

Factors associated with multi-drug resistant tuberculosis in Kenya

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DECLARATION

This is my original work and has not been presented for a degree in any other University.

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DEDICATION

To my wife Rachel Njeri, daughters Neema and Joy and my mother Rispa Weyenga for bearing with my long absence from them encouragement and support they accorded me throughout this study

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LIST OF ABBREVIATIONS

AFB	Acid Alcohol Fast Bacilli
AIDS	Acquired Immunodeficiency Syndrome
AOR	Adjusted Odds Ratio
BCG	Bacille Calmette Guerin
CD4	Cluster Differentiation Cluster of Differentiation 4
COR	Crude Odds Ratio
DLTLD	Division of Leprosy Tuberculosis and Lung Disease
DOT	Directly Observed Therapy
DOTS	Directly Observed Therapy Short Course
DRTB	Drug Resistant Tuberculosis
DST	Drug Susceptibility Test
FELTP	Field Epidemiology and Laboratory Training Program
HIV	Human Immune Deficiency Virus
IQR	Inter quartile range
KEMRI	Kenya Medical Research Institute

MDR-TB	Multi Drug Resistant Tuberculosis
MSF	Médecins Sans Frontières International
NAA	Nucleic acid amplification
OR	Odds Ratio
PLHIV	People Living with HIV
PTB	Pulmonary Tuberculosis
SD	Standard deviation
TB	Tuberculosis
TST	Tuberculin skin test
USD	United States Dollar
WHO	World Health Organization
XDR-TB	Extensively Drug Resistant Tuberculosis
Z-N stain	Ziehl Neelsen stain

ABSTRACT

Multi-drug resistant tuberculosis (MDR-TB) and weak health systems threaten global tuberculosis control. Kenya is ranked 13th among the 22 high TB burden countries worldwide, and currently has an estimated 2,300 MDR-TB patients. A case-control study to determine factors associated with MDR-TB in Kenya was conducted to inform policy in designing public health interventions that are best suited to the country's needs.

This was an unmatched case control study conducted in 41 health facilities in 20 districts across the eight provinces in Kenya from September 2009 to January 2010. Cases were confirmed MDR-TB (resistance to at least rifampicin and isoniazid) patients while controls were sputum- smear positive TB patients with clinical response and negative sputum smear at the fifth month of treatment with first-line anti-tuberculosis drugs. Study approval was sought and obtained from relevant institutions. Using the health facility TB register as the sampling frame, MDR-TB patients and two randomly selected unmatched controls per case were enrolled. A pretested structured interviewer administered questionnaire was used for patient interviews and to abstract information from records. Data on socio-demographic, behavioural, and clinical exposure history were obtained. Data were entered and analyzed using *Epi-info* and Stata versions 3.5 and 9.0 software respectively.

A total of 81 cases {mean age: 32 years (SD: 10), 62% males} and 162 controls {mean age: 35 years (SD: 13), 59% males} there was no statistically significant difference with respect to baseline socio-demographic characteristics. Six (7.4%) of

the MDR-TB cases having no previous history of TB, reported living in the same house with a known MDR-TB patient. Cases were more likely to have history of previous exposure to first line anti-Tuberculosis drugs (OR= 85, 95% CI=29.7-243.3; P<0.0001) and be non Kenyan (OR=5.5, 95% CI=1.4-21.8; P=0.007). Case-patients with positive HIV status (OR=0.34, 95% CI= 0.1-0.9; P=0.025) and those who had received TB treatment under the Directly Observed Therapy program (DOT) (OR=0.23, 95% CI= 0.1-0.6; P=0.002) were less likely to have MDR-TB.

The study established that MDR-TB was associated with previous TB treatment, and being non Kenyan while use of DOT was protective. MDR-TB could be transmitted to otherwise healthy individuals. The protective association with HIV positive sero-status may reflect selective survival of HIV negative MDR-TB and thus need to be investigated.

We recommend strengthening of MDR-TB surveillance among previously treated TB cases and refugees and active MDR-TB case finding among HIV infected TB patients. More rapid MDR-TB diagnostic tests should be used among the HIV infected patients. Access to TB care services by all population groups including immigrants, implementation of DOT, MDR-TB contact tracing and screening and infection prevention should be strengthened in Kenya.

CHAPTER ONE

INTRODUCTION

1.1 Background

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* (*M. tuberculosis*) and occasionally *Mycobacterium bovis*, *Mycobacterium canettii* and *Mycobacterium africanum* (David, 2004). It can affect all tissues in the body except teeth hair and nail but the most commonly affects the lungs (David, 2004). Once known as “the captain among all men of death” and later “the white plague” due to high mortality (40-50%) in the pre-antibiotic era (Laster, 1998) TB is preventable and curable. TB is mainly transmitted through the airborne route (Rieder, 1999). Therefore its control requires early detection of infectious pulmonary TB (PTB) cases and effective treatment to sterilize the sputum in order to break the chain of transmission (Thuridur, 2009; David, 2004). Although anti-TB drugs have been available for the past 50 years, TB remains a major cause of death all over the world (Harries, 2004). Weak health systems, the HIV pandemic and emergence of *M. tuberculosis* strains that are resistant to conventional drugs used in TB treatment threaten global TB control (Thuridur, 2009).

An estimated 32% of the world’s population is infected with *M. tuberculosis* (Eker *et al.*, 2008). *M. tuberculosis* infection is a state where a person carries mycobacteria in small numbers in a dormant state, under control of the immune system, without disease. TB is a state in which one or more organs of the body become diseased as a result of mycobacterial infection giving rise to clinical symptoms and signs (Harries,

2004). Disease occurs when there is multiplication of the bacteria to a number that overwhelms the immune system (Harries, 2004).

Majority (90%) of *M. tuberculosis* infected untreated people without HIV infection remain asymptomatic throughout life. In these individuals, a positive tuberculin skin test is the only evidence of TB infection. About 10% of those initially infected develop TB disease with 50% of these developing disease within the first two years of infection. Young infants and the older people have the highest risk of developing disease. Transmission of TB is promoted by among other factors; PTB, congestion, poor living conditions, and HIV/ AIDS. It has been postulated through mathematical modelling that detecting 70% of all sputum smear positive TB cases and curing 85% of the detected cases would effectively reduce TB transmission (Styblo & Rouillon, 1991). These figures were adopted by WHO as targets for global TB control. To achieve high cure rates TB patients are treated with a combination of drugs. The World Health Organization (WHO) recommends the use of Directly Observed Therapy (DOT), which requires patients to take all their TB drugs under direct supervision. This has been effective in achieving high TB cure rates and preventing emergence of drug resistance (Rieder, 2002). However in the past two decades, drug resistant strains of *M. tuberculosis* have emerged and been reported in all parts of the world (Amor, 2008). Multi drug resistant tuberculosis (MDR-TB) defined as *M. tuberculosis* with resistance to at least Rifampicin and Isoniazid, the two key drugs in TB treatment, is estimated to account for about 2% of global TB cases (David, 2004). In 2006 an estimated 500,000 new cases of MDR-TB existed globally. However, less than 5% of these were notified to the World Health Organization (WHO) and less

than 10% of the notified cases put on treatment in WHO approved projects globally (Dye *et al.*, 2008).

In Kenya the Division of Leprosy Tuberculosis and Lung Disease (DLTLD) in collaboration with the Tuberculosis Central Reference Laboratory (CRL) is mandated to conduct TB, and drug resistant TB surveillance and control. According to the WHO report of 2009, Kenya has achieved 70% case detection and 85% treatment success targets (Ciceri, 2009). However it faces MDR-TB as a major challenge. So far since the initiation of MDR-TB surveillance among previously treated TB patients in 2003, 451 cases have been notified in Kenya (DLTLD, 2009).

1.1.1 Aetiology of tuberculosis

Mycobacteria that cause human and or animal tuberculosis are grouped together within the *Mycobacterium tuberculosis* complex (MTC) (Richard *et al.*, 2006). The classical species of the MTC include *Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium bovis* along with the widely used vaccine strain *M. bovis* Bacillus Calmette-Guerin (BCG) and *Mycobacterium canettii* (Murray, 2003; Richard *et al.*, 2006; Abebe & Bjune, 2006). The human tubercle bacillus (*M. tuberculosis*) is the main cause of tuberculosis all over the world (Crofton, 2009). First described in Senegal in 1968, *M. africanum* is a cause of human tuberculosis in tropical Africa (Baril *et al.*, 1995). It is predominantly isolated in West Africa and is responsible for up to half of smear-positive pulmonary tuberculosis cases in the region (Haas *et al.*, 1999; de Jong *et al.*, 2009). In other parts of the world *M. africanum* has only been isolated among West African

immigrants (de Jong *et al.*, 2009). *Mycobacterium bovis* has an exceptionally wide range of hosts. Most susceptible species, including man, are spill over hosts in which infection is not self-maintaining (O'Reilly and Daborn, 1996). *M. bovis* infection was recognized as a major public health problem when this organism was transmitted to man via milk from infected cows. The introduction of pasteurization of milk and other dairy products helped eliminate the problem (O'Reilly and Daborn, 1996; Rieder *et al.*, 2009). However sporadic cases of *M. bovis* tuberculosis continue to be reported (Hlavsa *et al.*, 2008). *M. microti* has commonly been documented to cause pulmonary tuberculosis in immunocompromised people (van Soolingen *et al.*, 1998) but more recently has been reported among immunocompetent persons (Horstkotte *et al.*, 2001; Frank *et al.*, 2009) while *M. canetti* has been isolated in immunocompromised patients (Somoskovi *et al.*, 2009).

1.1.2 Microbiology of tuberculosis causative agent

Mycobacteria are non-spore forming, non-motile, non-capsulated straight or slightly curved slender rods, measuring 1-10µm × 0.2-0.6 µm. They have a Gram positive cell wall although it does not stain well due to its waxy surface and may appear as clear zones or 'ghosts' in gram stained smears (Cheesbrough, 2000; Murray, 2003). In the natural state *M. tuberculosis* can grow only within the cells of the host organism, but *M. tuberculosis* can be grown on artificial media. *M. tuberculosis* grows aerobically on a protein rich medium e.g. Lowenstein Jensen egg medium at an optimal temperature of 35-37°C (Cheesbrough, 2000). It divides every 20 hours on

commonly used media which is a much slower compared to other bacteria which divide in less than an hour (Cox, 2004; Murray, 2003). Mycobacteria are able to survive for weeks to months on inanimate objects if protected from direct sunlight. They are easily killed by heat $>65^{\circ}\text{C}$ for at least 30 minutes and by UV light, but not by freezing or desiccation. They are more resistant to acid, alkali and some chemical disinfectants than are most non spore forming bacteria (Murray, 2003). Mycobacteria have special staining characteristics due to the cell wall that resists discoloration by alcohol and acid. The cell wall is rich in mycolic acid, a waxy substance that makes them difficult to stain with conventional stains. Staining of mycobacteria is done using the Ziehl–Neelsen (Z-N) staining method. In this method, a smear is prepared and fixed with 70% alcohol or heat and then flooded with strong carbol fuchsin solution, this is then heated to steam and kept steaming for 5-10 min at 60°C . Phenol and heat at this temperature allows the stain to penetrate through the waxy wall. The slide is then washed with water to remove excess stain. Mycobacteria are able to form stable complexes with certain aryl methane dyes like fuchsin and auramine. This yellowish-brown compound is leached easily from tissue and this process is even faster if alcohol is used. The cell wall mycolic acid residues retain the primary stain even after exposure to 20% sulphuric acid 95% alcohol (acid alcohol). The stain that is retained by mycobacteria makes them appear red. Counter staining is done for three minutes with methylene blue to colour the background and the slide finally washed with water to remove the excess counter stain. Malachite green may be used for counter staining but not widely applied due to the red-green colour blindness that exist in at least 30% of men (Akhtar *et al.*, 2000).

