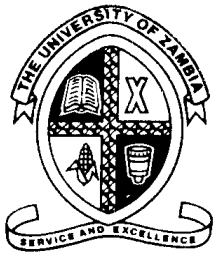

University of Zambia
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Distribution of Drug-Resistant Tuberculosis in Zambia, 2008-2011

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DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF PUBLIC HEALTH

The University of Zambia

Lusaka

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By Thandiwe Ngoma

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Abstract

Background: *Mycobacterium tuberculosis* is treated with four drugs: Isoniazid (INH), Rifampicin (RIF), Pyrazinamide (PZA) and Ethambutol (E). The emergence of drug resistant strains of *Mycobacterium tuberculosis* threatens the success of national tuberculosis (TB) programmes. Zambia has recorded a reduction in the notification rates of all forms of tuberculosis over the years but the burden of multiple drug resistant TB (MDR TB) which is resistance to the drugs isoniazid and rifampicin, with or without resistance to other drugs, is not well defined. This study aimed to describe the distribution of drug resistant TB in Zambia over the four year period 2008-11.

Methodology: This study was a retrospective review of national tuberculosis sputum analysis data from the Chest Disease Laboratories and the Topical Diseases Research Centre for the period 1st January 2008 to 31st December 2011. The variables in the anonymised database were: location of case (province), age, gender, and drug sensitivity test (DST) result to the four first-line anti-TB drugs. Analysis of the data was performed using STATA 11.0.

Results: Of the sputum specimens from 7,579 cases collectively received at the two laboratories, 811 cases had complete DST results. From these 811 cases, 404 (49.8%) were susceptible to all 4 drugs, and 407 (50.2%) had resistance to one or more of the anti-TB drugs. Mono-resistance was most common to isoniazid (INH) (5.3%) and streptomycin (SM) (3.3%). MDR TB was found in 256 (31.6%) of the cases. Over the four years under investigation, MDR TB cases increased from 24.2% in 2008 to 30.9% in 2011. Analysis of MDR TB by gender, location and age showed more cases in males than females (57% vs. 37.9%); more cases in Lusaka (49.3%) and the Copperbelt (30.1%) provinces; and the highest proportion of cases in the 35-49 years age group (31.3%). Three MDR TB cases were recorded for the age group <20 (of 25 cases tested). Being age <20 years was less likely to be associated with MDR TB (p=0.0337). However, MDR TB was not statistically significantly associated with gender or location.

Conclusions: The study showed significant proportions of mono and MDR-TB among the study sample that included drug sensitivity test results on all four anti-TB drugs. Drug resistant TB in Zambia will continue to be a challenge to management and control of TB if not properly managed. Studies on treatment outcomes of mono resistant TB patients need to be performed to inform the current treatment regimens.

Acknowledgements

I thank the Ministry of Health for permitting the use of Ministry data for this study, the Chest Disease Laboratories and Tropical Diseases Research Centre for providing access to their data and to my supervisor Dr. Yusuf Ahmed for his support towards my research.

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Abbreviations

AFB	Acid Fast Bacilli
CDL	Chest Disease Laboratory
DOTS	Direct Observed Treatment Short Course
DRS	Drug Resistance Survey
DR-TB	Drug Resistant Tuberculosis
DST	Drug Sensitivity Test
EMB	Ethambutol
HIV	Human Immunodeficiency Virus
INH	Isoniazid
IUATLD	International Union Against Tuberculosis and Lung Disease
LJ	Lowenstein-Jensen media
MDR-TB	Multi-Drug Resistant Tuberculosis
MGIT	Mycobacteria Growth Indicator Tubes
MoH	Ministry of Health
NTM	Non-Tuberculosis Mycobacteria
PTB	Pulmonary Tuberculosis
PLWHIV	People Living with Human Immunodeficiency Virus
PZA	Pyrazinamide
RIF	Rifampicin
SM	Streptomycin
TB	Tuberculosis
UTH	University Teaching Hospital
WHO	World Health Organization
XDR-TB	Extremely Drug Resistant Tuberculosis
ZN	Ziehl-Neelsen stain

Dedication

I dedicate my work to my parents Mr and Mrs Ngoma, to my brothers Maunga, Reuben and Nephart, and to my good friend Mwansa Lumpa.

CHAPTER ONE: BACKGROUND AND INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by the bacterium *Mycobacterium tuberculosis.* TB is responsible for about 2 million deaths per year with nearly 10 million infections (Levinson and Jawetz 1998; WHO 2012). TB takes two forms based on the location of the disease. Pulmonary TB occurs when the bacterial infection is localised in the lungs and extra pulmonary TB occurs when the infection occurs outside the lungs. It is important to know that not all cases of infection with the TB bacterium result in disease. When infection is present but there are no symptoms of the disease, this is called latent TB. Generally, healthy adults with TB infection (latent TB) have a 10-15% likelihood of developing TB disease in their lifetime and for infants up to 50% will develop disease after 3-9 months of infection, for children 1-5 years of age and adolescents, up to 25% and 15% respectively will develop disease with 1-3 years of infection (Salazar-Vergara, Sia, Tupasi et al. 2003). The risk of developing disease is however increased in cases of compromised immunity and poor nutrition. Over 50% of TB patients with a documented status are HIV positive (WHO 2012).

Pulmonary TB is more infectious of the two forms of TB and is of greater public health concern. It is spread through the air when a person with active pulmonary TB coughs or sneezes. TB infection and disease occurs in people of all ages, gender and ethnicity and is the second most common infectious disease worldwide, second to the Human Immunodeficiency Virus (HIV). It is estimated that one third of the worlds population has TB infection (Zhang 2004). An estimated 8.6 million people developed TB in 2012 (WHO 2013 report). The co-morbidity of TB and HIV was responsible for an estimated 320,000 deaths among people living with HIV (PLWHIV) in 2012 (WHO report 2013). TB is the number one opportunistic disease among PLWHIV resulting in an increase in TB notification rates in areas with high HIV prevalence. This increase in the number of TB cases notified due to the HIV epidemic is a threat towards the successful control of TB.

Another challenge to successful management and control of tuberculosis is drug resistance. This problem of strains of the mycobacterium resistant to treatment regimen has become a worldwide problem (WHO 1997; WHO 2000). Resistance of the bacterium to drugs was first shown in the 1940s when streptomycin was used as a single drug for TB treatment. Following resistance to streptomycin it was clear that when combinations of

drugs are used resistance to anti-mycobacterial drugs was not common and today when a new case of TB is diagnosed, a combination of four drugs, namely Isoniazid (INH), Rifampicin (RIF), Pyrazinamide (PZA) and Ethambutol (EMB), are administered as the first line TB treatment drug regimen (WHO, 2010). With treatment, TB is curable and death can be prevented.

Mycobacteria resistance occurs by random spontaneous chromosomal mutations of drug target sites. No plasmids have been found (Villarino, Geiter and Simone 1992; Levinson and Jawetz 1998; Rich 2006; Marahatta 2010) ruling out any possibility of vertically transmitted mutations. Selective pressure imposed on a population of the mycobacteria in a host organism by a therapeutic agent results in continued multiplication of the small populations of the mycobacteria that have acquired resistance to the agent while the drug susceptible strains of the mycobacteria are suppressed enabling large populations of the drug resistant strain to be present in the host organism. It has been shown that the probability of mutation of the mycobacteria to drug resistance is directly proportional to the size of bacteria population. The rates of spontaneous mutation for first line drugs are; 1 in 10^6 for INH, 1in 10^8 for RIF, 1 in 10^6 for E and 1 in 10^5 for streptomycin (Villarino, Geiter and Simone 1992).

Because resistance occurs by random mutations, a single bacterium can be resistant to more than one drug and resistance to TB drugs is therefore classified as mono (to one drug only) or poly (to more than one drug) (TBPolicy 2011), and it is expected that treatment of mono and poly-drug resistance to first line drugs requires individualised treatment based on the drug resistance profile to the anti-TB drugs.

The development of drug resistant TB (DR-TB) occurs through three processes; transmitted resistance, acquired resistance and amplified resistance. Transmitted resistance occurs when resistant mycobacteria strains are transmitted to an uninfected person. Acquired resistance is a result of conversion of wild type mycobacteria strains to resistant strains by spontaneous mutations and amplified resistance is a result of progressive acquisition of resistance to more drugs by drug-resistant strains during repeated treatment incidences. It is important to note that the different types of mycobacterial drug resistance are not clinically distinguishable as the exact causative nature of resistance in a patient is not always possible to assess especially because drug sensitivity testing (DST) is not usually performed at point of diagnosis for new TB patients. (Blower and Chou 1994;

Mwinga 2001; TBPolicy 2011). When resistance occurs to the drugs INH and RIF, with or without resistance to other drugs, this is called multi-drug resistant tuberculosis (MDR-TB). MDR-TB results in treatment failure with the first line treatment regimen. A second line treatment regimen is used to treat MDR-TB. Second line TB treatment regimen is administered for a longer period and the treatment comes with challenges in that it is expensive and the drugs cause severe side effects. Side effects to second line drugs include hepatitis, renal failure, nausea, vomiting, and seizures (Zambia TB Manual).

TB disease can be diagnosed by microscopic techniques but to detect drug resistance, a culture needs to be made. Cultures are however not routinely performed on new cases of TB and because the presence of drug resistant TB in new cases is an indication of levels of transmission of resistant strains of M. tuberculosis, National TB programmes are placed at a disadvantage when systems in place do not allow them to effectively monitor trends of drug resistance risking setbacks towards the progress made by the National TB Programme.

The presence of DR-TB strains in new TB cases is an indication of the levels of transmission of resistant *M. tuberculosis* strains and it can only be detected if culture and DST are routinely performed at diagnosis stage for all TB patients (new and re-treatment). The availability of data on the levels of resistance in new TB patients and in re-treatment TB cases is important at national level for all countries faced with a high TB burden because this data can be used to monitor TB control programmes.

Apart from a study by Mulenga et al (2010) that described TB-drug resistance on 361 sputum samples in a select group of patients in Ndola, and another one on inmates (Habeenzu et al 2007) where 168 isolates were tested for TB-drug resistance, there is no comprehensive data on the extent of drug resistant TB in Zambia. This study aims to address this knowledge gap by assessing the data from the whole country over a period of 4 years from 2008 to 2011.

Statement of the Problem

According to Kapata et al (2011), in their study on 'Trends of Zambia's tuberculosis burden over the past two decades' the burden of the disease remains high in spite of the implementation of the WHO recommended DOTS strategy. Similarly, apart from the Ndola study by Mulenga et al (2010), the epidemiology of drug resistance to TB is

undefined. It is unclear what the nature and extent of drug resistant TB is in Zambia, and it is not known whether there is any relationship with geographical area, age or gender.

