RH was 60(21.6%) of all tested isolates. Overall XDR-TB was observed in 1/278 (0.4%). Among 278 MDR-TB isolates tested, 60 were new cases and 217 were retreated cases (Table 4.5)

4.8 PREVALENCE OF XDR-TB AMONG MDR-TB ISOLATE

At CTRL in Muhimbili national hospital, a total of 278(100%) isolates were successfully sub cultured on LJ media and subsequently tested for second line drug resistance. Only 1 isolate was resistant to Kanamycin and Ofloxacin, representing the prevalence level of 0.36% (95% CI = (0.0 – 1.1%) and met the definition of XDR-TB. Origin of the isolate was found to come from Kilimanjaro region (Table 4.6).

Table 4. 6 Proportion of DST second line profile

DST Second Line Profile	(sample against DST)	Percent (%)	95% CI
Isolates sensitive to Second line Drugs	277	99.64	(98.9 – 100.0)
Isolates Resistant to Second line Drugs	1*	0.36	(0.0 - 1.1)
Total	278	100	

Note: * = *Drug resistance was observed against Kanamycin & Ofloxacin; XDR-TB=1/278(0.36% 95% [CI 0.0-1.1%])*

4.9 RISK FACTORS ASSOCIATED WITH MDR-TB

4.9.1 UNIVARIABLES ASSOCIATED WITH MDR-TB OCCURRENCE

Generally MDR-TB was seen to vary markedly among the 20 regions P = 0.0001, with Dar-es-Salaam contributing over 55.4% of the cases screened to the CTRL, followed by Kilimanjaro at 23.4%. The prevalence of XDR-TB was calculated as 0.36% [95% CI: 0.0-1.0%] and this was associated with second highest TB cluster of

Kilimanjaro region. Apart from regions, other key factors linked to the TB prevalence included sex P =0.041, with males being twice likely to have TB than females (OR = 1.85, 95%CI [1.03-3.35], P = 0.041). Age was another factor associated with high prevalence with the reproductive active group between 20 to 40 years being more likely to be associated with MDR-TB than any other age category (OR= 0.5, 95%CI [0.29-1.0] P = 0.05. Other key factors found to hinge on MDR-TB included the patients' treatment history. Patients with history of re-treatment were less likely to be positive for MDR-TB (OR = 0.93, 95%CI [0.89 -0 .978]) P = 0.004.

4.9.2 CRUDE DESCRIPTION OF RISK FACTORS FOR MDR/XDR-TB OCCURRENCE

Patients from Dar-es-Salaam and Kilimanjaro regions combined and then compared to other regions were found to be significantly associated with MDR-TB cases ($\chi 2$ = 9.21) P = 0.001). Crude odds ratio of occurrence of MDR-TB under univariate analysis revealed a higher proportion of males being positive than their female counterparts (cOR 1.85, 95% [CI 1.03-3.35]) P = 0.026 whilst age range of between 21– 40 was similarly significant. Isolates from patients with retreated cases of TB treatment (cOR 0.38, 95% [CI 0.19 - 0.79]) P = 0.009 were highly associated with region of origin. On risk ratio univariate analysis, age range in the reproductive group were found to be two times more likely to test positive for MDR-TB than those outside this category (Relative risk = 2.98; CI = 1.82-4.29); P = 0.0016, just like area of origin/region (Relative risk = 2.50; CI = 1.36-4.76); P = 0.0014.

4.9.3 MULTIVARIABLE LOGISTIC REGRESSION MODEL

Variables considered to be significant on univariate analysis were assessed via a multivariable logistic regression model. All the main variables with their interaction-variables were included as predictors in the model, as each variable or its interaction could affect the odds of a patient being positive for MDR-TB. All the variables and their interactions were previously analyzed using contingency tables and those that were statistically significant (P < 0.05) on univariate analyses were included in the full model (Table 4.7 and table 4.8).