1.1.3 Pathology of tuberculosis

The disease process in an individual who is successfully infected with *M. tuberculosis* depends on the number and virulence of the bacilli as well as the ability of the host's cell mediated immunity to contain the bacilli (Bouke *et al.*, 2008). Primary infection occurs in individuals who have never been exposed to *M. tuberculosis*. Infection begins with inhalation of infectious droplet nuclei into the alveoli. In the alveoli mycobacteria multiply and attract immune cells which induce inflammation to form a lesion referred to as the Ghon focus. In majority of cases, the immune response stops multiplication of bacilli. However, multiplication may continue in few cases causing disease (Harries, 2004).

The pathogenesis of primary tuberculosis can be described in four stages. In the first three to eight weeks after *M. tuberculosis* infection, the bacilli multiply and are drained to the hilar lymph nodes leading to hilar lymphadenopathy. Hilar lymphadenopathy and the Ghon focus constitute the primary or Ghon complex. Immune response (delayed hypersensitivity and cell-mediated immunity) with a positive tuberculin skin test also manifest during this period. The second stage which lasts about three months is associated with hematogenous spread of bacilli to other parts of the lungs and body organs (Crofton, 2009). In this stage acute and fatal disease may occur as a result of disseminated disease, for example miliary or meningeal tuberculosis (Crofton, 2009). In the third stage, inflammation of the pleura accompanied with severe chest pain may occur. This results from hematogenous or contiguous spread of bacilli from the sub pleural lesions into the pleural space where the bacilli or their antigens react with sensitized CD4 T lymphocytes that are

attracted to the site and release inflammatory cytokines (Smith, 2003). This stage lasts 3 to 7 months but may be delayed up to 2 years. Resolution of the primary complex marks the beginning of the fourth stage which may last up to three years. In this stage more slowly developing subclinical lesions for example in bones joints may appear in some individuals (Smith, 2003). In many cases the primary lesion commonly heals with no residual changes except occasional pulmonary or tracheo-bronchial lymph node calcifications (Crofton, 2009).

Secondary tuberculosis occurs after a latent period of months or years following a primary infection. Most adult TB in non-HIV infected adults is thought to result from reactivation of dormant, endogenous tubercle bacilli in a sensitized patient who has had previous contact with the tubercle bacillus but re-infection can occur. It may also follow an unresolved primary infection. Reactivation of dormant mycobacteria from the primary infection results from a weakening of the immunity. HIV infection is the most important trigger of progression of infection to disease. HIV infected individuals are not only at a higher risk of getting infected but also tend to rapidly progress to active disease and death. The life time risk of developing active TB in these individuals is 50%. Regardless of the immune status adults tend to develop pulmonary disease. In post primary TB, the immune response characteristically results in localized lesions with caseation, tissue destruction, and cavitation of the lungs. These cavities promote multiplication and shedding of bacilli in sputum. Cavitory pulmonary disease with smear positive sputum is the most important source of *M. tuberculosis* transmission (Harries, 2004). In secondary tuberculosis, hypersensitivity to *M. tuberculosis* enhances resistance and induces a more rapid

response by activated macrophages and fibroblasts. This varies in spectrum from cure, continuous progression to death. If left untreated about 65% of non-HIV infected sputum smears positive pulmonary tuberculosis patients die within 5 years most within the first 2 years. (David, 2004).

1.1.4 Tuberculosis Intervention Strategies

The aim of tuberculosis intervention is to minimise the impact of epidemiological risk factors that promote progression from exposure to *M. tuberculosis* to infection, development of disease, and death. This can be achieved through four approaches including; treatment (chemotherapy), prophylactic treatment, vaccination with BCG and preventive chemotherapy (Rieder, 2002).

The main tuberculosis control strategy aims at reducing the incidence of tuberculosis infection. This can be accomplished by promptly identifying sputum smear positive cases as they are the most infectious source of TB transmission. Sputum smear positive patients should undergo adequate chemotherapy to quickly render them non infectious while at the same time preventing emergence of drug resistance. Prophylactic treatment of people at risk of being exposed but who are not infected also reduces the risk of infection. Another aim is to reduce the prevalence of infection in the population since tuberculosis cases will continue to emerge from the pool of people that harbour *M. tuberculosis*. Preventive chemotherapy targeted at populations with high risk of morbidity is effective in reducing the risk of progression of infection to TB disease. Vaccination with BCG is useful in reducing the risk of

progression from infection to disease and thus in the long run reduces the prevalence of infection (Rieder, 2002).

Many national TB control programs worldwide have adopted the DOT strategy for tuberculosis control (Amukoye, 2008). For successful TB control, all elements of the DOT strategy should be implemented. These include; securing political commitment with adequate and sustained financing; ensuring early case detection and diagnosis through quality assured bacteriology; providing standardized treatment with supervision and patient support; ensuring effective drug supply and management and monitoring and evaluating performance and impact of the TB control programs. The DOT strategy together with other components of the WHO Stop TB strategy including addressing; TB-HIV, MDR-TB and the needs of the poor and vulnerable population among others are approaches proposed by WHO for global TB control. The DOTS strategy is the most cost effective strategy for TB control (Rob *et al.*, 2005)

1.1.4.1 Chemotherapy

Early case finding and appropriate management of Tuberculosis patients is key in TB control. Chemotherapy is the most powerful weapon for tuberculosis control. It has both individual benefit by reducing morbidity and mortality and epidemiologic impact by breaking the chain of transmission if the cure is assured and drug resistance prevented (Crofton, 2009). Tuberculosis treatment regimen should be designed and administered in a way to prevent the emergence drug resistant tuberculosis. The choice of a treatment regimen depends on the form of TB, age of

patient, previous TB treatment history, drug resistance pattern of the infecting strain among other factors. First line drugs used in treatment of tuberculosis include; isoniazid, rifampicin, pyrazinamide, ethambutol, streptomycin and thiacetazone. Isoniazid has a potent early bactericidal activity while rifampicin is bactericidal and has important relapse preventing property that has allowed treatment period to be shortened. Pyrazinamide also has relapse preventing properties that allow further shortening of the treatment period. Ethambutol, streptomycin and thiacetazone prevent emergence of drug resistance (Rieder, 2002). Rifampicin and isoniazid are the two most important drugs in TB treatment. Resistance to these two drugs necessitates the use of second line drugs that are toxic, poorly tolerated, expensive, require prolonged period of treatment, and have poor treatment outcomes. (Iseman, 1999; Amukoye, 2008)

Treatment of new (previously untreated) TB patient begins with a combination of four drugs isoniazid (H) rifampicin (R), pyrazinamide (Z), and ethambutol (E) given in formulations that contain the four drugs in a single pill. These four drugs (RHZE) should be swallowed for the first two months (intensive phase) under direct supervision by a health care provider or community/ family member followed by two drugs either (RH) for four months or (EH) given for six months (continuation phase) given under similar supervision. (Chakaya *et al.*, 2009)

Patients who have ever been treated for TB receive streptomycin (S) injection during the first 2 months in addition to RHZE followed by one month of RHZE then five months of RHE (Chakaya *et al.*, 2009). This combination of drugs constitutes the retreatment regimen. Treatment with this regimen takes eight months and must be

administered under the supervision of a health care provider. During TB treatment patients are monitored for clinical and bacteriological response respectively through monthly clinical examination and sputum smear examination at the completion of the intensive phase and subsequently at the fifth month and completion of treatment either at the sixth or the eighth month depending on the treatment regimen. Patients who remain sputum smear positive even after four months of treatment are said to be treatment failures (Chakaya *et al.*, 2009). Patients with treatment failure should be restarted on the retreatment regimen. However it has been argued that the retreatment regimen described above is inadequate and may result in MDR-TB since there is simply an addition of one drug (streptomycin) to an already failing regimen (Quy *et al.*, 2003).

Drugs for treatment of MDR-TB are classified into five groups. Group one includes first line anti TB drugs; isoniazid, rifampicin, ethambutol, and pyrazinamide. These are well tolerated drugs. Group two agents include injectable drugs like kanamycin, capreomycin, and amikacin. These are highly bactericidal and critical in treatment of MDR-TB. Fluoroquinolone including ciprofloxacin, ofloxacin, levofloxacin, moxifloxacin and gatifloxacin are group three agents. Group three agents have been shown to have excellent invitro activity against *Mycobacteria tuberculosis*. Group four agents including Ethionamide, prothionamide, cycloserine and paraaminoasalicic acid are mainly bacteriostatic but not well tolerated compared to first line drugs. The fifth group is composed mainly of reinforcing agents which include co-amoxiclav, clofazimine, clarithromycin and thiacetazone (Joia *et al.*, 2003).

Regimen for treatment of MDR-TB is preferably made of five drugs to which the *M. tuberculosis* strain is believed to be sensitive. Frequently more than five drugs are started when susceptibility pattern is not known. An injectable drug aminoglycoside or capreomycin is used for at least six months after culture for sputum has turned negative. An 18-24 months regimen is given but patients should get treatment for at least 18 months after culture conversion where conversion means two consecutive negative smears and culture results in samples taken two months apart. Each dose of MDR-TB medicine must be give under direct observation in what is called Directly Observed Treatment Plus DOT-Plus) for MDR-TB treatment (Jaime *et al.*, 2006).

There are various approaches to the choice of an MDR-TB treatment regimen. These include, use of empiric regimen based on the patients history of tuberculosis treatment, use of a standardized regimen based on the drug susceptibility pattern in the population from which the patients originate or individualized regimen tailored to the drug susceptibility pattern of each patient (Jaime *et al.*, 2006).

The design of a regimen for use in treatment of MDR-TB should be based on the drug susceptibility pattern but generally all agents in group one to which the strain is susceptible should be included in the regimen. All patients should receive an injectable agent in group two and a quinolone in group three to which the *M. tuberculosis* isolate is susceptible. Five drugs to which the isolate is susceptible should be used. Group four agents are added based on drug susceptibility. If five drugs cannot be obtained from the first 4 groups then agents in group five are added (Joia *et al.*, 2003). Drugs used in treatment of MDR-TB are toxic and require regular patient monitoring including creatinine for renal function, thyroid stimulating

hormone (TSH) for thyroid function, liver enzymes for liver toxicity among other tests. Patient clinical and bacteriological response is also monitored through regular clinical review and by use of sputum culture and microscopy. Chest radiographs should be taken at least every six months. Sputum smears and culture should be done every month until conversion of sputum.. After conversion sputum smears and culture should be done every three month until patient is cured. Surgical resection of lesions to remove the infected tissues is recommended in patients with localized disease as an adjuvant to chemotherapy (Ormerod, 2007).