This study was conducted to determine patterns of drug resistant TB in Zambia at provincial level and thereby provide knowledge of the epidemiology and importance of drug resistant TB in Zambia. The study was based on secondary data provided by the national reference laboratory-Chest Disease Laboratory (CDL) and from the regional Tropical Disease Research Centre (TDRC) laboratory.

Justification of Study

Knowledge of the epidemiology of drug resistant TB in Zambia will indicate whether the rates are increasing or decreasing, which drugs are affected and will show if there is any relationship to geographical area, age or gender. This knowledge has the potential to provide a better understanding of drug resistant-TB and plan to inform targeted interventions towards the effective prevention and control of TB.

Research Question

What has been the distribution of TB drug resistance by the demographic characteristics of age, sex, and locality (by province) in Zambia during the period 2008 to 2011?

Main Objective

To determine the distribution of TB drug resistance by the characteristics of age, sex, and locality (by province) in Zambia from 2008 to 2011.

Specific Objectives

1. To describe the distribution of cases undergoing drug sensitivity testing (DST) by province, age and by gender.
2. To determine the extent of different types of drug resistant TB cases.
3. To describe drug resistant TB patterns by:
    i. province
    ii. year and gender
    iii. age
4. To determine factors associated with multi-drug resistant TB.

CHAPTER TWO: LITERATURE REVIEW

The first and second global survey to determine global prevalence of DR-TB was conducted by the WHO in collaboration with the International Union Against Tuberculosis and Lung Disease (IUATLD) (WHO 1997; WHO 2000). It was evident from these surveys that DR-TB is a global concern with virtually all countries surveyed reporting cases of drug resistance. When mono drug resistance was considered for the first survey, it was observed that resistance to rifampicin (RIF) was not common because at the time of the survey most countries had been using the drug for less than a decade (RIF was a new addition to the treatment regimen). However, in countries like the Dominican Republic where RIF had been used for more than a decade at the time of the survey, resistance was high (6.9%). Resistance to RIF is of concern because patients infected with organisms with resistance to RIF have a higher risk of treatment failure for the standard six month treatment period (Frieden, Sterling, Pablos-Mendez et al. 1993)

According to the WHO/IUATLD 2008 report on TB drug resistance in the world, 489,139 incident cases were estimated in 2006 (WHO, 2008). The proportion of MDR-TB cases among new TB cases was 3.1% and among previously treated cases was estimated to be 19.3%. The higher rate of drug resistance in previously treated cases is in conformity with studies that have shown that previous treatment is a strong predictor of MDR-TB (WHO 1997; WHO 2000).

In their study on the emergence of drug resistant TB in New York City, Freiden et al (1993) estimated that up to 23% of patients who were resistant to one or more drugs had no previous history of TB treatment reflecting a high proportion of transmitted resistance in the city. The strongest predictor for the presence of drug resistant TB was a history of TB treatment. Other predictors were infection with HIV for cases that had never been treated for TB and injection drug users. In 1994, Bloch et al published a paper detailing the geographic distribution of drug resistant TB in the United States and findings were that drug resistance was detected in 14.2% of the cases and distribution was as follows:

- Drug resistance patterns showed that resistance to INH and/or RIF (detected in 9.5% cases) in 107 counties in 33 states.
- Drug resistant patterns showed MDR-TB (detected in 3.5% of the cases) in 35 counties in 13 states.

New York City accounted for 61.4% of the nation's MDR-TB cases with racial and ethnic minorities accounting for 91.2% of the cases (Frieden, Sterling, Pablos-Mendez et al. 1993; Bloch, Cauthen, Onorato et al. 1994).

Concerning drug resistance in Africa, Mwinga (2001) explains that despite having the highest incident rate of TB per capita, levels of DR-TB in Africa are relatively low compared to countries like Russia and Estonia and she alludes this to properly functioning TB control programmes in most African countries and to the recent introduction of RIF.

Drug resistance to second line drugs resulting in the development of extensive drug resistant tuberculosis (XDR-TB) has been reported. Drug resistant TB is a challenge to TB control programmes because of severe side effects to second line drug regimens and these drugs are very expensive especially for developing countries. In 1999 and 2000, the DOTS-Plus and Green Light Committee (GLC) were launched with the aim to increase the access of second line drugs to resource poor countries to supplement efforts for the control of MDR-TB (WHO 2006)

Risk factors for the development of DR-TB

The single most significant risk factor for the development of drug resistant tuberculosis is history of previous anti-tuberculosis treatment. Generally, the rate of DR-TB is always higher among this group of patients who serve as a source of infection if not timely diagnosed and medicated.

In a 2001 analysis of 11 of the countries included in the Global Project on Drug Resistance Surveillance conducted by WHO and IUATLD, the findings were that cases of drug resistant TB were significantly higher among previously treated TB patients for one drug (OR=2.5; $P<0.001$), two drugs (OR=4.6; $P<0.001$), three drugs (OR=11.5; $P<0.001$) and four drugs (OR=18.5; $P<0.001$) (Espinal et al 2001). The increased proportion of resistance among previously treated TB patients has been thought to be because of mismanagement on the part of clinicians but most importantly, it has been thought to be a result of noncompliance on the part of the patients when on TB treatment therapy which leads to conversion of the mycobacteria to drug resistance forms. Espinal and colleagues showed that the period of previous treatment is an important predictor of drug resistance in previously treated patients with patients who previously received treatment for a period of

six to eleven months recording greater proportion of resistance than those who received treatment for a period of three months (OR=7.6; $P<0.001$) (Espinal et al 2001).

Drug resistance patterns in Gujarat, India showed a high proportion of resistance to INH in isolates of MTB from both new and re-treatment patients (Ramachandran et al. 2009). The range of distribution was in new patients from 1.9% (95% CI 1.2-2.6) for ethambutol to 11.0% (95% CI 9.4-12.5) for INH and in re-treatment patients from 10.2% (95% CI 8.4-12.0) for ethambutol to 37.0% (95% CI 30.0-39.9) to INH. India as a country has the largest burden of TB disease worldwide, accounting for one fifth of the world's total burden, and record cases of MDR-TB as well as XDR-TB. For this study, the rate of MDR-TB among new patients was 2.4% (95% CI 1.6-3.1) and among re-treatment patients was 17.4% (95% CI 15.0-19.7. Among the first line drugs for TB treatment, INH has been used for the longest period, it is therefore expected that resistance to it be the most common.

When considered at molecular level, resistance to INH has been shown to be a risk factor for conversion to MDR-TB. Resistance to INH is a result of mutations at different genes of the mycobacteria chromosome and it has been reported that INH resistant strains with katG315Thr mutation is strongly associated with the development and successful transmission of MDR-TB. Hu et al (2010) reported that INH resistance is likely to be clustered in a community and can therefore propagate an epidemic of MDR-TB in communities with a high TB burden. It is therefore important, especially for high TB burden countries, to have an up to date profile of rates of drug resistance to prevent a possible propagation of an epidemic.

A search of literature showed that different studies show differences in the association of gender and MDR-TB with some studies showing greater risk among women, others among men and some showing no significance difference between men and women. In a study conducted in Georgia by Lomtadze and colleagues (2009), women had a higher risk for MDR-TB disease because of their role as care providers which predispose them to infection from relatives with MDR-TB disease as they spend longer hours attending to them than men do. A similar finding of higher risk among women was also demonstrated in South Africa according to the 2010 WHO global drug resistance survey report.

In some countries of the Soviet Union, MDR-TB disease prevalence was higher among men than among women. This difference was because alcohol dependency and

imprisonment status were more likely among men than women. Higher levels of DR-TB disease have been documented in prison settings compared to the general population placing both prison staff and inmates at an increased risk of infection with MDR-TB strains and subsequent disease. A study conducted in Zambian prisons showed a higher rate of TB among inmates than in the general population with the prisons having a 10 fold higher rate (4005/100,000) (Habeenzu, Mitarai, Lubasi et al. 2007). This high rate is an indication of rampant transmission in prisons owing to overcrowding and poor ventilation. Under such conditions, transmission of drug resistant strains will also be more rampant than in the general population. In the Zambia study by Habeenzu et al, overall resistance was in the range 11.9% for INH and 17.9% for RIF (poly-resistance for both), MDR-TB was detected in 9.5% of the inmates.

Olusoji and Elayeb (2011) in their study in Nigeria found no significant difference for DR-TB disease among males and females (OR=1.24 CI 0.49-3.4; *P*= 0.62). Mulenga and colleagues (2010) had similar findings (*P*=0.467) for a study conducted in an urban setting in Zambia.

Another risk factor to be considered is age. It is thought that the presence of primary infection with DR-TB in children is an indication of recent transmission of infection and inference is made that it is a measure of the magnitude of resistance in a community (Espinal, Laserson, Camacho et al. 2001).

In a study conducted by Mulenga and colleagues in Zambia (2010) there was no statistically significant different with regard to age (*P*=0.999). The study interestingly also showed no significance with regard to treatment history (*P*=0.999). The sample size for this study was small with a total number of 193 isolates used for the study and of these 156 had never received TB treatment, 31 were re-treatment cases and 6 had missing case type data. This small sample size can be attributed to reduced power of the study.

It is evident from the literature review that DR-TB is heterogeneously distributed globally and what stands out as an explanation for this is the difference in predisposing factors such as lifestyle, gender associated roles and period of time for which anti-tuberculosis drugs have been used in a region. Owing to this heterogeneity, it is important for every country especially those with a high TB burden to have knowledge of associated risk factors and to set up surveillance systems to monitor DR-TB trends to strengthen TB control programmes.

TB in Zambia

The first TB control programme in Zambia was launched more than 40 years ago. Today however, TB remains a disease of major public health concern causing morbidity and mortality in many people. An escalation in the TB notification rates was seen after the advent of the HIV pandemic such that in the year 2004, the number of TB cases notified (58,070) were more than tenfold those notified in 1964 (4,572) (MOH 2011). In 2007, 50,415 cases were notified. Two thirds of the national notification rates were accounted for by Lusaka, Copperbelt and Southern provinces (Zambia Annual Report, 2010). Below is a table showing notification of all forms of TB in Zambia for the period 2005-2010 at provincial level.