Table 4.7 Factors associated with MDR/XDR-TB

Variable	**MDR-TB=278**	**Univariate**			**Multivariate**		
	***n* (%)**	**cOR**	**95% CI**	**p-Value**	**aOR**	**95% CI**	**p-Value**
Sex	196(70.76)	1.85	1.03-3.35	**0.041**	2.01	1.09 -3.73	**0.026**
Age	149(53.6)	0.54	0.29-0 .97	**0.041**	0.05	0.27- 0 .93	**0.028**
Retreated cases	218(78.42)	0.38	0.19 - 0.79	**0.009**	0.50	0.6 -1.24	**0.07**
Region of origin	20(66.67	0.93	0.89-0 .978	**0.004**	0.93	0.894 -0 .98	**0.005**

cOR, crude odds ratio; aOR, adjusted odds ratio, adjusted for gender, age, treatment history and region of origin. Values in boldface indicate a significant (P<0.25)

The final multivariable logistic regression model identified sex, with males being more at risk than females (aOR 2.01, 95% CI [1.09 -3.73] P= 0.026, age 21–40 years (aOR 0.05, 95% CI [0.27–0.93]) P = 0.028, region of origin of the isolates (aOR 0.93, 95% CI [0.894-0.98]) P = 0.005, to be associated with MDR-TB. Others included previous history of TB treatment (aOR 0.5, 95% CI [0. 6 –1. 24]). In the final model, retreated cases were strongly influenced by area of origin. Most cases of MDR-TB came from the two regions of Dar-es-salaam and Kilimanjaro Tables 4.7 and 4.8.

Table 4.8 Final multivariate multiple regression analysis of MDR-TB positivity

Risk Factor	SE	Odds ratio	P	95% CI
Sex	0.01	2.01	0.026	1.09 -3.73
Age	0.34	0.05	0.028	0.27- 0 .93
Retreated cases	1.22	1.3	0.04*	1 .24 -1.9
Region of origin	0.05	1.0	0.005	0.894 -1.08

Not significant (p>0.05), Likelihood ratio = -36.72 Number of observations in the model = 287 Hosmer – Lemeshow chi2 (8) = 3.87, Prob > chi2 = 0.358