Adherence to treatment is critical in management of MDR-TB. It is recommended that patients should receive incentive, enablers and psychological support to ensure that they complete treatment.

1.1.4.2 Management of co-morbid condition

The current TB pandemic is driven by HIV/AIDS. Further evidence suggests convergence of the MDR-TB and HIV/AIDS pandemics (Wells *et al.*, 2007). Therefore management of TB and MDR-TB should include HIV care; HIV counselling and testing, cotrimoxazole preventive therapy and highly active antiretroviral therapy (HAART) can reduce mortality and progression of TB disease (Mermin *et al.*, 2004; Payam *et al.*, 2007). When taken as recommended HAART controls the course of HIV infection and prevents many life threatening complication of AIDS. It also serves as an incentive for HIV testing. On the other hand screening HIV patients for tuberculosis promotes early TB case finding among the HIV patients and reduces the burden of TB among HIV infected individuals (Harries *et al.*, 2004).

1.1.4.3 Preventive Chemotherapy

Isoniazid Preventive Therapy (IPT) for 6-12 months has been effective in preventing the progression of latent TB infection to TB disease in up to 90% of compliant individuals with TB susceptible to first line anti TB drugs (David, 2004). It however carries the danger of mono therapy and isoniazid resistance in adults with TB that is unrecognized. Isoniazid is the drug of choice in patients with TB susceptible to first line drugs. However this cannot be used in prevention of MDR-TB. Regimens that have been tried in preventive chemotherapy in MDR-TB were both ineffective and toxic (Papastavros *et al.*, 2002). Therefore only close observation of contacts is recommended. Generally, even with drug susceptible tuberculosis, preventive chemotherapy is an individual intervention not shown to have a great epidemiologic impact (Rieder, 2002).

1.1.4.4 Prophylactic Treatment

Prophylactic treatment can be used to prevent infection and development of disease in exposed individuals. However there is limited evidence to support large scale use of prophylactic treatment for tuberculosis. Nevertheless this is provided to newborn babies of mothers with infectious pulmonary tuberculosis.

Isoniazid is given to the baby when the infectious source is still smear positive and continued for at least three months after sputum conversion (Rieder, 2002).

So far no regimens have been recommended for prophylaxis against MDR-TB.

1.1.4.5 Infection prevention and control

Infection prevention and control programs are usually set up in settings where the risk of TB transmission is high. These include settings where health care is provided, prisons, the uniformed forces and where immunocompromised patients such as HIV-infected persons congregate. This is useful in reducing transmission of TB.

Educating the public on the mode of spread, methods of control, the importance of early diagnosis, continued adherence to treatment and reducing social conditions that increase the risk of infection should be included in the minimum package for the TB patient care. Training the patient on simple cough etiquette is a useful approach in preventing infectious cases from producing infectious droplets. Covering the mouth and nose during cough is efficient in reducing the number of droplets that reach the air. However this is best done by effective chemotherapy which reduces infectiousness even in smear positive cases within few weeks (Rieder, 2002)

1.1.4.6 Vaccination

Bacille Calmette- Guerin (BCG) vaccine reduces the risk of tuberculosis by 50% (Aronson *et al.*, 2004) and continues to be used to prevent tuberculosis around the world (David, 2004). Although it has shown inconsistent protective effect ranging from no protection to 80% protection, it reduces the risk of severe childhood TB by 70%. In other studies it has been shown to offer protection for up to 50-60 years (Aronson *et al.*, 2004). It has also been documented to offer protection against MDR-TB infection even after controlling for age, sex, race, purified protein derivative (PPD) status, and isoniazid prophylaxis (Kritski, 1996). With the emergence of

MDR-TB in many parts of the world, the use of BCG vaccine among adults with prolonged exposure to MDR-TB is recommended in settings where there is high TB prevalence and where BCG has been demonstrated to offer protection in adults (Kritski, 1996; Rieder, 2002).

1.1.5 Global Impact of TB and Multi Drug Resistant Tuberculosis

Tuberculosis is a major cause of morbidity in most of the world (David, 2004). It is estimated that 32% of the world's population is infected with *M. tuberculosis*, the causative agent for TB (Dye, *et al.*, 2008). Globally most of the TB cases (95%) and TB deaths (98%) are in developing countries. Majority (75%) of TB cases in developing countries are in economically productive age group 15-50 years (Harries, 2004). The annual incidence of tuberculosis increased rapidly between the year 1990 and 2005 (Dye, 2007). This is thought to be mainly due to the HIV/ AIDS pandemic (Dye, *et al.*, 2008). There were 9.2 million new cases including 4.1 million sputum smear positive cases and 1.7 million deaths from TB in the year 2006 globally (Dye, *et al.*, 2008). During the same period, an estimated 709,000 (7.7%) of the global notified TB cases were HIV co-infected, 85% of whom are in Africa (Dye, *et al.*, 2008). Other than causing an increase in number of TB cases, data from DOTS implementing zones worldwide indicate that HIV-infected patients have less favourable TB treatment outcomes compared to non HIV-infected patients (Dye, *et al.*, 2008). This is a result of higher defaulter, death, and out of control rates among these patients (Dye, *et al.*, 2008). The HIV/AIDS pandemic is a major challenge in tuberculosis control. The control of tuberculosis is further confounded by the recent emergence of drug resistant forms of disease. MDR-TB defined as TB strains that are resistant at least to isoniazid and rifampicin (Harries, 2004; CDC, 2006). Extensively Drug Resistant Tuberculosis (XDR-TB) is defined as resistance to Rifampicin and isoniazid and also to an injectable anti-TB (amikacin, kanamycin or capreomycin) and a fluoro-quinolone (Harries, 2004; Dye, *et al.*, 2008). These extreme forms of

TB have emerged in all regions of the world (Harries, 2004: Dye, *et al.*, 2008) and thus MDR-TB has been referred to by some scientists as the third epidemic (Neville *et al.*, 1994). In 2006, there were an estimated 489,000 incident cases of MDR-TB and 150,000 deaths globally, there were also 50,000 XDR-TB cases. However only 23,000 MDR-TB case were notified (Abigail & Zignol, 2008). MDR-TB diagnosis requires expensive laboratory procedures that are not readily available in low income countries (Ormerod, 2005). Most of the notified MDR-TB cases were reported in Europe and Asia (Dye, *et al.*, 2008). Surveillance data indicate that the number of MDR-TB cases is rising especially in the countries of the former Soviet Union, Israel and areas of China (Dye, *et al.*, 2008: Abigail & Zignol, 2008).

1.2 Statement of the Problem

MDR-TB is associated with high morbidity and mortality. It is difficult and expensive to treat and threatens to reverse the gains made in TB control. Described as the “third epidemic” (Neville *et al.*, 1994) it has emerged at a time when TB control programs in many parts of the world especially in resource poor countries are constrained by the HIV /AIDS pandemic. Surveillance of MDR-TB requires well developed laboratory networks, expensive equipment and trained personnel that are not widely available in many settings. Unfortunately the risk of transmission of MDR-TB is highest in resource poor settings.

The prevalence of MDR-TB among TB patients in Kenya is estimated to be less than 1% (Amor, 2008). In 2007, WHO estimates that there were about 2300 MDR-TB cases in Kenya (Ciceri, 2009). However, only 82 were captured by the MDR-TB

surveillance system during the same year (DLTLD, 2007). Although the prevalence of MDR-TB appears to be low, the potential for development and spread of MDR-TB in Kenya is high given the high prevalence of HIV/AIDS (45%) among TB patients, congestion in health care facilities in prisons and in informal settlement, poverty, and overwhelming increase in number of TB cases without matching increase resources for TB control. These factors coupled with limited access to MDR-TB treatment and limited coverage of the population under MDR-TB surveillance may lead to high MDR-TB morbidity and mortality. Currently the country has only been able to initiate treatment for 20% (93) of the 451 confirmed MDR-TB cases (DLTLD, 2009). This leaves 80% of the diagnosed cases untreated, in the community, interacting with the general population probably transmitting disease.

To forestall the potential disastrous consequence of MDR-TB epidemic, there is need to develop public health interventions that are economical, evidence guided, and tailored to meet the needs of individual countries. Understanding factors that are associated with MDR-TB in various settings is critical in developing interventions and formulating policies for MDR-TB control.

1.3 Justification of the study

Kenya's National TB control program faces the challenge of emerging MDR-TB, and an exploding number of TB cases due to the HIV pandemic in the face of shrinking resources. The country's MDR-TB surveillance system is limited to retreatment TB cases and is currently covering 60% of this population. So far since its institution in 2003, the system has been able to detect 451 cases (DLTLD, 2009). This is a low

number compared to an estimated 2,300 cases that existed in 2007 only (Ciceri, 2009). Of those who have been detected, only 93 (20%) of have access to treatment (DLTLD, 2009).

Understanding factors associated with MDR-TB is critical in designing evidence guided intervention strategies that are best suited for the country's situation. Although these factors have been studied extensively in many parts of the world, a knowledge gap still exists in Kenya. This study intends to explore factors that are associated with MDR-TB in Kenya. This information is crucial in formulating policies that will help in allocating resources, targeting surveillance activities, promoting early case finding, managing cases, preventing MDR-TB and its progression to XDR-TB. This will reduce the burden of MDR-TB on the healthcare system and the community.

1.4 Hypotheses

1.5.1 Null Hypothesis

There are no modifiable socio-demographic, behavioural and clinical factors associated with MDR-TB in Kenya.

1.5.2 Alternative Hypothesis

There are modifiable socio-demographic, behavioural and clinical factors associated with MDR-TB in Kenya.

1.5 Objectives

1.5.1 General Objectives

The general objective of this study was to determine socio-demographic, behavioural and clinical factors associated with MDR-TB in Kenya.

1.5.2 Specific Objectives

1. To determine socio-demographic factors associated with MDR-TB in Kenya
2. To determine behavioural factors associated with MDR-TB in Kenya
3. To determine clinical factors associated with MDR-TB in Kenya

CHAPTER TWO

LITERATURE REVIEW

2.1 Mode of Infection and Transmission of Tuberculosis

M. tuberculosis is commonly spread through exposure to tubercle bacilli in tiny airborne droplet 1-5 microns called droplet nuclei that are produced by people with pulmonary or respiratory tract tuberculosis during expiratory efforts for example coughing, singing, sneezing or speaking and are inhaled by a vulnerable contact into the pulmonary alveoli (David, 2004; Harries *et al.*, 2004). Another mode of TB transmission that was common before pasteurization was introduced is consumption of milk or dairy products from an animal infected with *M. bovis*. This was responsible for a large number of gastrointestinal *M. bovis* tuberculosis in children (Rieder, 1999; Crofton, 2009). Direct invasion of *M. tuberculosis* through mucus membranes or breaks in the skin may occur but rarely (David, 2004). In pulmonary transmission, procedures that generate aerosols such as bronchoscopy, intubation, autopsy or laboratory manipulation of specimen (David, 2004) may expose health care workers like does expiratory efforts.