All forms TB notification per province, 2005-2010

	2005	**2006**	**2007**	**2008**	**2009**	**2010**
Central	3,742	3,622	3,454	3,025	2,969	2,988
Copperbelt	13,213	10,949	10,863	9,860	10,586	10,612
Eastern	3,315	2,972	3,171	2,803	2,814	2,623
Luapula	2,969	2,694	2,318	1,954	1,956	1,710
Lusaka	17,601	18,068	17,157	16,624	17,649	18,276
Northern	2,466	1,988	2,141	2,035	1,833	1,720
North-western	1,891	2,216	2,132	1,966	1,887	1,616
Southern	5,939	6,128	6,147	6,051	5,988	5,773
Western	2,433	2,542	3,032	3,015	2,909	3,298
Total	53,267	51,173	50,415	47,333	48,591	48,616

Source: WHO Country Office, Zambia Annual Report 2010 (WHO, 2010)

The direct observed treatment short course (DOTS) strategy was adopted in Zambia as the primary approach to TB control in 1993 and by the year 2003 the country had attained 100% DOTS coverage in all of the then nine provinces. In 2006, the country adopted the STOP TB strategy for which the DOT remains the corner stone. The reduction in TB notification rates after 2004 was a positive output of TB control programmes though there is still much to be done if we are to attain the Millennium Developing Goal to reduce prevalence and death due to TB in adults by 50% by 2015 compared with baseline of 1990

(Mulenga, Mwakazanga, Vereecken et al. 2010; Kapata, Kapata, O'Grady et al. 2011; WHO 2011)

TB in Zambia affects all age groups with the majority of the notified cases falling within the age range 25-44 years. There are more male than female TB cases in Zambia and about 70% of notified TB cases are co-infected with HIV. According to the 2011 Ministry of Health (MoH) action plan (MoH, 2011), the country has an annual TB notification rate of approximately 50,000 new TB cases. Of the cases notified, when disaggregated by type of TB, there are more smear negative cases than there are smear positive cases, this can be attributed to the high prevalence of HIV among TB cases and the reduced sensitivity of smear microscopy in HIV infected people.

TB diagnosis in Zambian health facilities is mainly performed by microscopy because not all facilities are equipped with the necessary laboratory services to perform diagnosis by culture techniques. The country has 158 diagnostic facilities, of which three perform culture and DST and 1800 treatment facilities. The University Teaching Hospital (UTH) Laboratory, Chest Disease Laboratory (CDL) and Tropical Disease Research Centre (TDRC) perform culture and DST routinely on retreatment cases (Kapata et al., 2011).

While the rates of drug resistant TB in Zambia have not been extensively documented, the WHO (2004) estimates rates between 0 and 5.9% and 0 and 2.9% of MDR TB for previously treated cases and new TB cases respectively. There remains a need for a national survey to determine the rates of drug resistant TB in the Zambia general population.

CHAPTER THREE: METHODOLOGY

This study was a cross sectional study and involved the retrospective review of laboratory data provided by the two national tuberculosis culture laboratories - Chest Diseases Laboratory (CDL) in Lusaka and Tropical Disease Research Centre (TDRC) in Ndola.

The following sections explain how the specimens were received and processed at the laboratories, and how data was provided for purposes of this study.

Bacteriological Examination Protocols at the Chest Diseases Laboratory (CDL) in Lusaka and Tropical Disease Research Centre (TDRC) in Ndola.

Sources of sputa

The two national reference laboratories receive referred sputum specimen from: relapse patients; treatment failure patients; TB patients who remain positive or convert to positive after 5 months of treatment; TB patients who were in contact with MDR-TB patients; health workers who were exposed to MDR-TB patients; and persons from areas with high MDR-TB rates such as prisons.

Sputum specimens are received at the laboratories with an accompanying request form that contains information on health facility, patient name and clinical details, age and gender.

AFB Smear Microscopy

For every clinical specimen sent to the laboratories, the first test performed is microscopy of concentrated smear to check for the presence of AFB using Ziehl-Neelsen (ZN) stain or the fluorescent stain auramine-phenol. Results are recorded as: no AFB seen for smears with no bacilli; and for smears where bacilli are seen, results are recorded by intensity of observed bacilli.

Culture

Following microscopy, specimens are prepared for culture by first decontamination by the Petroff's method (Kent and Kubica 1985) then centrifugation after which the pellets are inoculated onto Löwensteing-Jensen (LJ) solid media and in Mycobacteria growth indicator tube (MGIT) 960 liquid media. The LJ media is then incubated at 37°C for a

period of up to 8 weeks with weekly assessment for growth. The MGIT liquid cultures are inoculated in BACTEC 960 MGIT machine under automated conditions for 42 days.

Identification of Cultures

Confirmation of species from MGIT growth positive tubes and LJ cultures that have colonies resembling *Mycobacterium tuberculosis* is by auramine-phenol stain or ZN stain and Capilia *Mycobacterium tuberculosis* identification Tests were performed.

Drug Sensitivity Testing (DST)

Cultures identified positive for the presence of *M. tuberculosis* are subjected to drug sensitivity testing (DST) to the four first line anti-TB drugs (streptomycin, isoniazid, rifampicin or ethambutol) using the proportion method (Canetti W et al., 1969) on LJ media or using the MGIT liquid culture system. Laboratory records for tests performed are stored in electronic databases and supplemented by paper records.

Recording of results by laboratories

The laboratories record for each specimen a number of variables related to the patient and includes health facility, demographics (age and gender), whether positive or negative on culture, and if culture positive whether resistance or susceptibile to any of the four first line anti-TB drugs.

Study Database

Creation of anonymised database

For purposes of this study, a separate anonymised dataset for all complete records was created in Excel spreadsheet and provided to the researcher for analysis by the two reference laboratories. Although a large number of variables exist, for purposes of this study, to prevent personal identification, the study database only contained the following five variables:

1. Date (year) of specimen collection
2. Age
3. Sex
4. Provincial location of health facility
5. Drug susceptibility results to any of the four first line anti-TB drugs

Sampling frame

The sampling frame for this research was all sputa specimen received during 2008 to 2011 by the two national referral laboratories. These constituted all specimens that underwent drug sensitivity testing in Zambia during the time period. The documented bacteriological examinations carried out on the sputa specimen were acid-fast bacilli (AFB) smear microscopy, culture and drug susceptibility testing if culture was positive. The anonymised dataset was created from this sampling frame after considering the eligibility of cases as described below.

Eligible cases

Eligible cases for the study were: all records for sputum specimens received at CDL and TDRC during the period 1st January 2008 to 31st December 2011 with drug sensitivity testing (DST) performed with complete susceptibility results (i.e. susceptibility results **for all four first line drugs**) in the database.

Contents of study database

The outcome measure (and hence **dependent variable)** was drug resistance of *Mycobacterium tuberculosis* to any of the four anti-tuberculosis drugs as recorded by the two reference laboratories (CDL and TDRC) during the period under review.

The following were the operational definitions and categorization:

Drug resistance on susceptibility testing:

> **Mono-resistance:** resistance to any one of the following: streptomycin, isoniazid, rifampicin or ethambutol
>
> **Multi drug resistant tuberculosis (MDR TB):** Resistance to **isoniazid** and **rifampicin** with or without resistance to any other drug
>
> **Poly-resistance:** resistance to more than one drug. This also includes MDR TB. For example, resistance to streptomycin, isoniazid and rifampicin would be both MDR and poly-resistance. Resistance to streptomycin, isoniazid and ethambutol is not MDR TB (since rifampicin is susceptible) but is considered poly-resistance.

The **independent variables** were age, sex and geographic location in Zambia by province.

Analysis

The created databases were exported into STATA v11.0 for the analysis. This was primarily a descriptive study and the analysis included tabulations to determine the rates of drug resistance to answer the research question and achieve the specific objectives.

An analytic component was used to calculate the unadjusted odds ratios which were calculated using chi square to test the association between MDR TB and the three independent variables (age, gender, and locality). Multivariate logistic regression was employed to explore any potential confounding. For this regression, all cases with missing variables were excluded.

Significance was set at $P< 0.05$ and all odds ratios were presented with the 95% confidence intervals.

Ethical considerations

This research was approved by the University of Zambia Biomedical Research Ethics Committee (Appendix 1). All data used were anonymised before analysis and no personal identifiers were used. Approval for use of the data was provided by the Ministry of Health (Appendix 2).

CHAPTER FOUR: RESULTS

Overview

The results presented in this section are from the analysis of cases that were eligible for analysis of this research. A total number of 15,715 sputum specimens were received collectively at the Chest Disease Laboratories and Tropical Diseases Research Centre during the period 1st January 2008 to 31st December 2011. The specimens came from a total of 7,579 cases. Of these, 811 cases (10.7%) were eligible for this study as defined by cases with drug sensitivity test (DST) results for all four anti-TB drugs (streptomycin, isoniazid, rifampicin or ethambutol).

The results are presented in five sections as follows:

1. Distribution of cases drug sensitivity test (DST) results by province and by gender
2. Drug resistance TB in Zambia and patterns by province
3. Patterns of MDR TB by year and gender (2008-11)
4. Drug resistant TB patterns by age
5. Association of MDR TB with age, gender and locality

Raw data are presented in tabular form and stratified as listed above. The relevant proportions are presented as percentages across rows and down columns. This is to enable a review across each province and separately down each stratum, respectively.

1. Distribution of cases drug sensitivity test (DST) results by province and by gender

The distribution of the 811 cases from each province is presented in table 1 stratified by gender. The proportions are expressed as a percentage. Almost half of the 811 cases came from Lusaka Province (400, 49.3%) followed by Copperbelt (244, 30.1%). Overall 58% of cases were males and 37.1% female; in 4.9% gender was not listed. Apart from the Western Province, all other provinces had more males than females contributing as cases (30.8% males vs. 53.8% females). This is illustrated in figures 1 and 2.