CHAPTER FIVE

5.0. DISCUSSION

The emergence of Multidrug resistant tuberculosis (MDR-TB) and extensive drug resistant tuberculosis (XDR-TB) have had devastating consequences and significant challenges on the progress made in terms of tuberculosis (TB) treatment as well as control and prevention programmes. This epidemiological scenario of TB is worsened by its worldwide distribution with a growing trend displaying new and highly antibiotic resistant strains. Further, the dual infection with HIV and TB has increased the morbidity and mortalities fuelled by this co-infection. It was against this background that this present study was formulated and carried out to investigate the alluded to emerging epidemiological trends of TB in the presence of MDR-TB and XDR-TB in Tanzania as well as to identify the likely risk factors linked to the occurrence and distribution patterns of anti TB drugs resistance. The findings of MDR-TB and XDR-TB in this study in Tanzania have proved and agreed with WHO assertion on the emergence of new epidemiological trends with regards to TB and the development of new and highly antibiotic resistant strains across the global. One important point worth noting is the spatial distribution and disease burden of MDR-TB and XDR-TB. From our results, two main areas are seen to have a high burden of these resistant forms of TB, namely; Dar-es-Salaam and Kilimanjaro regions. Whilst Dar-es-Salaam is the busiest and economic hub for Tanzania, being the commercial capital and a sea-port, Kilimanjaro is a tourist hub. Both these regions demographically are open to a lot of human movement, especially in the economically reproductive age categories. This was seen in our results from univariate analysis and multivariable analyses where middle age group of 21-40 years was a factor linked to MDR-TB occurrence together with region where the patients came from. Looking at a global stage, by 2011, global TB data reported an estimated 9.0% of all MDR-TB cases to be XDR-TB (WHO, 2014). However, very little data is available on DR-TB in Africa due to limited capacity of laboratories in this region to perform DST. Considering that Africa has 25% of the global TB burden, 80% of which is HIV co infected, data on DR-TB prevalence in the region is thus essential to guide planning of TB control and management policies. Under this current study in Tanzania, the TB patients whose MDR-TB isolates were analyzed were predominately young, with a mean age of 33 years (IQR: 27–45). This is in agreement with other studies in Africa that had a similar age distribution (O.Daniel *et al*., 2013, Cox *et al*., 2010) among MDR-TB patients. Study done in Zimbabwe also report similar findings with MDR-TB patients having a mean age of 33years (Sagonda *et al*., 2014). Drug resistance data stratified by age is very important in surveillance of drug resistant trends in a setting since a high proportion of drug resistant cases in young age groups could be indicative of recent transmission, while in older age groups could be an indicator of reactivation of old infections. Further, in this study, a high proportion of “request for examination” forms omitted data on gender (0.4%) and 33.33% data of the total region available were not reported at CTLR. This revealed poor data collection by hospital workers requesting TB examination for their patients. So we cautiously interpret our results with regards to sex owing to this omission. Nevertheless, remote deductions can be made on the results obtained with regards to the influence of sex on MDR-TB cases, which in our present study showed a higher proportion among males than females. This poor recording of demographical data presents a challenge to effective monitoring and surveillance of drug resistance patterns and trends, as patient biographical and clinical data is essential in understanding the factors driving TB and possible risk factors for the disease. Effective reporting of such data reduces the need for resource limited countries, such as Tanzania, to conduct periodical surveys, which are costly and labour intensive. Routinely collected data is easy and usually an efficacious way of monitoring TB drug resistance patterns and trends over time, without the need for expensive surveys. Additionally, our study showed that the largest group 78.42 %(95% CI: 73.46 - 83.22) of MDR-TB isolates was from previously retreated TB cases. This is a big concern as it may mean a high TB treatment failure rate and possible relapses of cases. The figure of close to 80% in Tanzania is extremely high compared to the 20% that of the WHO global estimates of MDR-TB cases coming from re-treated TB cases (WHO, 2014). The proportion of MDR-TB among re-treated cases observed in this study was higher than the global average and this could be explained by a number of factors. The MDR-TB isolates analyzed in this study were archived between January 2011 and December 2014, a period during which Tanzania was going through socio-economic changes after general election of 2010. New government directives, the resource constraints, constituted the major problems for not being able to cope adequately with health problems due to significant increase in the cost of providing health care services. Global financial crisis and change in technology to diagnose and treat tuberculosis especially among patients co-infected with HIV/AIDS and at the same time the emergence of drug resistant strains. Another key finding from this study was that close to 50% of the cases of the MDR-TB isolates showed resistance to all four first line TB drugs (isoniazid, rifampicin, streptomycin, and ethambutol). This scenario coupled with poor patient adherence compounded further by frequent interruptions in drug supplies can be partly blamed to have contributed to the emergence of MDR-TB strains in Tanzania. Patients with such organisms pose a challenge for management and treatment through normal National TB programs which basically rely on the same first line TB drugs. The internationally accepted practice when treating MDR-TB cases is to base the second line regimen on DST results (Sia and Wieland, 2011). Having a large proportion of MDR-TB patients resistant to all first line drugs places a huge financial burden on the nation as second line drugs are often more expensive, requiring extended treatment periods, and are often toxic (Drobniewski *et al*, 2004, Schaaf *et al*, 2009). In this study, the drugs evaluated were ofloxacin and aminoglycosides, kanamycin. Therefore in the context of our study, an XDR-TB strain is the one with resistant either to kanamycin and ofloxacin. Our study identified such resistance patterns, and so there were XDR-TB strains. A large proportion (99.64%) of the MDR-TB isolates showed susceptibility to all second line drugs. The remaining 0.4% of the isolates was shown to be resistant to the second line drugs and met the definition of XDR-TB. Regionally, by late 2012, most of Tanzania's neighbours (Zambia, Mozambique, Kenya, Uganda and Congo DR) had identified at least one XDR-TB strain (WHO, 2012). Tanzania is the economic hub where a large population moves to and from her neighbouring countries, and nearby countries especially South Africa which is one of the countries with the highest MDR-TB burdened countries and has reported a number of XDR-TB outbreaks (Kvasnoslay *et al*.,2011,Shenoi *et al.*, 2012). Therefore this study hypothesizes that XDR-TB strains could have been imported into the country during this period and thus would be identified among MDR-TB isolates archived during that time. However, this study failed to identify more of XDR-TB strains could be due to a number of factors. There has been much debate on the diagnostic accuracy and reproducibility of some of the methods used to perform second line DST (Kahlmeter and Hoffner, 2012; Kahlmeter *et al*., 2006; Pasipanodya and Srivastava, 2012).This lack of consensus has led to the absence of an absolute gold standard for second line DST. Second line DST is especially difficult since the critical concentrations of some of the second line drugs are very close to the minimum inhibitory concentrations (MICs); hence the changes in MIC associated with resistance are very small (Kahlmeter and Hoffner, 2012; Kahlmeter, *et al.*, 2006; Kim, 2005).A lot of research is needed towards standardizing the second line MICs for the various DST methods available. The proportion method on 7H10 Middlebrook has been the gold standard for 2nd line DST in Europe and in America in the past 20 years, but recent WHO guidelines on 2nd line DST recommend the MGIT based DST method (Martin *et al*., 2008, Martin *et al.,* 2009, O'Grad *et al.*, 2011). Regarding MDR-TB this is in line with the findings of an earlier study performed by (Kruuner, 2001). In generating extremely resistant strain, the duration of re-treated TB case treatment seem to play a major role. This is because a risk factors study for XDR-TB was performed only on re-treated patients (Lea, 2007).The presence of XDR-TB was associated with cumulative duration of the re-treatment. In our study among the MDR-TB isolates re-treated cases account for 78.8 %(95% CI: 73.46-83.22) and the only XDR-TB 0.4 %(95%CI: 0.0-1.10) is from the re-treated cases of MDR-TB isolates used in our study. We currently showed that in age group less than 41years, male patients were at higher risk of becoming ill with MDR-TB. Our results are the same to the results of Meta analysis that male gender was found to be a determinant risk factors to MDR-TB (Faustini *et al*., 2006). Contrary to our results an association between MDR-TB and female gender in the whole study population has also been described in a few previous studies (Clark *et al.,* 2005, Lockman; *et al.,* 2001, Mdivan *et al.,* 2008). But in particular High MDR-TB risk in young males has been, to our knowledge, noticed first in our study. Taking into account the type of the society where male and female have equal chance for accessibility to health services; an explanation to this phenomenon is that male patients have poor health seeking behaviour than women; this has been observed in most studies. In a Meta analysis the exposure to difficult to access to health services was found to be one of the strongest risk factor for re-treated cases (Brasiel, *et al.*, 2008). Contrary to this in our study re-treated cases was strongly associated with living in Dar-es -salaam and Kilimanjaro one of hypothesized explanation for this experience is the huge amount of enticement in urban environment that interfere with treatment adherence, despite of better accessibility of medical attention itself.