Once released into the air, liquid droplets containing *M. tuberculosis* evaporate rapidly to reach a critical size that remain suspended in the air for long period and disperse throughout the air volume (Riley and Nardell, 1989). A single cough produces 3000 droplet nuclei. For transmission to occur, the droplet nuclei must be small enough to reach the alveoli at the periphery of the lung. Transmission of tuberculosis depends on infectious cases producing airborne droplets thus limiting

TB transmission to patients with tuberculosis of the respiratory tract. The concentration of bacilli in the sputum and level of smear positivity correlate well with degree of infectivity and severity of disease in the infectious source (Liipo *et al.*, 1993; Akhtar *et al.*, 2000). Heavily smear positive cases are more likely to transmit tuberculosis. It requires some 5000 bacilli in 1 millilitre of sputum to yield a positive sputum smear (Toman *et al.*, 1979). Consequently the risk of transmission from someone with a sputum smear-positive pulmonary is higher than those whose sputum smears are negative but culture positive and even lower from someone with extra pulmonary TB (Herna'ndez-Gardun'õ *et al.*, 2004; Harries, 2004). In fact although sputum smear positive TB cases constitute about 50% of all TB cases, they are responsible for 95% of TB transmissions (Rieder, 2002). The risk of transmission of TB decreases with increasing distance between the sputum smear positive TB case and the contact (Veen *et al.*, 1992).

The probability that an infection occurs given exposure depends on the concentration of infectious droplet nuclei in the air and the duration of exposure of the susceptible person (Rieder, 1999). Ventilation rapidly reduces the concentration of infectious droplets while direct sunlight kills bacilli in 5 minutes. However, bacilli can survive in dark places for long periods (Harries, 2004). Transmission therefore generally occurs indoors. Infection rates are highest among people who closely interact with an infectious case in poorly ventilated areas for prolonged periods of time and low among casual contacts (Rieder, 1999).

At the peripheral alveoli, establishment of an infection depends on the host immune response. Bacilli are either removed completely or develop a latent TB infection

(LTBI). The risk of infection does not appear to be related to genetic factors (David, 2004). HIV infection dramatically increases the risk of TB infection in susceptible individuals. Other factors that influence the risk of infection include; young age, gender, previous exposure to *M. tuberculosis*, malnutrition, underweight, debilitating condition e.g. chronic renal failure, cancer, silicosis, diabetes or gastrectomy and drug abuse (Crofton, 2009). The life time risk of active tuberculosis disease in non HIV infected individuals given infection is only 10%. One half of these will develop disease in the first 12-24 months of the infection while the rest will develop over a life time. Low socioeconomic status young age below three years, advanced age, malnutrition concurrent infections all increase the risk of progression of infection to active disease (Crofton, 2009). HIV infection increases the life time risk of TB from 10% to 50% and dramatically raises the annual risk to 5-15% depending on the CD4 cell count and antiretroviral therapy (David, 2004). HIV infected patients with high CD4 cell counts are less likely than those with low counts to develop active disease. Antiretroviral therapy reduces both the risk of progression to active disease given infection and risk of death given disease. The risk of death given disease depends on the form and site of disease and appropriateness and timeliness of the intervention provided. Death is certain if patients with meningeal TB are left untreated (Crofton, 2009).

2.2 Diagnosis of Tuberculosis and Multi-Drug Resistant

Tuberculosis

The diagnosis of tuberculosis is based on several approaches including; medical history and physical examination, bacteriologic examination, imaging techniques, tuberculin skin test (TST), and nucleic acid amplification tests.

History taking is critical in the initial diagnostic workup of a suspected tuberculosis patient. This should include a proper history of the presence and duration of symptoms of tuberculosis including; cough, weight loss, night sweats, loss of appetite, chest pain, as well as exposure to other risk factors for developing tuberculosis disease. The definitive diagnosis of tuberculosis is made through laboratory demonstration of acid fast bacilli in sputum smear preparations. Isolation of *M. tuberculosis* in cultures is the gold standard for TB diagnosis. It allows for genotyping for epidemiological purposes as well as drug susceptibility testing (DST) for definitive chemotherapy.

A tuberculin skin test is administered to identify those with TB infection among patients presenting with TB symptoms as well as to screen for TB among close contacts of known TB cases. Chest X-ray is useful in diagnosing TB in patients with a pulmonary disease but with negative sputum smears and in those with a positive (TST). X-ray is also used in diagnosing TB of bony tissues. e.g. tuberculosis of the spine (David, 2004).

In clinical diagnosis, cough, cough with sputum, hemoptysis, weight loss, fever and sweating are the most important symptoms of tuberculosis (Crofton, 2009). In a study

conducted among patients attending services for diagnosis of PTB in Sudan, cough for three weeks or more was the most sensitive symptom for identifying TB patients (El-Sony *et al.*, 2003). Other general symptoms to look for in the history include chest wall pain, breathlessness, localized wheeze, frequent colds, tiredness and loss of appetite. Patients who visit health facilities late may have more symptoms than those who present early. However in all cases symptoms appear gradually over weeks or months (Crofton, 2009). History of previous TB treatment should capture information on the duration of treatment, the outcome of treatment, source and types of drugs used and if available, the patient should be asked to provide packets of the drugs to identify possible monotherapy. A good medical history can identify suspects of drug resistant tuberculosis for example, patients with failed previous TB treatment, poor compliance, relapsing TB and close contacts of MDR-TB cases. Physical examination does not often help in diagnosis (Crofton, 2009) but useful signs may be elicited e.g. deformities of the spine due to vertebral collapse.

Diagnosis of TB with a chest X-ray has high sensitivity in HIV negative patients however with reduction in radiological findings in HIV positive patients sensitivity reduces with advanced HIV disease (Wood, 2007). High resolution computerized tomography (CT) scanning on the other hand can detect TB with minimal exudation and early bronchogenic spread (Hatipoglu *et al.*, 1996).

The sensitivity of Clinical and X-ray diagnosis of tuberculosis is high and can identify patients with a lower number of bacilli per millilitre of sputum compared to other diagnostic tests (Akhtar *et al.*, 2000). However TB symptoms are none specific and unable to differentiate TB from other chronic lung diseases particularly in

children (Schaaf *et al.*, 1994; Lockman *et al.*, 2003). Therefore all patients with cough for three weeks or more should undergo further investigations for example sputum smear examination for acid fast bacilli. Combining several approaches makes TB diagnosis procedure more sensitive. In Nairobi, routine TB diagnostic approach which includes history taking chest X- ray and three sputum smears for microscopy was able to detect 92% of culture positive tuberculosis (van Cleeff *et al.*, 2003).

Sputum smear microscopy is the cornerstone for diagnosis of pulmonary tuberculosis in adults. It identifies the most powerful sources of infection, can be performed quickly, and is highly specific in high TB prevalence countries (David, 2004; Wood, 2007). It has high accessibility and in settings with good microscopy, if done well sputum microscopy can detect 83% of TB transmitters (Akhtar *et al.*, 2000). However, its sensitivity in detecting all cases of tuberculosis even in good settings is only 60% (Tessema *et al.*, 2001; Bruchfeld *et al.*, 2000; Siddiqi, *et al.*, 2003). It is tedious, and, cannot distinguish live from dead bacilli. Fluorescence microscopy offers higher sensitivity and the same specificity as Z-N staining, but its value in HIV infected patients is not known (Steingart *et al.*, 2006). It is recommended that two or three sputum samples including a morning sample should be examined and the number of bacilli seen reported using the International Union Against Tuberculosis and Lung Disease (IUATLD) criteria (Appendix 3). Useful as it is, sputum smear microscopy requires at least 5000 bacilli per ml of sputum to test positive (Cook and Zumla, 2003). To detect as few as 10^1 to 10^2 viable organisms per ml specimen culture of sputum sample is required. (Murray, 2003; Akhtar *et al.*, 2000).

Culture is the gold standard for TB diagnosis but it requires expertise, expensive equipment and careful handling of specimen. It is also slow and has a high risk of contamination by fast growing bacteria. Culture has a sensitivity and specificity of 80-85% and 98% respectively (Ichiyama *et al.*, 1993). Broth or solid media are available for *M. tuberculosis* isolation. Broth media are preferred for rapid initial isolation of *M. tuberculosis* since large inoculums can be added improving the probability of isolating *M. tuberculosis* (Cook & Zumla, 2003). They also take a shorter time 1-3 weeks to grow *M. tuberculosis* compared with 3-8 weeks for traditional egg based solid medium e.g. Lowenstein Jensen (L-J). Nevertheless they have higher contamination rates (Murray, 2003; Morgan *et al.*, 1983) Agar based media can also be used for growth of *M. tuberculosis*. Colonies can be detected on agar based media after 10-12 days compared with 18-24 days for egg based solid media (Murray, 2003). It therefore offers an alternative to liquid media for faster isolation of *M. tuberculosis*. Solid media require 6-8 weeks to confirm diagnosis and a further 4-6 weeks for drug susceptibility testing. Liquid culture is faster with results of diagnosis and DST received within 7 days (Wood, 2007). Growth detection methods based on detection of radioactivity (Bactec 460-TB), fluorescence (Bactec Growth Indicator Tube (MGIT 960)), phage-based tests e.g. FASTPlaque TB-RIF and Microscopic Observation drug susceptibility Assay (MODS) are faster diagnostic methods.

Drug resistant tuberculosis is diagnosed by demonstration of invitro growth of mycobacteria in media containing drugs under test. In this test, it is assumed that lack of mycobacteria growth is due to inhibition by the drug contained in the media.

Nucleic acid amplification (NAA) tests have improved detection and identification of *M. tuberculosis*. They have a high specificity but variable sensitivity and have the advantage of providing faster results however they should be used in combination with clinical and microscopy findings to confirm TB diagnosis. These methods are also useful in identifying drug resistance by identifying sequence in *rpoB* and *katG* genes which code for rifampicin and isoniazid resistance. Some commercially available kits amplify nucleic acid directly from clinical samples and can give results within 6-7 hours (Wood, 2007). These test however cannot differentiate dead from live bacteria, are expensive, require high laboratory technical capacity and quality control.