Table 1: Distribution of cases with drug sensitivity testing (DST) by province and gender

Province	Male (n)	Percent (Row, Column)	Female (n)	Percent (Row, Column)	Missing Sex (n)	Percent (Row, Column)	All (N)	Percent (Row, Column)
Lusaka	**220**	55.0, 46.8	**159**	39.8, 52.8	**21**	5.2, 52.5	**400**	100, 49.3
Central	**19**	67.9, 4.0	**9**	32.1, 3.0	**0**	0, 0	**28**	100, 3.5
Copperbelt	**152**	62.3, 32.3	**84**	34.4, 27.9	**8**	3.3, 20	**244**	100, 30.1
Eastern	**10**	55.6, 2.1	**8**	44.4, 2.7	**0**	0, 0	**18**	100, 2.2
Luapula	**5**	62.5, 1.1	**3**	37.5, 1.0	**0**	0, 0	**8**	100, 1.0
Northern	**7**	77.8, 1.5	**1**	11.1, 0.3	**1**	11.1, 2.5	**9**	100, 1.1
N.Western	**10**	66.7, 2.1	**5**	33.3, 1.7	**0**	0, 0	**15**	100, 1.8
Southern	**30**	58.8, 6.4	**19**	37.3, 6.3	**2**	3.9, 5	**51**	100, 6.3
Western	**4**	30.8, 0.9	**7**	53.8, 2.3	**2**	15.4, 5	**13**	100, 1.6
Missing province*	**13**	52.0, 2.8	**6**	24.0, 2.0	**6**	24.0, 15	**25***	100, 3.1
Total	**470**	58.0, 100.0	**301**	37.1, 100.0	**40**	4.9, 100	**811**	**100** **100**

*Province data for 25 cases were not available

Figure 1. Percent distribution of cases by Province, Zambia 2008-11

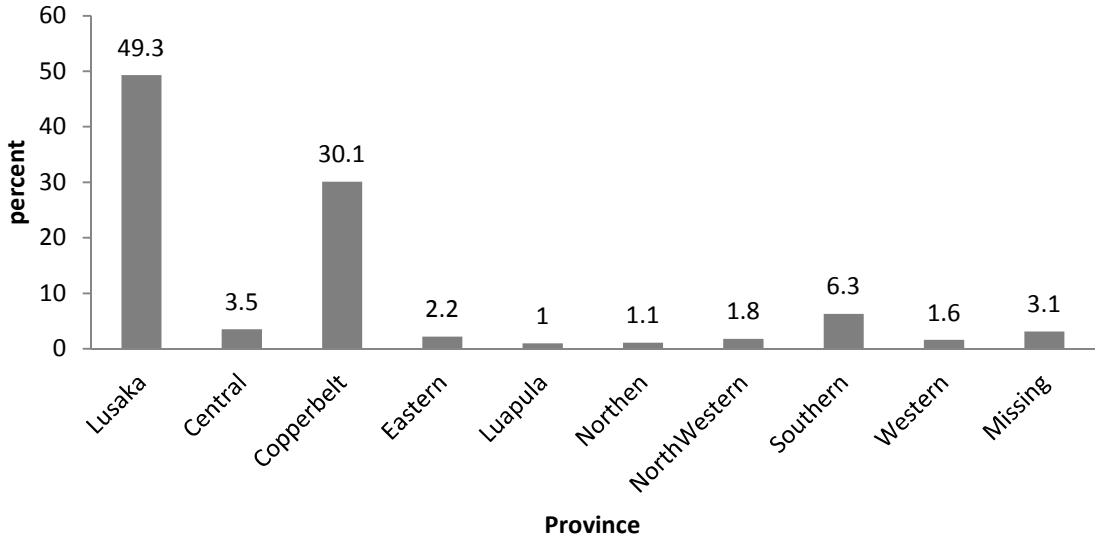
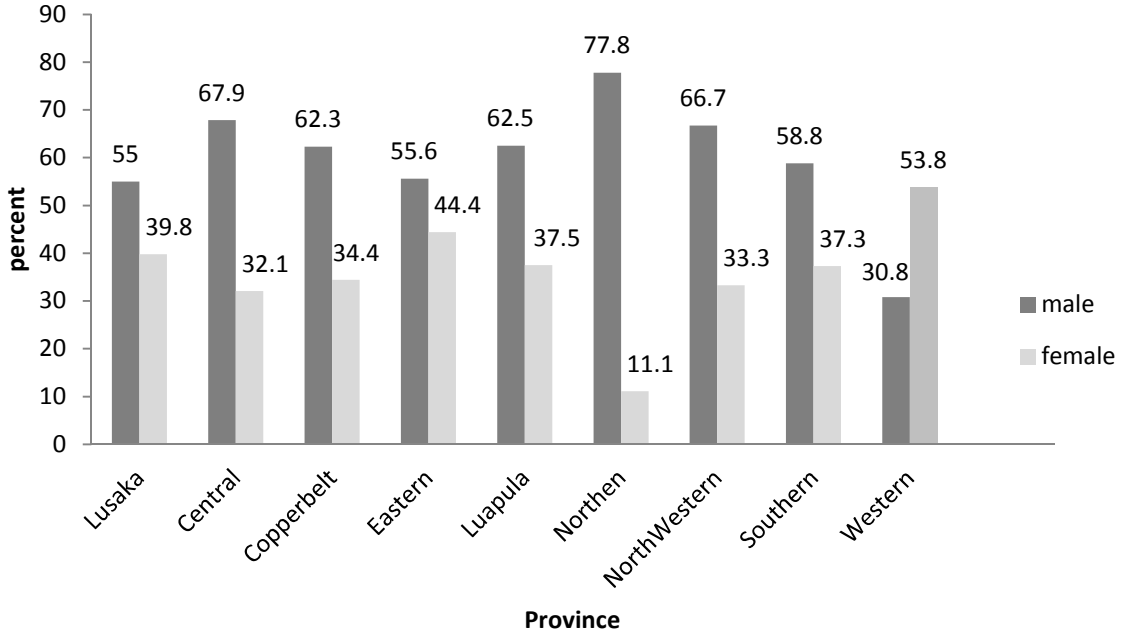


Figure 2. Percent distribution of cases by gender and province, Zambia 2008-11



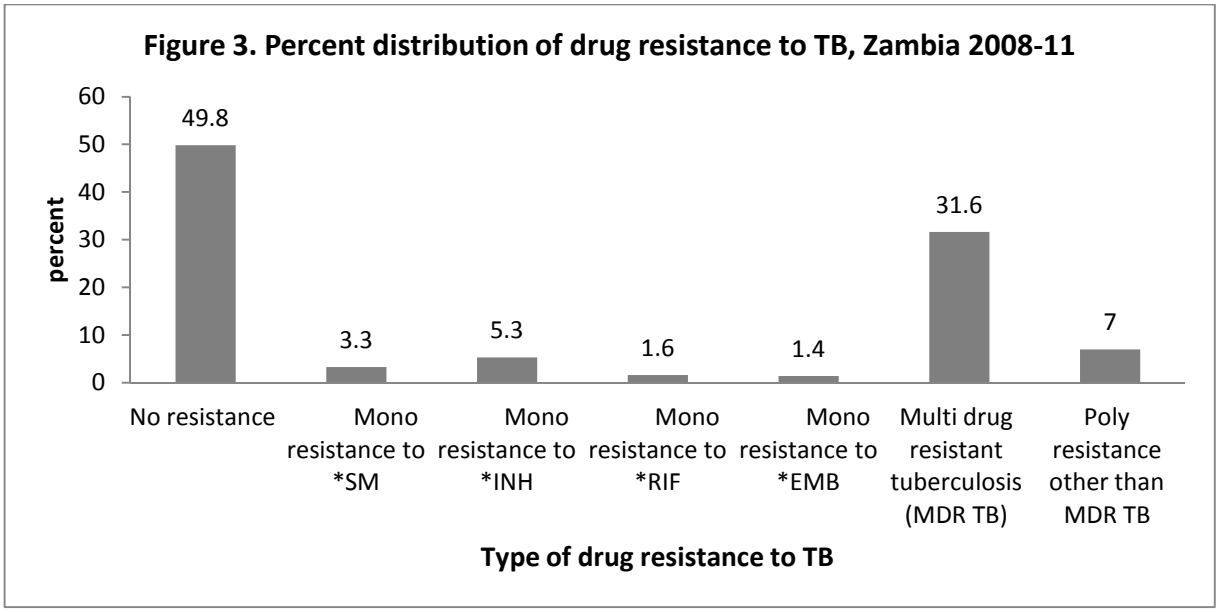
2. Drug resistance TB in Zambia and patterns by province

Of the 811 cases on whose specimens drug sensitivity testing (DST) was performed, 407 (50.2%) had resistance to one or more of the anti-tuberculosis drugs while the remaining 404 (49.8%) were susceptible (sensitive) to all four anti-TB drugs. (Table 2 and Figure 3).

Of the 407 (of 811 total cases) with drug resistance, 256 (31.6%) had MDR TB, 94 (11.6%) had mono resistance to one of the 4 drugs and 57 (7%) had poly resistance (i.e. resistance to more than one drug other than MDR TB). Most of the mono resistance was to isoniazid (INH) (5.3%) and least to ethambutal (1.4%).

Table 2. Drug sensitivity test patterns by province

Province	Mono drug resistance				Any Mono resistant TB	MDR TB	Poly resistance (other than MDR TB)	*Any resistance*	No resistance (sensitive to all 4 ATT)	Total
	SM (n)	INH (n)	RIF (n)	EMB (n)	n (% row) (% column)	n (% row) (% column)	n (% row) (% column)	n (% row) (% column)	n (% row) (% column)	N (% row) (% column)
Lusaka	12	19	7	4	42 (10.5) (44.7)	112 (28.0) (43.8)	10 (2.5) (17.5)	*164 (4.01) (40.3)*	236 (59.0) (58.4)	**400 (100) (49.3)**
Central	0	2	0	0	2 (7.1) (2.1)	17 (60.7) (6.6)	3 (10.7) (5.3)	*22 (78.6) (5.3)*	6 (21.4) (1.5)	**28 (100) (3.5)**
Copperbelt	10	18	1	6	35 (14.3) (37.2)	84 (34.4) (32.8)	27 (11.1) (47.4)	*146 (59.8) (35.9)*	98 (40.2) (24.3)	**244 (100) (30.1)**
Eastern	1	0	1	0	2 (11.1) (2.1)	5 (27.8) (2.0)	3 (16.7) (5.3)	*10 (55.6) (2.5)*	8 (44.4) (2.0)	**18 (100) (2.2)**
Luapula	1	0	0	0	1 (12.5) (1.1)	1 (12.5) (0.4)	2 (25.0) (3.5)	*4 (50.0) (1.0)*	4 (50.0) (1.0)	**8 (100) (1.0)**
Northern	0	0	0	1	1 (11.1) (1.1)	2 (22.2) (0.8)	1 (11.1) (1.8)	*4 (44.4) (1.0)*	5 (55.6) (1.2)	**9 (100) (1.1)**
N.Western	1	0	0	0	1 (6.7) (1.1)	3 (20.0) (1.2)	0 (0.0) (0.0)	*4 (26.7) (1.0)*	11 (73.3) (2.7)	**15 (100) (1.8)**
Southern	0	3	2	0	5 (9.8) (5.3)	16 (31.4) (6.3)	10 (19.6) (17.5)	*31(60.8) (7.6)*	20 (39.2) (5.0)	**51 (100) (6.3)**
Western	1	1	2	0	4 (30.8) (4.3)	5 (38.5) (2.0)	1 (7.7) (1.8)	*10 (76.9) (2.5)*	3 (23.1) (0.7)	**13 (100) (1.6)**
Missing Province	1	0	0	0	1 (4.0) (1.1)	11 (44.0) (4.3)	0 (0.0) (0.0)	*12 (48.0) (2.9)*	13 (52.0) (3.2)	**25 (100) (3.1)**
Total	**27**	**43**	**13**	**11**	**94 (11.6) (100)**	**256 (31.6) (100)**	**57 (7.0) (100)**	***407 (100)***	**404 (49.8) (100)**	**811 (100) (100)**