CHAPTER SIX

6.0. CONCLUSION AND RECOMMENDATIONS

6.1. CONCLUSION

Despite the small sample size to generalize the study results, this study identified at least 0.36% of XDR-TB among 278 MDR-TB isolates. A large proportion 48.93% of the MDR-TB isolates showed resistance to all four first line drugs (isoniazid, rifampicin, streptomycin, and ethambutol), a possible indicator of the future emergence of XDR-TB in Tanzania. The results of this study provide preliminary information about potential risk factors that were found to be associated with MDR-TB status. Further, although the prevalence of XDR-TB seems low, nevertheless, its potential risk to public health is important based on traditional methods of diagnosis found in most of the major Hospitals coupled with poor sanitary measures thus a likelihood of nosocomial spread cannot be ruled out given a general lack of understanding about multidrug resistant strains. The study also identified young age, region of origin and male gender to be associated with MDR-TB. The proportion of XDR-TB in the study population is low 0.4.

6.2. RECOMMENDATIONS

- Second-line anti-TB drugs are required at this point where the resistance pattern in the area is increasing and progressively leading to MDR-TB. This would be appropriate putting into consideration the prevalence of multiple drug resistance among new cases which is greater than 35%.

- It would be appropriate to follow patients from whom Mycobacterial isolates were studied and correlate their treatment response pattern with their drug resistance pattern.
- Current findings on MDR/XDR-TB in Tanzania are essential in guiding the planning & policy implementation on TB control, management and surveillance.
- The government through the Ministry of Health and Social Welfare should strengthen the DOTS services in TB Management.

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