A variety of immunodiagnostic test based on the recognition of the specific host response to infection with *M. tuberculosis* are available. TST is the oldest however it has limited value in diagnosis of TB in areas with high TB and HIV prevalence and where BCG is given at an early age (Siddiqi *et al.*, 2003) this test yields false negative results in immunosuppressed HIV infected individuals and gives false positive results due to BCG vaccination, interaction with environmental mycobacteria and previous TST (Rieder, 1999). It also lacks the ability to differentiate active from past sensitization. Newer immunodiagnostic tests based on antibody detection are available however the specificity of these test is less than 80% (Wood, 2007) Elisa tests for detecting lipoarabinomannan (LAM) an *M. tuberculosis* specific antigen and cytokine detection assays e.g. QuantiFERON-TB GOLD which uses peripheral blood and T-SPOT TB assay which uses isolated peripheral monocytes are based on INF- γ detection and therefore not subject to

interaction with environmental mycobacteria or exposure to *M.bovis* strains used for BCG vaccination. The sensitivity and specificity of LAM ELISA test is unknown. While the specificity and sensitivity of QuantiFERON-TB GOLD and T-SPOT TB is 75%-95% and 90-100% respectively in none HIV infected. The sensitivity may be lower in HIV infected (Pai *et al.*, 2007)

2.3 Emergence of Tuberculosis Drug Resistance

Emergence of drug resistance in *M. tuberculosis* occurs mainly through mutations. Several mechanisms that favour emergence of drug resistant strains of *M. tuberculosis* during chemotherapy have been described. These include effective or functional mono therapy, difference in bactericidal activity, mono therapy during sterilization of special populations, sub inhibitory concentration, and difference in post antibiotic lag phase (Rieder, 2002)

The use of a single drug to treat TB (effective mono therapy) has been documented as a cause of TB drug resistance. In a large population of susceptible bacilli present in a TB patient, spontaneous mutations occur with a given probability for each drug that convey resistance to that drug. (Rieder, 2002) This leads to selection and replication of the drug resistant mutants giving them an opportunity to become the dominant strain while susceptible organisms are being killed off. If another drug is added to the one to which resistance has developed, the bacilli that are resistant to the initial drug quickly develops resistance to the second drug and same cycle repeats with addition of a single drug to the already failing ones.

This phenomenon can be prevented by use of multiple drugs in combination started at the same time. Even with the use of drug combination, drug resistance may still occur due to the difference in early bactericidal activity (EBA) of the drug in the combined pill. Isoniazid has a higher EBA than rifampicin (Jindani *et al.*, 1980) therefore if started together; mutants that are resistant to isoniazid will be selected since they will only be killed by rifampicin. This however only happens for the first 2 days as later than this the EBA of the two drugs are similar. Owing to the fact that at the beginning of treatment the bacillary number is high, there is a high probability of having mutants that are already resistant to single drugs. If a patient takes drugs irregularly, for example for 2-3 days followed by a period when no medication is taken, this may allow selection of strains that are resistant to multiple drugs even with the use of a combination of drugs (Mitchison, 1998)

Drug used in TB treatment have different sterilization ability on of different *M. tuberculosis* populations. Rifampicin has a high bactericidal activity on semi-dormant bacilli while pyrazinamide has the highest bactericidal activity on bacilli in acidic media. During treatment, none of the other drugs has effect on these groups of *M. tuberculosis* therefore a high risk of selecting resistant mutants.

In periods when the serum concentration of drugs is low and only slows the growth of susceptible organism but does not kill them, drug resistant mutants are selected as the bacilli re-grow. In these circumstances, resistance tends to emerge to drugs with a long half life that persist in serum after the other drugs have been cleared.

Following the first few doses of anti tuberculosis drugs, different drugs inhibit bacterial growth for different periods of time after the last dose of the drugs (lag period). At the end of the lag period, there is bacterial re-growth. If for example isoniazid which has a long lag period is given in combination with rifampicin with a short lag period, there will be a time when the bacilli will be exposed to isoniazid only after the end of rifampicin lag period. As the bacilli re-grow, isoniazid resistant mutants will be selected. This mechanism is more likely to result in resistance to drugs with long lag periods e.g. streptomycin and isoniazid (Donald, 1997).

Prescribing of single drugs or inadequate drug regimen by the care providers, adding a single drug to an already failing regimen can contribute to an increase in drug resistance (Quy *et al.*, 2003). Poor compliance to treatment and use of drug preparations that do not achieve optimal serum concentration may promote development of resistance through any of the stated mechanisms.

2.4 MDR-TB Situation in Kenya

In 2008, Kenya was 13th among the 22 high tuberculosis burdened countries that collectively contribute to 80% of the global TB cases (Dye, *et al.*, 2008). Although Kenya has achieved the WHO targets of detecting 70% infectious sputum smear positive TB cases and successfully treating 85% of the detected cases, it continues to face challenges in TB control. The most disturbing of the challenges is the emergence of MDR-TB. In the global anti-TB drug resistance survey conducted in 1993-94, Kenya did not report any multi-drug resistant TB (WHO, 1997); However MDR-TB was reported in a refugee population in the North Eastern province. Since initiation of MDR-TB surveillance in 2003, 451 MDR-TB cases have been identified from, 14,000 retreatment TB cases for whom sputum was submitted for drug susceptibility testing (DLTLD, 2009). In 2007, 82 cases were detected by the MDR-TB surveillance system although according to WHO estimates, 2300 cases existed in the same year (Ciceri, 2009). WHO estimates the prevalence of MDR-TB among all TB patients in Kenya to be less than 1% (Abigail & Zignol 2008), however other studies suggest that, MDR-TB may be underestimated in African countries (Amor *et al.*, 2008; Ellen *et al.*, 2008). Although a large number of MDR-TB cases have been diagnosed in Kenya since 2003. It is worth noting that the MDR-TB surveillance is limited to retreatment TB cases and therefore a large number of patients may die undiagnosed. This could occur particularly among HIV infected patients who tend to rapidly develop TB and die within a period shorter than it takes to complete drug susceptibility tests. Furthermore treatment for MDR-TB was not available in the public sector in until 2008. Even after introduction of MDR-TB treatment, most of

the diagnosed patients are left in the community untreated, and an unknown number of diagnosed MDR-TB patients in Kenya may have died. Complicating TB control further is the recent diagnosis of XDR-TB in a seventeen year old HIV positive girl. This girl had neither suffered from TB nor had prior exposure to anti-TB drugs. However the girl had a history of contact with a foreigner who was undergoing treatment for TB. This is disturbing as it suggests that there is transmission of drug resistant tuberculosis in the community. Under the prevailing circumstances, the risk of spread of MDR-TB in Kenya is high given the high HIV/ AIDS prevalence (45%) among TB patients and 7.4 % in the general population nationally (Odhiambo *et al.*, 1999; DLTLD, 2007; NASCOP, 2008). Poor living condition, poverty, congestion in hospitals in prisons and the mushrooming informal settlements are all in favour of the growth of the MDR-TB epidemic. Despite the high mortality and morbidity of MDR-TB, the chronic nature of TB allows some infectious cases to survive for long periods spreading MDR-TB to a population that is rich in susceptible individuals (Odhiambo *et al.*, 1999; David, 2004).

2.5 Risk Factors for Developing MDR-TB

MDR-TB has been described as a man-made problem resulting from the use of inadequate drug regimen that select drug resistant tubercle bacilli, poor supervision of treatment by TB control programs and non-adherence by the patient (Chakaya *et al.*, 2006). Studies to determine risk factors for developing drug resistant tuberculosis have been conducted in several parts of the world. Most of these studies consistently report history of previous treatment as a risk factor for development of MDR-TB (Clark *et al.*, 2005; Espinal *et al.*, 2001, Anastasis *et al.*, 1997). A case-control study conducted in Hong Kong indicates that frequent travel and young age are independent predictors of MDR-TB (Law *et al.*, 2008). In the same study, MDR-TB patients were found to be less likely to have received directly observed treatment (DOT) during the previous treatment. Young age was also reported to be a risk factor for MDR-TB in British Columbia where reactivated TB and pulmonary TB (PTB) were also found to be associated with MDR-TB (Moniruzzaman *et al.*, 2006). Another study conducted in Brazil reported previous TB and hospitalization within 24 months of diagnosis to be significantly associated with MDR-TB (Telles *et al.*, 2005). Smoking, recreational drug use and imprisonment were found to be associated with drug resistance in a survey carried out in Samara region Russia (Ruddy *et al.*, 2005). Mass incarceration has been implicated in explosion of MDR-TB in Asia and former Soviet Union states (Stuckler *et al.*, 2008).

Low CD4 cell count and advanced HIV disease were risk factors for MDR-TB in a study conducted among HIV infected patients in Mozambique (Nunes *et al.*, 2005). Although other studies have failed to demonstrate HIV as a risk factor for MDR-TB,

surveillance data suggests convergence of MDR-TB and HIV/ AIDS (Wells *et al.*, 2007; Abigail and Zignol. 2008). Several other studies, indicate that development of rifampicin resistance may be spurred by HIV infection (Burman *et al.*, 2006, Li *et al.*, 2005, LoBue & Moser, 2005).

MDR-TB is associated with high morbidity and mortality in high HIV prevalence settings. In these settings, the rate of spread of MDR-TB is high, and outbreaks may occur (Wells *et al.*, 2007). HIV-infected individuals who develop MDR-TB tend to progress rapidly to fatal disease.

Failure to control MDR-TB may lead to progression to XDR-TB, a virtually untreatable form of TB. (Chakaya *et al.*, 2006) XDR-TB in HIV infected individuals has been reported to have case fatality rates as high as 98% (Ghandhi *et al.*, 2006). Treatment for MDR-TB and XDR-TB is prolonged, expensive, associated with heightened adverse drug reactions and low cure rates. It is also not widely available in resource-limited countries.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design

This was an unmatched case-control study. This study design was chosen because it allowed for assessment of any association between multiple factors and MDR-TB to be made between cases and controls. The study design was also suitable since it allowed the study to be conducted within a relatively short period of time and limited resources.

3.2 Study Site

The study was conducted in 41 sites in 20 districts across the eight provinces of Kenya listed in Appendix 10. Health facilities were chosen based on the presence of surviving MDR-TB patients.

3.3 Study Period

The study was conducted between September 2009 and January 2010.

3.4 Study Population

The study targeted all surviving MDR-TB patients confirmed by culture and drug susceptibility testing between 2006 and 2009 and selected ordinary sputum smear positive TB patients at the same site as the MDR-TB patients.

3.4.1 Inclusion criteria

Cases: These were patients confirmed by culture and drug susceptibility testing to have *M. tuberculosis* strains that were resistant at least to rifampicin and isoniazid

(MDR-TB). MDR-TB patients were identified from line lists available at Provincial TB coordinators offices and traced to their respective districts.

Controls: These were patients on treatment for sputum smear positive pulmonary tuberculosis, who had shown clinical and bacteriological response to treatment with first line anti tuberculosis drugs. Bacteriological response was based on conversion of sputum smears from positive at initial diagnosis to negative at the fifth month as recorded in the facility treatment register for diagnosis and treatment monitoring respectively. Ziehl- Neelsen staining method is used in routine TB diagnosis and treatment monitoring.

For both cases and controls, an informed consent was a prerequisite for enrolment into the study.

3.4.1 Exclusion criteria

Patients who did not consent to participate in the study, those with sputum smear positive TB at initial diagnosis but fail to achieve sputum conversion by the fifth month, those with sputum smear positive TB at initial diagnosis but without laboratory confirmation of sputum conversion at the fifth month and patients with sputum smear negative and extra pulmonary TB at diagnosis were excluded from the study.