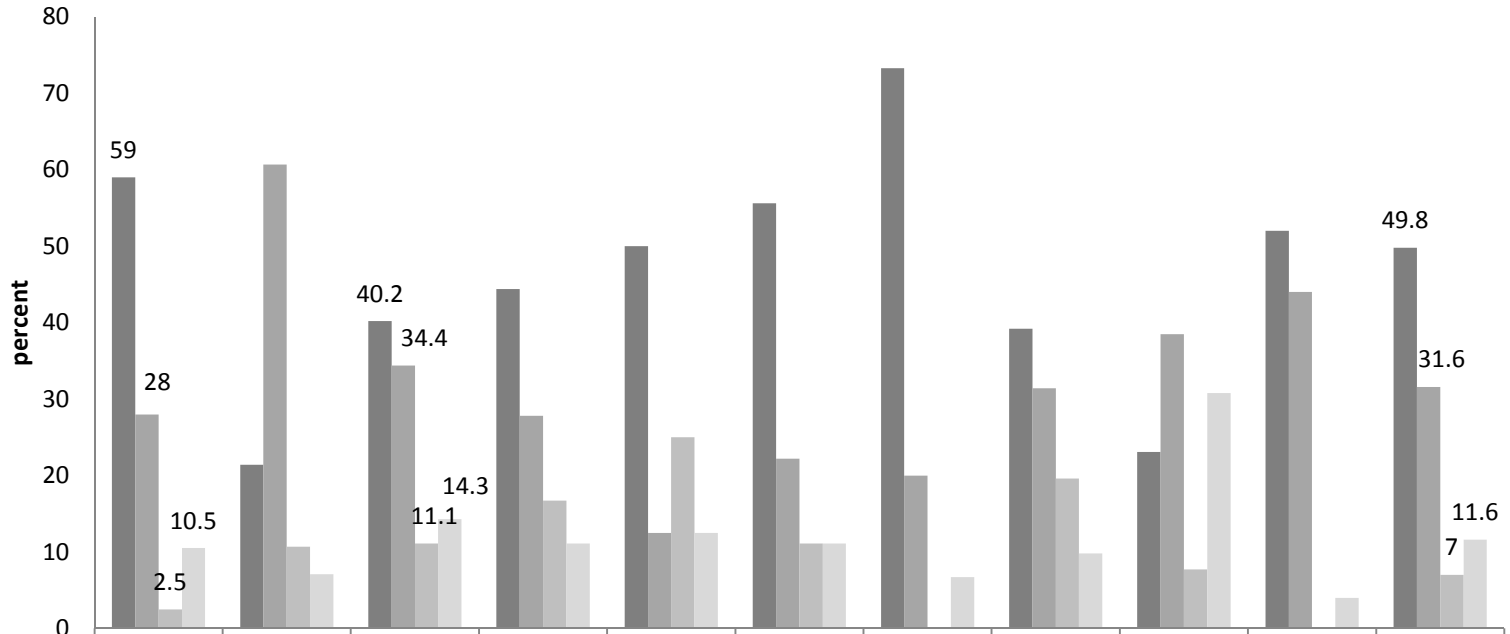


*SM= streptomycin, INH= isoniazid, RIF= rifampicin EMB= ethambutol

Note: MDR TB indicated resistance to isoniazid and rifampicin

The drug sensitivity pattern for Lusaka Province showed that 59% were sensitive to all drugs, 10.5% had mono-drug resistance, 28% had MDR TB and 2.5% had poly resistance other than MDR TB. (Figure 4). Copperbelt Province was the other province with large number of drug sensitivity tests performed and there were fewer cases with sensitivity to all drugs (40.2%); mono resistance was 14.3% and MDR TB was 34.4%. The other provinces had few cases and the resistance pattern within these provinces is not discussed.

Figure 4. Percent distribution of drug sensitivity and resistant pattern of TB by province, Zambia 2008-11



	Lusaka	Central	C/Belt	Eastern	Luapula	Northern	NW	Southern	Western	Missing Province	All
sensitive to all 4 ATT	59	21.4	40.2	44.4	50	55.6	73.3	39.2	23.1	52	49.8
MDR TB	28	60.7	34.4	27.8	12.5	22.2	20	31.4	38.5	44	31.6
Poly resistant TB	2.5	10.7	11.1	16.7	25	11.1	0	19.6	7.7	0	7
any mono resistant	10.5	7.1	14.3	11.1	12.5	11.1	6.7	9.8	30.8	4	11.6

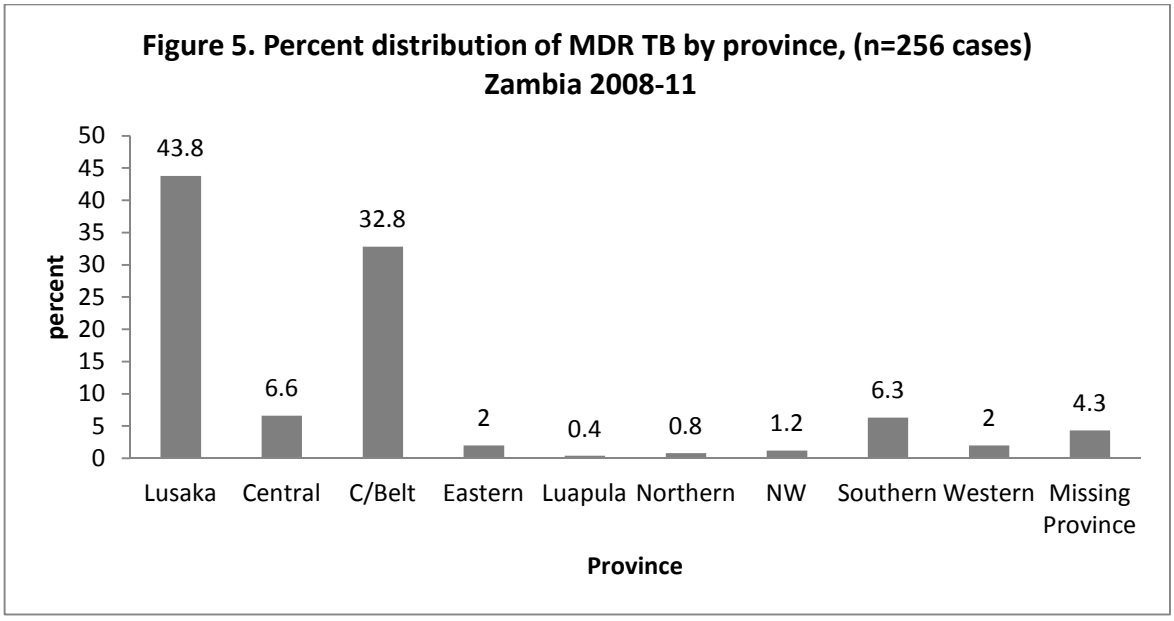


Figure 5 illustrates that most of the cases of MDR during this study period were from Lusaka Province (112 of 256 cases with MDR, 43.8%), followed by Copperbelt Province (84 of 256 cases with MDR, 32.8%).

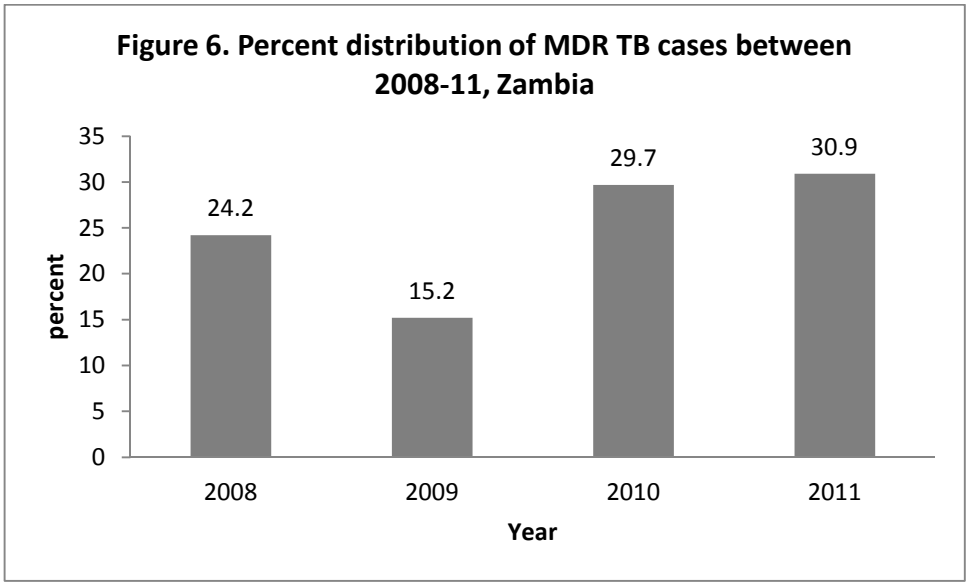
All the other provinces had few cases of MDR TB (between 1 and 17) with the highest percentage being 6.3% in Southern Province.

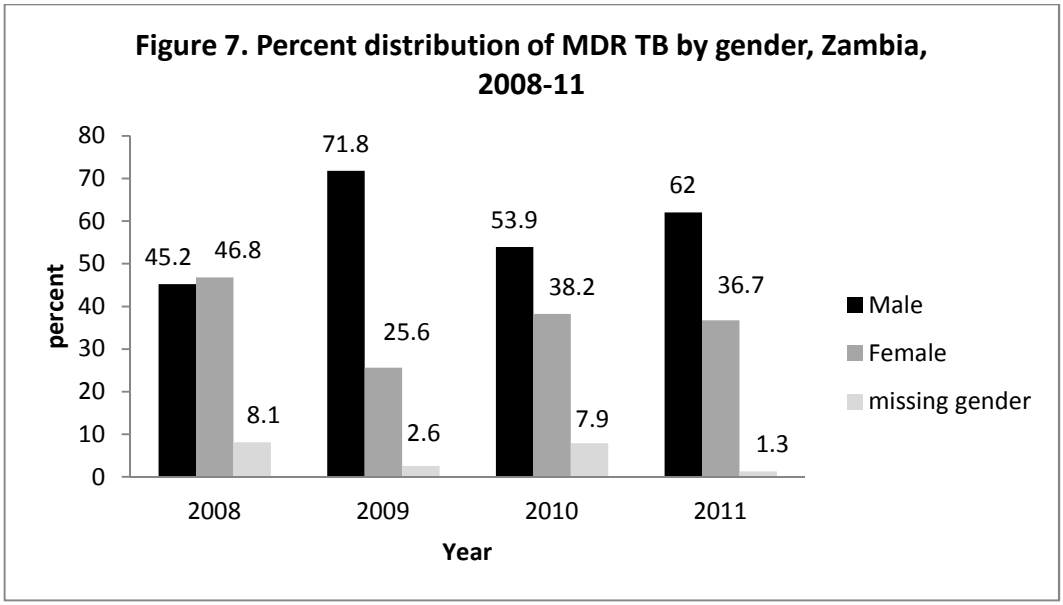
3. Patterns of MDR TB by year and gender (2008-11)

The proportion of MDR TB had increased from a 24.2% in 2008 to 30.9% in 2011, though there was a reduction in 2009 (15.2%). (Table 3 and Figure 6). Overall, proportionally there were more male cases of MDR TB than female (57.0% vs. 37.9%) and this pattern was noted in all years except 2008 when there were slightly more female cases of MDR TB than male (45.2 vs. 46.8%). (Figure 7).

Table 3: MDR TB patterns by year and stratified by gender

Year	Cases	MDR TB	%	Male	% MDR Row column	Female	% MDR Row column	Gender missing	% MDR Row column	Total N	Total % Row column
2008	186	62	24.2	**28**	45.2 19.2	**29**	46.8 29.9	**5**	8.1 38.5	62	**100** **24.2**
2009	159	39	15.2	**28**	71.8 19.2	**10**	25.6 10.3	**1**	2.6 7.7	39	**100** **15.2**
2010	247	76	29.7	**41**	53.9 28.1	**29**	38.2 29.9	**6**	7.9 46.2	76	**100** **29.7**
2011	219	79	30.9	**49**	62.0 33.6	**29**	36.7 29.9	**1**	1.3 7.7	79	**100** **30.9**
Total	**811**	**256**	**100**	**146**	**57.0** **100**	**97**	37.9 100.0	**13**	5.1 100.0	**256**	**100** **100.0**





4. Patterns of drug resistant TB distribution by age (2008-11)

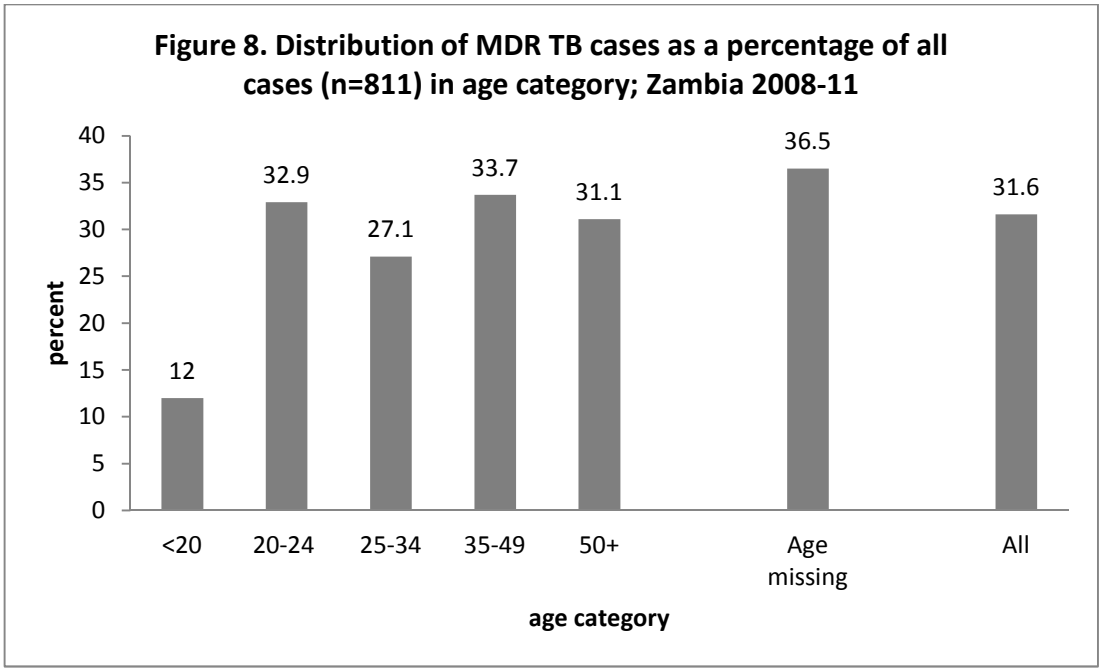
The distribution of MDR TB cases (n=256) over the four years 2008-11 is tabulated in Table 4, stratified by age. There were three cases of MDR TB under 20 years of age (1.2%) and the highest number was in age category 35-49 years (n=80, 31.3%). It is noteworthy that for 58 cases of MDR-TB (22.6%) the age was not available in the dataset.

As a percentage of all cases, the 3 cases in the under age 20 years category were from 25 cases (12%) and for the other age categories it ranged from 32.9% (20-24 years), 33.7% (35-49 years) and 31.1% (50+ years). (Table 4 and Figure 8).

Table 4: Age distribution of MDR TB cases recorded during the period 2008-2011

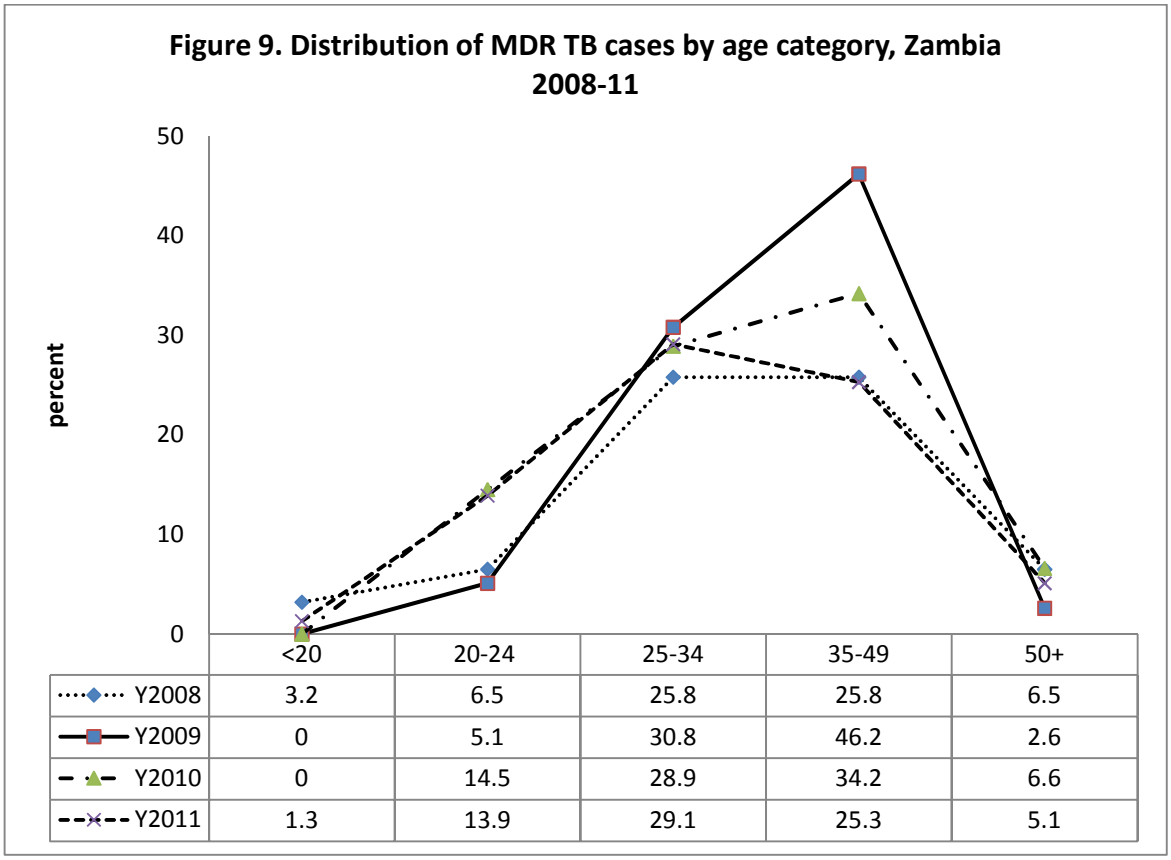
AGE (years)	**<20 n (% row) (% column)**	**20-24 n (% row) (% column)**	**25-34 n (% row) (% column)**	**35-49 n (% row) (% column)**	**50+ n (% row) (% column)**	**Age missing n (% row) (% column)**	**All N (% row) (% column)**
Cases in age category	**25 (3.1)**	**85 (10.5)**	**260 (32.1)**	**237 (29.2)**	**45 (5.5)**	**159 (19.6)**	**811 (100)**
MDR TB cases (2008-11)	3 (1.2) (12)	28 (10.9) (32.9)	73 (28.5) (27.1)	80 (31.3) (33.7)	14(5.5) (31.1)	58 (22.7) (36.5)	256 (100) (31.6)
2008	2 (3.2) (66.7)	4 (6.5) (14.3)	16 (25.8) (21.9)	16 (25.8) (20.0)	4 (6.5) (28.6)	20 (32.3) (34.5)	62 (100) (24.2)
2009	0 (0.0) (0)	2 (5.1) (7.1)	12 (30.8) (16.4)	18 (46.2) (22.5)	1 (2.6) (7.1)	6 (15.4) (10.3)	39 (100) (15.2)
2010	0 (0.0) (0)	11 (14.5) (39.3)	22 (28.9) (30.1)	26 (34.2) (32.5)	5 (6.6) (35.7)	12 (15.8) (20.7)	76 (100) (29.7)
2011	1 (1.3) (33.3)	11 (13.9) (31.5)	23 (29.1) (40.0)	20 (25.3) (25.0)	4 (5.1) (28.6)	20 (25.3) (34.5)	79 (100) (30.9)

Proportions as percentages in parentheses. First percentage represents across row and second down column.



Patterns of MDR TB across age categories (2008-11)

Reviewing the data for each year across age categories, most cases of MDR TB were in the 25-34 and 35-49 years age groups. (Figure 9).



	<20	20-24	25-34	35-49	50+
Y2008	3.2	6.5	25.8	25.8	6.5
Y2009	0	5.1	30.8	46.2	2.6
Y2010	0	14.5	28.9	34.2	6.6
Y2011	1.3	13.9	29.1	25.3	5.1

5. Association of MDR TB with age, gender and locality

In order to explore the association of MDR TB with age, gender and provincial location, the unadjusted odds ratios (OR) of the three variables are first presented below using the 2 x 2 contingency tables and administering chi-square test (or Fisher's exact test if cell values <5):

- Age (as categories)
- Gender: male or female
- Province: urban or rural. Urban provinces included Lusaka and Copperbelt; the rest were considered rural.