3.5 Sample Size Determination

A minimum sample size of 231 (77 cases and 154 controls) was reached using the Fleiss formula (Fleiss, 1981) the following assumptions were made (Appendix 9) for detailed symbol description):-

The proportion of MDR-TB cases and controls with smear positive TB relapse was 23% and 9% respectively (DLTLD, 2007)

Minimum Odds ratio worthy detecting by this study was 3.0

2- Tailed level of significance (α) = 5%

Power of the study ($1-\beta$) = 80%, (β) = 0.20

Sample is independently and randomly selected.

Fleiss formula

$$n_1 = \frac{\left[z_{\alpha/2} \sqrt{(r+1)\bar{p}\bar{q}} + z_{1-\beta} \sqrt{rp_1q_1 + p_2q_2} \right]^2}{r(p_1 - p_2)^2},$$

$$n_2 = r \times n_1 \quad \text{and} \quad p_1 = \frac{p_2(OR)}{1 + [p_2(OR - 1)]}$$

$$\bar{p} = \frac{p_1 + r \times p_2}{r + 1} \quad \text{and} \quad \bar{q} = 1 - \bar{p}$$

In order to maximize the power, and given that the total number of known surviving MDR-TB patients on the provincial line lists in the country was only 186, all MDR-TB patients who accepted to participate in the study were enrolled.

3.6 Sampling Method

All 186 MDR-TB patients on the provincial line lists by September 2009 were tracked to their respective districts and the living accessible ones who consented progressively enrolled until eighty one cases were enrolled.

Two controls were selected per case in the same facility as the case using simple random sampling method. A table of random numbers was used to select control from the facility register. In facilities where adequate suitable controls were not available, more controls were selected from randomly selected facilities that treat, ordinary TB patients referred from the MDR-TB managing health facility. The facility TB register was used as the sampling frame for all controls. A total of 40 facility TB registers were used.

3.7 Data Collection Methods and Instruments

A pre-tested structured interviewer administered questionnaire (Appendix 1) was used to collect data from each study participant (cases and controls). The same questionnaire was used to extract and abstract data from patients' records. The BCG scar was checked on all participants.

District TB coordinators were notified of the patient interviews and requested to provide a schedule of the monthly clinic days for the facilities they supervise. They were also requested to trace MDR-TB cases prior to the interviewers' visit. Interviewers were trained on data collection procedures before the beginning of data collection to ensure consistency. Information on general social demographic, behavioural and clinical, characteristics was collected through interviews. Additional

data on patient clinical category, DOT status, patients' HIV serostatus, CD4 cell count and antiretroviral therapy were abstracted from the patient record card and TB register.

At the end of each interview, the principal investigator crosschecked the questionnaire to ensure completeness and data accuracy.

3.8 Data Handling and Management

Data were coded during collection and *Epi info* version 3.5 statistical software (free software by WHO/CDC) used for both data entry and analysis. Double data entry was done on daily basis to minimize errors by identifying inconsistently entered files. Data cleaning was done prior to analysis. Each questionnaire was assigned a unique identifier to allow validation. To ensure confidentiality, access to data was restricted by use of passwords only available to the principal investigator. Questionnaires were kept under lock and key by the principal investigator and only availed to authorized persons.

3.9 Data Analysis

A descriptive analysis was done based on frequency distribution of selected socio-demographic characteristics. Means, standard deviations and quartiles of selected study variables were obtained. Dummy tables were used to guide the analysis. The respondents were categorized into those that have MDR-TB (cases) and those who do not but are also TB patients (controls).

Crude Odd Ratio (OR), Yates corrected chi square test and Fishers exact test (where the count in some cells of the tables was less than 5) for categorical variables

(nominal data) at 95% confidence interval (CI) and alpha level of significance set at 0.05 were used as measures of association in the analysis of factors associated with MDR-TB. T-test at the same confidence interval and significance level was used for numerical variables. An odds ratio (OR) of < 1 was considered protective while odds ratio of > 1 was considered a risk factor. An odds ratio equal to 1 indicated that there was no difference between cases and controls. Confidence interval was used to assess variability of the odds ratio. A 95% confidence interval that included 1 was interpreted to be not significant.

Risk factor variables with $P < 0.05$ were considered as having significant association with MDR-TB. Stratified analysis was carried out to determine any confounding factors and effect modifiers. Effect modification was identified by determining whether the Chi-square for differing Odds Ratios by stratum (interaction) is significant (i.e. $P < 0.05$). Stratified analysis was used to identify socio-demographic factors to be included in unconditional logistic regression. Risk factors with less than 10% level of probability ($P < 0.1$) in bi-variate analysis were entered into unconditional logistic regression model. Stepwise backward elimination logistic regression was used to come up with the final “Best” model. The final model achieved thus contained only statistically significant factors at an alpha level of 0.05. All the variables initially removed from the model were henceforth re-entered into the model for retesting and subsequently removed if they did not contribute to improving the model.

3.10 Ethical Considerations

Approval and clearance was sought and obtained from the Ministry of Higher Education Science and technology, JKUAT Board of Postgraduate and the ethical review committee and the Ministry of Public Health and Sanitation (Appendixes 4, 5 and 6). Informed consent was a prerequisite for enrolment for all participants.

Data collectors were trained in infection prevention. Respirators and ordinary surgical masks were provided to all interviewers and study participants respectively for use during the interviews. The interviews were conducted in open areas with free circulation of air to reduce the risk of TB transmission. Information obtained was treated with confidentiality. Copies of the questionnaires are under lock and key at least for the next three years and only available to authorized persons.

CHAPTER FOUR

RESULTS

4.1 Demographic Characteristics of Study Participants

In this study done from September, 2009 to January, 2010, a total of 243 participants were enrolled comprising of 81 cases and 162 controls. These participants were recruited from 41 TB treatment health facilities in 20 districts across the eight provinces in Kenya viz: Coast, Nairobi, Rift Valley, Western, Nyanza, North Eastern, Central and Eastern (Figure 1). Although majority of patients were in Nairobi, the proportions of cases sampled in most provinces were similar. (Appendix 10)

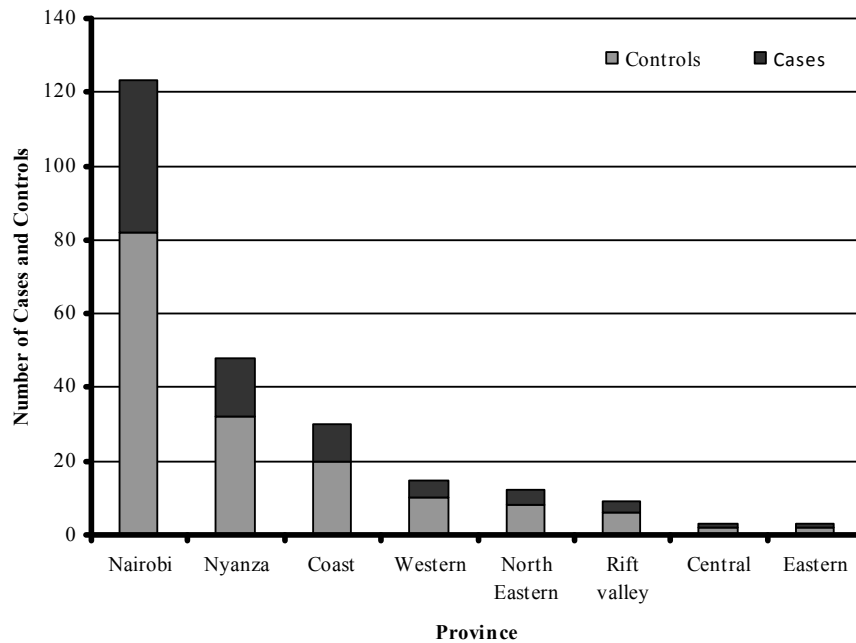


Figure 4. 1: Distribution of MDR-TB cases and controls by Province, Kenya 2009

Cases had a mean age of 32.4 (SD*=10.4) years, a median of 31 (IQR**=26.0-37.0) years, while the controls had a mean age of 34.7 (SD=12.6) years and median of 32.0, (IQR=26.0-40.0) years. Majority of the study participants were in the economically productive age group, 15-44 years (Figure 4.2).

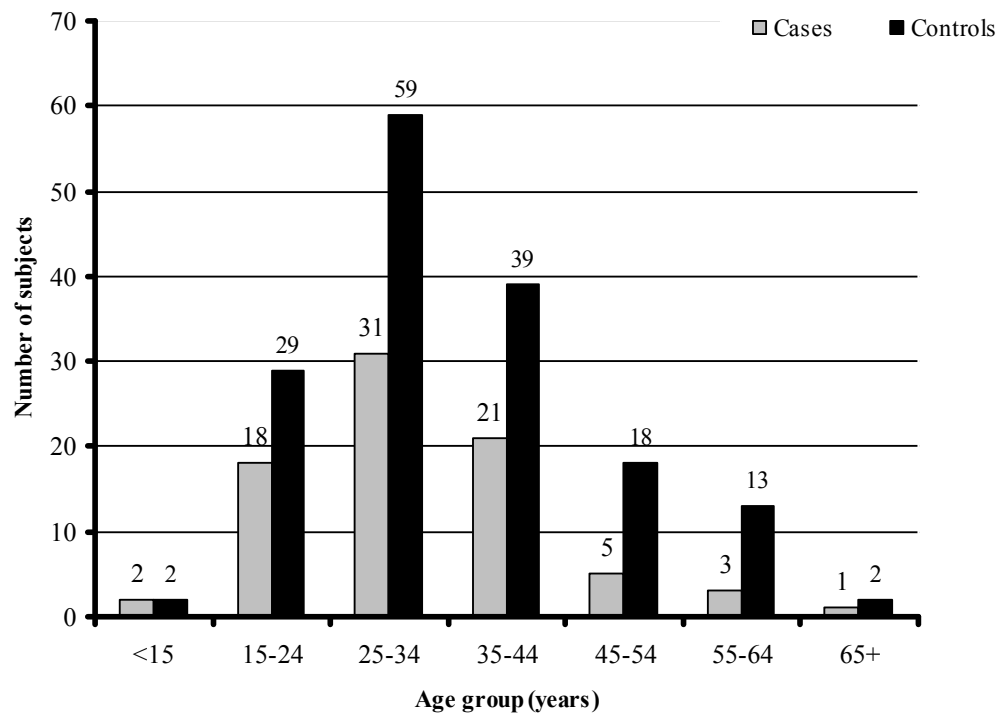


Figure 4.2: Age distribution of MDR-TB cases and controls Kenya, 2009

*SD** Standard deviation. *IQR*** Interquartile range.

One hundred and forty six (60%) study participants were male. A total of 130 (54 %) of the study participants were living in some form of marital union (married or cohabiting) while, 138 (91.5%) had completed at least the primary school education. No statistically significant differences were observed in these variables among cases and controls, although a larger proportion 65 (80.2%) of cases were unemployed at the time of the interview compared to the time before illness (Table 4. 1).