The only significant association was in age less than 20 years; this appeared not to be associated with MDR-TB (using Fisher exact test).

	MDR TB	No MDR TB	
Female	75	153	228
Male	117	239	356
	192	392	**584**

Unadjusted Odds Ratio = 1.001 (95% confidence interval = 0.70 to 1.43), P = 0.9923, ns

	MDR TB	No MDR TB	
Age >20	3	20	23
Age <20	189	372	561
	192	392	**584**

Fisher exact test p=0.0337 (the two classifications are significantly different)

	MDR TB	No MDR TB	
Age >25	161	321	482
Age <25	31	71	102
	192	392	**584**

Unadjusted Odds Ratio = 1.15 (95% confidence interval = 0.73 to 1.84), P = 0.5632, ns

	MDR TB	No MDR TB	
rural	42	60	102
urban	150	332	482
	192	382	**584**

Unadjusted Odds Ratio = 1.55 (95% confidence interval = 0.99 to 2.40), P = 0.0534, ns

Multivariate analysis

Two multivariate logistic regression models are presented below.

1. In the first one, the age variable is dichotomised around 25 years (i.e. age <25 or age >25 years).
2. In the second model, all age categories are separately dichotomised and maintained in the model.

1. Logistic regression (with age dichotomized as >25 or <25 years)

Deviance goodness of fit chi-square =	18.133824	P = 0.0012
Deviance (likelihood ratio) chi-square =	4.07597	P = 0.2534

Intercept	b0 = -0.917198	z = -3.771573	P = 0.0002
sex female=1	b1 = 0.027587	z = 0.15021	P = 0.8806
>25=1	b2 = 0.135435	z = 0.56482	P = 0.5722
rural =1	b3 = 0.436073	z = 1.945675	P = 0.0517

logit MDR=1 = -0.917198 +0.027587 sex female=1 +0.135435 >25=1 +0.436073 rural =1

Logistic regression - odds ratios

Parameter	Estimate	Odds Ratio	95% CI
Constant	-0.917198		
sex female=1	0.027587	1.027971	0.717218 to 1.473367
>25=1	0.135435	1.145035	0.715671 to 1.831994
rural =1	0.436073	1.546622	0.996803 to 2.399712

Interpretation: Using the available data, MDR TB was not associated with gender, age or locality. However, there was a tendency for cases from rural areas to be more likely to be associated with MDR TB, though this was not statistically significant.

(There were too few cases if age was dichotomised around <20 years, though this model is presented in the Appendix 3).

2. Logistic regression (with all age variable categories dichotomized)

Logistic regression

Deviance goodness of fit chi-square =	23.353128	P = 0.0249
Deviance (likelihood ratio) chi-square =	10.105347	P = 0.1203

Intercept	b0 = -0.832614	z = -5.576848	P < 0.0001
sex female=1	b1 = 0.067778	z = 0.364957	P = 0.7151
<20 =1	**b2 = -1.151832**	**z = -2.191716**	**P = 0.0284**
20-24 =1	b3 = 0.120622	z = 0.515946	P = 0.6059
25-34 =1	b4 = -0.088142	z = -0.505674	P = 0.6131
35-49 =1	b5 = 0.157837	z = 0.908616	P = 0.3636
>50 =1	b6 = 0.1289	z = 0.423064	P = 0.6722
rural =1	b7 = 0.413047	z = 1.832462	P = 0.0669

logit MDR=1 = -0.832614 +0.067778 sex female=1 -1.151832 <20 =1 +0.120622 20-24 =1 -0.088142 25-34 =1 +0.157837 35-49 =1 +0.1289 >50 =1 +0.413047 rural =1

Logistic regression - odds ratios

Parameter	Estimate	Odds Ratio	95% CI
Constant	-0.832614		
sex female=1	0.067778	1.070128	0.743626 to 1.539986
<20 =1	**-1.151832**	**0.316057**	**0.11283 to 0.88533**
20-24 =1	0.120622	1.128198	0.713485 to 1.783962
25-34 =1	-0.088142	0.915631	0.650656 to 1.288515
35-49 =1	0.157837	1.170976	0.833075 to 1.645932
>50 =1	0.1289	1.137577	0.626086 to 2.066938
rural =1	0.413047	1.511416	0.971669 to 2.350985

Interpretation: Using the available data, being age <20 years was less likely to be associated with MDR TB. However, MDR TB was not associated with gender or locality. Furthermore, there was a tendency for cases of MDR TB to be from rural areas, though this was not statistically significant. Further,

Summary

There was little by way of confounding between the three variables (age, locality and gender). Since there were too few cases if age was dichotomised by <20 or not, this is not presented in Table 5.

Table 5. Summary of unadjusted and adjusted Odds Ratios of candidate variable associated with MDR TB.

		MDR TB (n)	No MDR TB (n)	N	**Unadjusted Odds ratio** (95% CI) P value	**Adjusted Odds ratio** (95% CI) P value
gender	Female	75	153	228		
	Male	117	239	356	OR= 1.001 (95% CI = 0.70 to 1.43), P = 0.9923	OR= 1.03 (95% CI = 0.72 to 1.47), P = 0.8806
age	>25	161	321	482		
	<25	31	71	102	OR= 1.15 (95% CI = 0.73 to 1.84), P = 0.5632	OR= 1.15 (95% CI = 0.72 to 1.83), P = 0.5722
Province	Urban	42	60	102		
	Rural	150	332	482	OR 1.55 (95% CI = 0.99 to 2.40), P = 0.0534	OR 1.55 (95% CI = 0.99 to 2.40), P = 0.0517

CHAPTER FIVE: DISCUSSION

This retrospective study aimed to provide data on the patterns of drug resistant tuberculosis in Zambia and the associations with provincial location (rural/urban), age and sex.

Findings from this study were that resistance to at least one or more tuberculosis drugs was recorded in 50.2% of the cases. Mono drug resistance was observed in 11.6% of the cases and was most common for isoniazid at 5.3%. MDR TB was observed in 31.6% of the total cases for the study.

The rates of drug resistance documented in this study show an increase in MDR TB cases from a rate of 24.2% in 2008 to 30.9% in 2011 with a drop in the year 2009 to 15.2% (Table 3 and figure 6). Though this change in rates has to take into account that data was not available on all cases, this steady increase in MDR TB rates is of public health concern. MDR TB has a lower cure rate, about 50% or less, than susceptible forms of the disease, which has a cure rate of 90% or more. In addition to the lower cure rate, second line treatment medication has severe side effects and is more expensive than first line treatment (American Lung Association, 2013; WHO 2014) . In a similar study documenting MDR TB in Zambia, Kapata et al (2013) reported an overall MDR TB rate of 22%. The difference in the two rates can be attributed to the difference in study periods and data sources. The Kapata study covered the period 2000 to 2011 and had used data not only from CDL and TDRC, but also from the University Teaching Hospital (UTH) laboratory; (UTH data was not available for this study as it was not part of the National Reference Laboratory database at the time). An important finding that is consistent for both studies is that there has been a steady increase of the rates of MDR TB over time in Zambia.

The rate of mono drug resistance was 11.6%. Drug resistance amplification and high treatment failure rates are associated with mono drug resistance. Studies conducted to determine treatment outcomes of isoniazid mono resistance have revealed a need for early identification and rigorous follow up to improve treatment success. In a study conducted in South Africa by Jacobson, Theron, Victor et al. (2011), 61% of isoniazid mono resistant patients later developed MDR TB. Mono resistance to isoniazid or isoniazid or streptomycin has been documented to be a strong risk factor for relapse, treatment failure and acquisition of MDR TB. (Quy, Lan, Borgdorff et al. 2003; Menzies, Benedetti, Paydar

et al. 2009; Menzies, Benedetti, Paydar et al. 2009; Gegia, Cohen, Kalandadze et al. 2012). The rate of isoniazid and streptomycin mono resistance for this study (5.3% and 3.3%, respectively) is similar to previously documented work (Kapata et al. 2013)

Zambia treatment guidelines provide a specific treatment regimen, including doses, for confirmed TB patients and confirmed MDR TB patients; but are not clear on the TB treatment regimen to be given to confirmed mono resistant or poly resistant cases. The guidelines leave this decision to experience and skill of the physician and recommend that this approach should be done only by specialized physicians with the appropriate expertise. There has been no study conducted in Zambia to determine treatment outcomes among confirmed new TB patients with mono resistance and/ or poly resistance. From the work of Quy et al (2003), it is clear that there is need to have this information known for Zambia, as the current treatment plan may be inadequate for the management of mono and/or poly drug resistance cases.

Other findings from the study were that Lusaka and the Copperbelt provinces have the highest numbers of drug resistant TB. It is known that these two provinces also record the highest TB case notification rates (WHO 2010). What could not be established from this study, however, is the comparison of drug resistant TB cases among new TB cases and retreatment TB cases. This was a limitation of the study; the cases were not identified as such in the data set. The age group 35-49 years has the largest number of drug resistance cases and there were more male than female drug resistant cases. Jacobson, Theron, Victor et al. (2011) found that patients aged 33-43 had increased odds of poor treatment outcomes. This study found only three cases under the age 20 with drug resistant TB, two of them being less than 18 year. All three cases had MDR TB. WHO estimates child TB at 10-15%. In a study conducted by Bates, O'Grady, Maeurer et al. (2012) at the University Teaching Hospital over a period of one year, two children under the age of 15 had MDR TB, an indication that there is the possibility of under diagnosis of MDR TB cases among children. Diagnosis of TB in children is a challenge because the disease can be bacteriologically negative in children, but with the use of new molecular biology techniques like the WHO endorsed Xpert MTB/RIF, detection rates of TB and cases of drug resistance in children can be improved (WHO 2010; Nicol, Workman, Isaacs et al. 2011). Active case finding of children at risk of drug resistant TB and the use on new diagnostic techniques, such as the WHO endorsed MTB/RIF that have been shown to have increased sensitivity for detection of drug resistant TB in children can improve diagnosis

and consequently increase treatment success rates among children with drug resistant TB. This will also improve diagnosis and treatment outcomes for susceptible forms of the mycobacterium. (Olson, Lebovitz and Claiborne 2011; WHO 2011; Bates, O'Grady, Maeurer et al. 2012)

Sixty seven (8.3%) cases from this study had different drug resistance patterns for the sputum samples submitted for drug sensitivity testing. A large proportion (70.2%) of these cases had MDR TB. From this study, it is not certain whether this difference was a result of laboratory cross contamination or whether this is an indication of different strains of the mycobacterium. To determine if the strains are different, molecular studies to investigate the mycobacterium DNA need to be conducted. The documentation of mixed infection, which is 'infection with multiple *M. tuberculosis* strains' has recently increased with documented ranges from 2.8% to 19% (Huang, Tsai, Lee et al. 2010; Hanekom, Streicher, Berg et al. 2013). Mixed infection influences diagnosis if a patient is infected with both a resistant and a susceptible strain. The implications of mixed infection include the emergence of drug resistant TB because of undetected strains and the continued transmission of undetected and therefore untreated strains. There is a need to conduct an investigation of the levels of mixed infection in the country. This knowledge will also serve as an indication of the rates of TB infection, information that can inform national planning towards TB prevention and control (Fang, Li, Li et al. 2008; Cohen, Wilson, Wallengren et al. 2010; Cohen, Helden, Wilson et al. 2012).