Table 4. 1: Demographic characteristics of MDR-TB cases and controls, Kenya 2009

Variable	Cases		Controls	
	(N=81)		(N=162)	
Mean age in Years (SD)	32.4	(10.4)	34.7	(12.6)
Gender				
Male	50	(62%)	96	(59%)
Female	31	(38%)	66	(41%)
Level of Education				
None	11	(13.6%)	8	(4.9%)
Primary	34	(42.0%)	74	(45.7%)
Secondary	24	(29.6%)	54	(33.3%)
Tertiary	12	(14.8%)	26	(16.0%)
Marital Status				
Single	27	(33.3%)	49	(30.8%)
Separated	10	(12.3%)	9	(5.6%)
Divorced	3	(3.7%)	4	(2.5%)
Widowed	4	(4.9%)	7	(4.3%)
Married	36	(44.4%)	91	(56.2%)
Cohabiting	1	(1.2%)	2	(1.2%)
Current Employment				
Employed	15	(18.5%)	68	(42.0%)
Not employed	65	(80.2%)	89	(54.9%)
In school	1	(1.2%)	5	(3.1%)
Employment before illness				
Employed	35	(43.2%)	70	(43.2%)
Not employed	45	(55.6%)	88	(54.3%)
In school	1	(1.2%)	4	(2.5%)

4.2 Socio-demographic Factors Associated with MDR-TB

The distance travelled to the TB treatment facility by the cases during treatment for ordinary TB was approximately twice that travelled by controls ($P < 0.001$) a similar observation was made in the duration of travel among the two groups ($P < 0.001$). Living within 3 kilometres from the TB treatment facility was significantly protective against MDR-TB ($P=0.002$). No statistically significant difference was observed in the means of transport used by cases and controls ($P= 0.353$) although case travelled a longer distance.

In bivariate analysis, the cases were five times more likely to be Non-Kenyan ($OR= 4.7$, 95% CI 2.1-10.6; $P= 0.0002$). with majority of cases being from the war torn Somalia.

While unemployment before illness was not associated with MDR-TB, there was a statistically significant threefold increase in unemployment among patients with MDR-TB disease after developing illness ($OR=3.31$, 95% CI= 1.67-6.65; $P<0.001$).

Cases were three times more likely to lack formal education ($OR= 3.0$, 95% CI=1.2-7.9; $P=0.030$). However, receiving messages concerning TB during treatment of the last episode of ordinary TB was protective ($OR=0.28$, 95% CI=0.16-0.50 $P=0.001$). This remained significant regardless of the level of formal education attained by the study participants (Table 4.2).

Table 4.2: Socio-demographic factors, for cases and controls, Kenya, 2009

Exposure variable	Cases		Controls		COR	95% CI *LL- *UL	P-Value
	No	(%)	No	(%)			
Mean distance (Km) travelled to hospital (SD)	10.0	(11.6)	5.5	(8.3)	–	–	< 0.001
Mean duration (minutes) of travel to hospital (SD)	42.0	(41.0)	25.0	(22.3)	–	–	0.001
Mean Rent in **Ksh (SD)	3944	(3050)	3080	(2628)	–	–	0.480
Mean No. of family members (SD)	3.4	(3.7)	3	(2.7)	–	–	0.104
Use of public transport (Yes)	29	(35.8%)	47	(29.0%)	1.36	0.77-2.41	0.353
Travelling < 3km to hospital (Yes)	29.0	(35.8%)	93.0	(57.4%)	0.41	0.23-0.72	0.002
Non Kenyan National *** (Yes)	19	(23.5%)	10	(6.2%)	4.66	2.05-10.58	< 0.001
Marital status (married)	37	(45.7%)	93	(57.4%)	0.62	0.36-1.07	0.111
Living with family (Yes)	53	(65.4%)	92	(74.7)	0.64	0.36-1.14	0.170
Religion (Christian)	50	(61.7%)	114	(70.4%)	0.68	0.39-1.19	0.226
Gender (Female)	31	(38.3%)	66	(35.8%)	0.90	0.52-1.56	0.820
Employment before illness (None)	24	(29.6%)	42	(25.9%)	1.20	0.67-2.18	0.650
Current employment (None)	65	(80.2%)	89	(54.9%)	3.31	1.67-6.65	0.001
Type of house lived in (Permanent)	43	(53.1%)	83	(51.9)	1.07	0.63-1.83	0.890
Homelessness (Yes)	10	(12.3%)	11	(6.8%)	1.9	0.78-4.76	0.220
Formal Education (None)	11	(13.6%)	8	(5.8%)	3.03	1.17-7.85	0.030
Received TB Messages (Yes)							
All participants (Yes)	43	(53.1)	130	(80.2)	0.28	0.16-0.50	< 0.001
Education ≤ Primary (Yes)	21	(46.7)	59	(72)	0.34	0.16-0.73	0.009
Education ≥ Secondary (Yes)	19	(52.8)	67	(83.8)	0.22	0.09-0.52	0.001

T test used for numerical variables and Yates corrected chi square test for categorical variables.

NB: *LL= Lower limit *UP = Upper limit **Ksh: Kenya shilling. *Non Kenyans were, 25 (86.2%) Somalis, 2(6.9%) Ugandans, 1(3.4%) Tanzanian, and 1(3.4 %) Ethiopian.**

4.3 Behavioural Factors Associated with MDR-TB

A lower proportion of cases (40.7%) compared to controls (49.7%) reported ever taking alcohol in life. However the difference was not statistically significant (P=0.260). Similarly there was no statistically significant difference in the history of ever smoking cigarettes although a larger proportion of cases reported smoking (P=0.688). An equal proportion of cases and control reported abuse of any substance (Table 4.3).

Table 4.3: Behavioural factors for cases and controls, Kenya 2009

Exposure variable	Cases		Controls		COR	95% CI		P-Value
	No	(%)	No	(%)		*LL-	*UL	
Alcohol intake (Yes)	33	(40.7)	76	(49.74)	0.71	0.42-1.24	0.26	
Ever smoked (Yes)	30	(37.5)	54	(33.3)	1.17	0.69-2.10	0.688	
Smoking in past 2 years (Yes)	15	(18.5)	37	(22.8)	0.77	0.39-1.50	0.542	
Living with smoker (Yes)	34	(42.0)	59	(36.4)	1.26	0.73-2.17	0.480	
Any Substance abuse (Yes)	17	(21.0)	34	(21.0)	1.00	0.52-1.93	1.000	
Marijuana and heroin abuse (Yes)	5	(6.2)	9	(5.6)	1.12	0.36-3.45	0.920	
Ever imprisoned (Yes)	25	(30.9)	44	27.2	1.20	0.66-2.15	0.880	
Family size less than 3 (Yes)	32	(56.1)	84	(67.2)	0.62	0.32-1.19	0.20	
Travel out of Kenya (Yes)	21	(27.3)	29	(20.8)	1.60	0.85-3.04	0.380	
Window in residential house (Yes)	11	(13.6)	11	(6.8)	2.16	0.89-5.2	0.133	
Practicing Cough hygiene (Yes)	45	(60.8)	89	(59.3)	1.03	0.58-1.83	0.961	

T test used for numerical variables and Yates corrected chi square test for categorical variables.

NB: *LL= Lower limit *UP = Upper limit

4.4 Clinical Information

The mean duration between diagnosis of tuberculosis and date of interview was longer for cases than controls 21.8 months (SD=13.2 months) and 5.6 months (SD=0.9 months) respectively. While the median was 18 months (IQR= 12.0-28.0 months) and 6 months (IQR= 5-6 months) respectively for cases and controls. Thirty seven (45.7%) of the cases and 25 (16.4%) of the controls had relapsing sputum smear positive TB (Figure4.3).

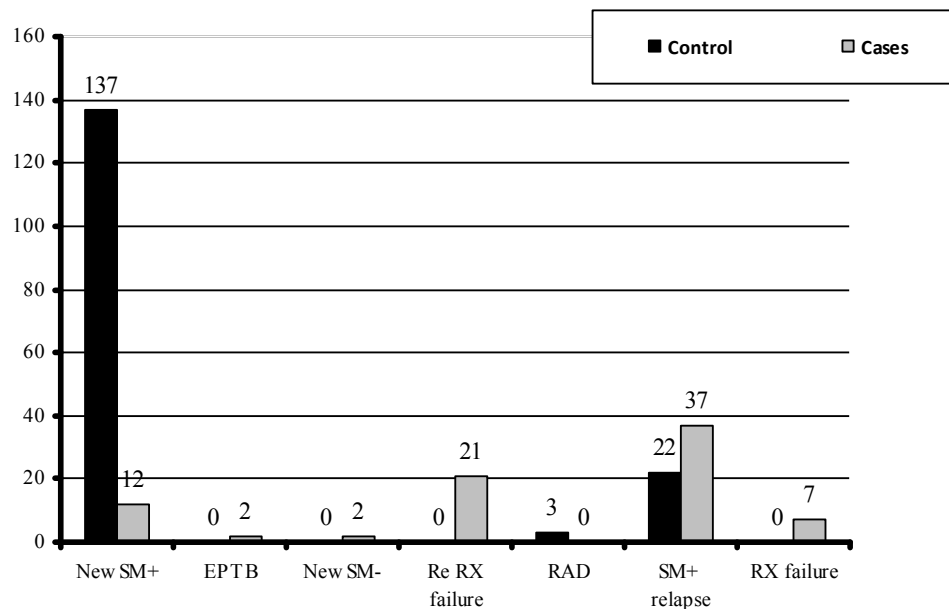


Figure 4.3: Distribution of cases and controls by clinical category Kenya, 2009.

Abbreviation

New SM+: New smear positive TB

EPTB: Extra-pulmonary TB)

New SM- : New smear negative TB)

Re RX failure: Retreatment Failure

RAD: Returnee after default)

SM+ relapses: Smear positive relapse

RX failure: Treatment Failure

Sputum smear results in the facility TB treatment register were reported in four categories depending on the bacillary load; scanty, 1 +, 2+ and 3+ (Appendix 3). Cases were four times more likely to have a bacillary load of 2+ or more (OR, 4.1, 95% CI= 1.9-9.8; P= 0.002). Conversely cases were less likely to have received directly observed therapy than controls (OR= 0.38, 95% CI= 0.21-0.66; P <0.001). Controls were more likely to be HIV sero-positive compared to cases (OR=0.42, 95% CI= 0.23-0.77; P=0.007) (Table 4.4).

Table 4.4: Clinical factors among MDR-TB cases and control Kenya, 2009

Exposure variable	Cases		Control		COR	95% CI		P-Value
	No	(%)	No.	(%)		*LL-	*UL	
Bacillary load ($\geq 2+$) (Yes)	38	(84.4)	76	(57.1)	4.07	1.70-9.80	0.002	
Recorded DOT (Yes)	52	(100)	157	(97.5)	–	–	0.646	
Self reported DOT (Yes)	37	(48.7)	116	(71.6)	0.38	0.21-0.66	<0.001	
Getting DOT once a week (Yes)	52	(64.2)	60	(37.0)	3.1	1.75-5.31	<0.001	
Skipped TB medicine >1week (Yes)	17	(23.0)	21	(13.8)	1.8	0.69-2.9	0.120	
HIV sero-status positive (Yes)	19	(23.5)	65	(42.2)	0.42	0.23-0,77	0.007	
On ART before TB diagnosis (Yes)	16	(88.9)	33	(55.9)	6.30	1.33-29.9	0.020	
Hospital admission (Yes)	16	(19.8)	21	(13.0)	1.65	0.81-3.37	0.230	
Known Diabetic (Yes)	2	(2.5)	3	(1.9)	1.3	0.22-8.19	0.873	
Chronic chest condition (Yes)	4	(5.0)	4	(2.5)	2.1	0.50-8.43	0.52	

T test used for numerical variables and Yates corrected chi square test / Fishers exact test for categorical variables.