CHAPTER SIX: CONCLUSIONS

The study showed significant proportions of mono and MDR-TB among the study sample that included drug sensitivity test results on all four anti-TB drugs. Drug resistant TB in Zambia will continue to be a challenge to management and control of TB if not properly managed. Studies on treatment outcomes of mono resistant TB patients need to be performed to inform the current treatment regimens.

Limitations

The study population for this study was drawn from national TB data from the two reference laboratories - Chest Diseases Laboratory and the regional TB laboratory at the Tropical Diseases Research Centre. Not all cases have samples sent to the laboratories and not all samples were tested for drug sensitivity at all times. Hence, a selection bias exists and it cannot be assumed that the drug resistant TB situation in the general population will be the same as reported. Nevertheless, triangulating from the Kapata et al study (2013) and others regionally and worldwide, this study shows similar patterns. In addition, the data set provided by the laboratories did not have comprehensive demographic information for cases. TB-HIV co-infection for instance is of public health concern but this information is not collected on the laboratory request forms and was not available in the data set. Other variables that were not available were marital status and home address. This information is captured at the clinic and is available only in the clinic files.

The province variable was based on the location of the clinic that sent the sputum samples to the laboratories. The implication of this is that transient cases (though not permanently living in that province) were allocated to the province from which their sputum samples were received and not the province from which they reside. This may have resulted in under-representation of some provinces and over-representation of others. The data sets also did not provided linkages for patients who submitted sputum for drug resistance investigation more than once during the period of study. For this reason, the samples are referred to as coming from cases and not from patients as there was no way of verifying duplicate patients for the period under study.

Recommendations

Though not statistically significant, the steady increase in the rate of MDR TB cases from 2008 to 2011 is an indication for a need to strengthen drug resistant TB surveillance systems.

The National TB Programme (NTP) needs to be strengthened to improve and standardise data management at the reference laboratories. With new diagnostic techniques that have greater sensitivity to detect TB in children the NTP should also be strengthened to provide the service to children at risk of drug resistant TB infection.

Treatment outcomes among patients with drug resistance other than multi drug resistance need to be investigated to evaluate the current treatment guidelines for patients with drug resistance other than multi drug resistance tuberculosis.

There is also a need to have a standardised treatment regimen for mono resistance cases and the poly resistance cases that are not MDR TB cases. Currently the national treatment guidelines do not have treatment plan for such cases, the treatment given is left to the discretion of the attending physician.

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APPENDICES

Appendix 1. University of Zambia, Biomedical Research Ethics Committee Approval letter

THE UNIVERSITY OF ZAMBIA

BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
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Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia

Assurance No. FWA00000338
IRB00001131 of IORG0000774

26th May, 2015.

Your Ref.: 011-02-13.

Ms. Thandiwe Ngoma,
University of Zambia,
School of Medicine,
Department of Community Medicine,
Lusaka.

Dear Ms. Ngoma,

RE: REQUEST FOR CONTINUING REVIEW FOR THE PROPOSAL ENTITLED:
"TRENDS OF DRUG-RESISTANT TB IN ZAMBIA, 2008-2011"
(REF. No. 011-02-13)

We acknowledge receipt of your request for continuing review of the aforementioned protocol. We have taken note that the study did not commence.

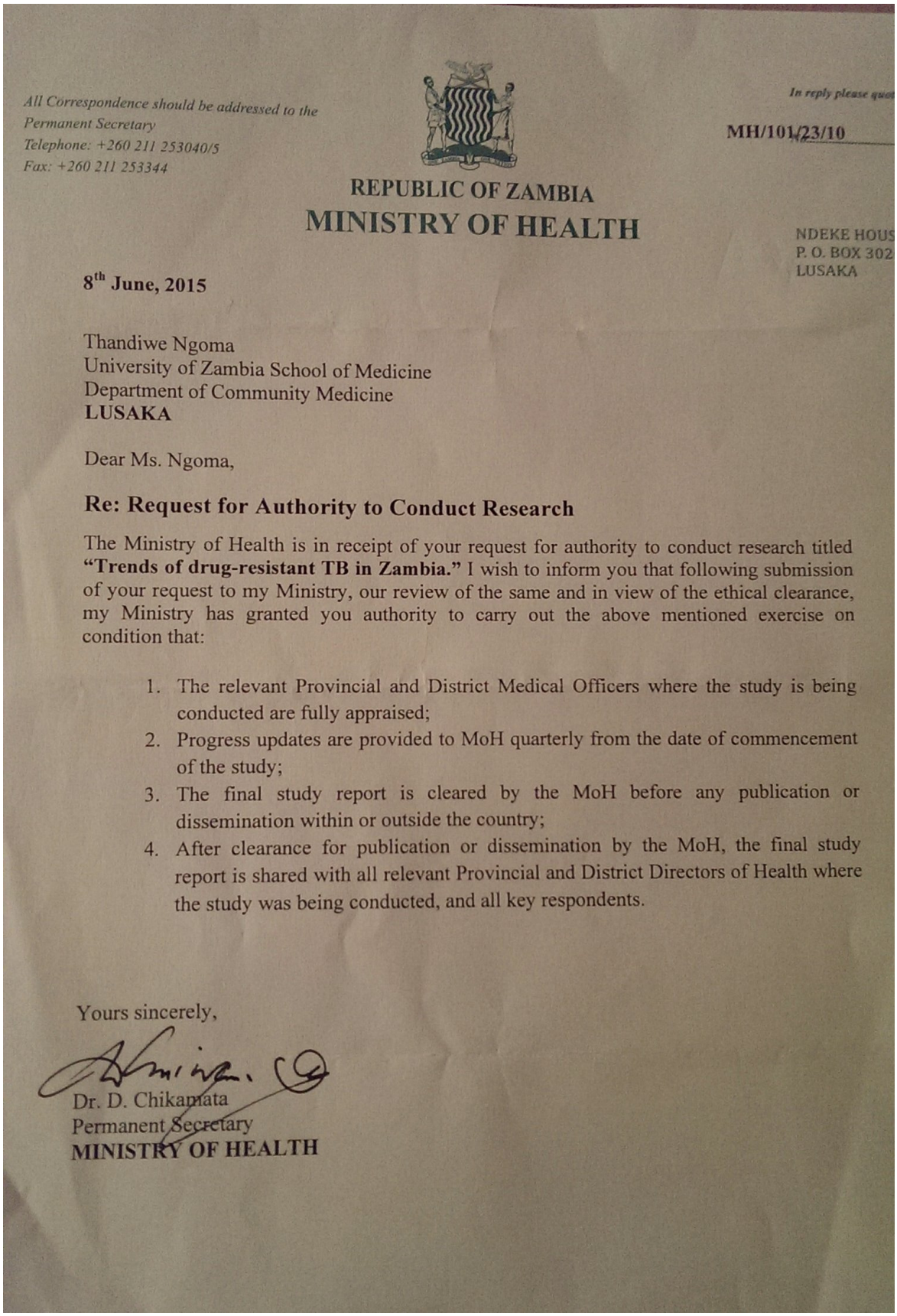
Your request was reviewed and the study period is renewed for a further one year. The new study period is:

- From 29th May, 2014 to 28th May, 2015 and
- From 29th May, 2015 to 28th May, 2016.

Yours sincerely,

Dr. M.C Maimbolwa
CHAIRPERSON

Appendix 2. Ministry of Health, Approval letter



All Correspondence should be addressed to the
Permanent Secretary
Telephone: +260 211 253040/5
Fax: +260 211 253344

In reply please quot

MH/101/23/10

REPUBLIC OF ZAMBIA
MINISTRY OF HEALTH

NDEKE HOUS
P. O. BOX 302
LUSAKA

8th June, 2015

Thandiwe Ngoma
University of Zambia School of Medicine
Department of Community Medicine
LUSAKA

Dear Ms. Ngoma,

Re: Request for Authority to Conduct Research

The Ministry of Health is in receipt of your request for authority to conduct research titled **"Trends of drug-resistant TB in Zambia."** I wish to inform you that following submission of your request to my Ministry, our review of the same and in view of the ethical clearance, my Ministry has granted you authority to carry out the above mentioned exercise on condition that:

1. The relevant Provincial and District Medical Officers where the study is being conducted are fully appraised;
2. Progress updates are provided to MoH quarterly from the date of commencement of the study;
3. The final study report is cleared by the MoH before any publication or dissemination within or outside the country;
4. After clearance for publication or dissemination by the MoH, the final study report is shared with all relevant Provincial and District Directors of Health where the study was being conducted, and all key respondents.

Yours sincerely,

Dr. D. Chikamata
Permanent Secretary
MINISTRY OF HEALTH

Appendix 3. Logistic regression (with age dichotomized as >20 or <20 years)

Logistic regression

Deviance goodness of fit chi-square =	11.338002	df = 3	P = 0.01 *
Deviance (likelihood ratio) chi-square =	8.465963	df = 3	P = 0.0373

Intercept	b0 = -1.976277	z = -3.123475	P = 0.0018
sex female=1	b1 = 0.054907	z = 0.300482	P = 0.7638
>20=1	b2 = 1.19982	z = 1.908142	P = 0.0564
rural =1	b3 = 0.419005	z = 1.864795	P = 0.0622

logit MDR=1 = -1.976277 +0.054907 sex female=1 +1.19982 >20=1 +0.419005 rural =1

Logistic regression - odds ratios

Parameter	Estimate	Odds Ratio	95% CI
Constant	-1.976277		
sex female=1	0.054907	1.056442	0.738425 to 1.511419
>20=1	1.19982	3.319519	0.96794 to 11.384188
rural =1	0.419005	1.520447	0.978843 to 2.361727

Interpretation: Using the available data, MDR TB was not associated with gender, age or locality. However, there was a tendency for cases from rural areas and those aged >20 years to be more likely to be associated with MDR TB, though this was not statistically significant.