NB: *LL= Lower limit *UP = Upper limit

Previous treatment for tuberculosis was strongly associated with MDR-TB (OR= 68.5, 95%CI=26.91-174.39; P <0.001) while cases were two and a half time more likely to lack a BCG scar (OR=2.5, 95% CI= 1.35-4.63; P=0.005). Having an unfavourable treatment outcome (treatment failure or default from treatment) during the previous TB treatment was associated with MDR-TB but did not achieve statistical significance (OR=3.7, 95% CI -1.01-14.03; P=0.07).

There was no statistically significant difference in reported history of contact with known TB or chronic cough among cases and controls OR=1.24 95% CI=0.71-2.15; P= 0.536). However six (7.4%) of the MDR-TB patients who had neither suffered from TB nor been exposed to drugs used in treatment of TB before diagnosis of MDR-TB reported contact with a household case of MDR-TB (Table 4.5). Their case reports are described below.

A thirty year old HIV positive man and a 10 year old HIV negative girl both BCG vaccinated lived in the same single room with a known MDR-TB case. The MDR-TB case-patient had been treated for TB over a period of two years and had defaulted twice. A HIV positive 23 year old non BCG vaccinated man shared a room with his MDR-TB infected wife that had been treated for sputum smear positive TB twice without bacteriological response. A 15 year-old HIV negative BCG vaccinated girl shared a room with her HIV positive auntie who later died during the first month of MDR-TB treatment. A 24 year old HIV negative non BCG vaccinated man of foreign origin living in a refugee camp also reported contact with MDR-TB. There was no statistically significant difference in clinical socio-demographic or behavioural

factors among the cases with known exposure to MDR-TB and those without exposure.

Table 4.5: Factors in the past medical history for cases and control, Kenya 2009

Exposure variable	Cases		Controls		COR	95% CI		P-Value
	No.	(%)	No	(%)		*LL-	*UL	
Treated Previously for TB (Yes)	75	(92.6)	25	(15.4)	68.5	26.91-174.39	<0.001	
Absence BCG Scar (Yes)	28	(34.6)	28	(17.4)	2.51	1.35-4.63	0.005	
Unfavourable outcome (Yes)	25	(37.3)	3	(13.7)	3.7	1.01-14.03	0.07	
Ever Skipped TB drugs (Yes)	21	(31.3)	8	(34.8)	0.9	0.31-2.33	0.960	
Skipped drugs > 1 week (Yes)	6	(30)	1	(20)	1.7	0.16-18.7	0.900	
DOT in previous treatment (Yes)	32	47.8	14	(60.9)	0.59	0.22-1.54	0.400	
Contact with Known TB (Yes)	31	(38.3)	54	(33.3)	1.24	0.71-2.15	0.536	
Contact with MDR-TB (Yes)	6	(7.4)	0	(0)	–	–	–	

T test used for numerical variables and Yates corrected chi square test for categorical variables.

NB: *LL= Lower limit *UP = Upper limit

4.5 Multivariate Analysis Results

A multivariate analysis was done by entering the variables that were found to be associated with MDR-TB at p-value ≤ 0.1 in the bivariate analysis into an unconditional logistic regression model. The variables included nationality distance from health facility to patients home, TB messages given, HIV status, Bacillary load, Previous treatment with anti-tuberculosis drugs, unfavourable outcome in previous TB treatment, having a DOT observer lack of formal education, and absence of a BCG scar. (Appendix 7)

From the model, four variables were independently associated with MDR-TB in the study. History of previous treatment with ant-TB drugs was the strongest factor associated with MDR-TB in Kenya. Receiving TB treatment under the DOT program, Being Non-Kenyan and a HIV positive status were protective against MDR-TB at 0.05 significance level alpha. (Table 4. 6)

Table 4.6: Final “Best Fit” model of unconditional logistic regression on factors associated with MDR-TB in Kenya 2009

Term	AOR	95% C.I.		Z-Statistic	P-Value
On DOT	0.2310	0.0923	0.5783	-3.1298	0.0017
Non Kenyan	5.5151	1.3944	21.8133	2.4338	0.0149
Positive HIV	0.3416	0.1333	0.8756	-2.2366	0.0253
Previous TB treatment	85.0237	29.7063	243.3497	8.2809	0.0001

Stepwise backward elimination logistic regression method was used.

CHAPTER FIVE

DISCUSSION

It has been estimated that one third of the world's population is infected with *M. tuberculosis*. Although anti TB drugs have been available for the past 50 years, tuberculosis remains a major cause of morbidity and mortality all over the world (Harries, 2004). Weak health systems, the HIV pandemic and emergence of *M. tuberculosis* strains that are resistant to conventional drugs used in TB treatment remain major threats to TB control (Thuridur, 2009). Poverty, poor living conditions and congestion are among factors contributing to the tuberculosis epidemic (Rieder, 1999). This study was conducted to determine factors associated with MDR-TB in Kenya to bridge the gaps in knowledge and help inform policy on development of interventions that are best suited to the country's needs.

5.1 Socio-demographic Factors Associated with MDR-TB

The distribution of MDR-TB cases across age groups was similar to that of controls. It is also similar to that in the national TB program data for smear positive TB patients (DLTLD, 2007) suggesting similarities in the epidemiology of MDR and ordinary TB. However, a significantly large proportion of cases was unemployed after developing disease ($P < 0.001$) compared to the period before illness when there was no difference in unemployment ($P = 0.650$). Loss of employment among cases may result in poorer living conditions that favour transmission of MDR-TB.

Living in close proximity to a health facility has been associated with greater access to care and adherence to treatment. In this study, living within three kilometres of the

TB treatment health facility was protective against MDR-TB although it did not achieve statistical significance in multivariate analysis. Living in close proximity to the chest clinic was associated with improved compliance to anti-tuberculosis drugs in a study in Nigeria (Erhabor *et al.*, 2000) while in another study in rural South Africa living far from the hospital was associated with increased risk of death (Barker *et al.*, 2002). Patients who live near the hospital are less likely to fail to collect their drugs or totally abandon their treatment.

Several studies have shown an increased risk of MDR-TB among foreign born people and refugees (Lambregts-van *et al.*, 1998; Casal *et al.*, 2005; Faustini *et al.*, 2006; Oeltmann *et al.*, 2008). In this study, being Non-Kenyan was a risk factor and an independent predictor of MDR-TB. This could be attributed to the fact that a large proportion of Non-Kenyans (86.2%) in the study were from the war torn Somalia, where there is high prevalence of TB, low case detection and, poor access to treatment. (Dahle *et al.*, 2003; Mauch *et al.*, 2010). TB treatment completion and cure rates are also low in Somalia. Similarly a large proportion of MDR-TB cases in Norway were immigrants from Somalia (Dahle *et al.*, 2003). This relationship could also explain the observed weak association between religion and MDR-TB given that majority of the Somali population are of non Christian religion.

Receiving formal education has been associated with good health seeking behaviour. In this study having any level of formal education was associated with a decreased risk of MDR-TB in the bivariate analysis. Similarly receiving targeted TB messages was significantly associated with a reduced risk of MDR-TB in the bivariate analysis. However in the multivariate analysis these were not statistically significant. The

purpose of patient education is to increase compliance and adherence to TB treatment in order to prevent emergence of drug resistance. In a randomized clinical trial health education was found to improve adherence to anti-TB drugs (Morisky *et al.*, 1998). Similar findings were described in a systematic review of randomized controlled trials of strategies to promote adherence to tuberculosis treatment (Volmink & Garner, 1997). The protective effect of receiving TB messages in the bivariate analysis was stronger among those who had secondary and tertiary education compared to those with no formal education and primary education suggesting a difference in the level of understanding. Targeting this population of patients with relevant messages may have some impact in reducing the risk of MDR-TB (Carolyn *et al.*, 2009).

5.2 Behavioural Factors Associated with MDR-TB in Kenya

In this study, most of the behavioural factors including, imprisonment, alcohol intake, family size, drug abuse, smoking, travel outside of Kenya, living conditions, were not statistically significant.

5.3 Clinical Factors Associated with MDR-TB in Kenya

The strongest risk factor for MDR-TB in this study was a history of previous treatment for tuberculosis ($P < 0.001$). This finding is consistent with a case control study in New York (Clark *et al.*, 2005) a population based survey in 11 countries (Espinal *et al.*, 2001) and a retrospective study in South Africa (Anastasiadis *et al.*, 1997). Exposure to first line anti TB tuberculosis drugs is thought to select strains that are resistant to some drugs during treatment (Mitchison, 1998). This allows

multiplication and increase in number of drug resistant mutants to a level where they become the dominant strains as the drug susceptible strains get killed off in what is called “rise and fall phenomenon”. Proliferation of resistant strains during treatment may lead to treatment failure (a patient having culture or smear positive sputum at or after 5 months of treatment) or relapse after completion of treatment. Patients who fail or relapse after the initial treatment with the recommended WHO first line drugs have a 10–15 fold increase in risk of having MDR-TB (Hayward *et al.*, 1996). Of patients who had had prior treatment with first line drugs, 25 (37.3%) of the cases had what was termed as an unfavourable treatment outcome during the first treatment. Unfavourable treatment outcomes included treatment failure and default from treatment. In this study having an unfavourable outcome during the first TB treatment was not an independent predictor of MDR-TB. This contrary to a study conducted in Samara Oblast, Russia where this factor was significantly associated with MDR-TB (Balabanova *et al.*, 2006).

Coming in contact with a known case of tuberculosis was significantly associated with MDR-TB in a Case-control study in four countries in Europe (Casal *et al.*, 2005). In the current study, there was no statistically significant difference in the proportion of cases and controls that came in contact with known TB or chronic cough ($P=0.536$). It has been suggested that MDR-TB is less easily transmissible than drug susceptible TB (Attamna *et al.*, 2009). Loss of virulence has been reported in organisms that become resistant to antimicrobials. This adaptation allows the host to survive long enough for to facilitate multiplication and spread of the drug resistant strains. In nationwide cohort study in Israel, there was no generation of secondary

cases of MDR-TB (Attamna *et al.*, 2009), similarly in another study in San Francisco USA, it was demonstrated that drug resistant strains including MDR-TB were less likely to result in secondary cases. Despite the findings of low risk of transmission of MDR-TB in the stated studies, it is worth noting that, loss of virulence does not appear to occur in Beijing strains of *M. tuberculosis*. These strains exhibit increased virulence, drug resistance and risk of transmission (Ordway *et al.*, 1995; Drobniewski *et al* 2005; Abebe and Bjune, 2006). In applying these findings to Kenya it should be considered that the stated studies were conducted in settings that are significantly different from the local setting with regard to social, economic, behavioural factors as well as the prevalence of HIV and AIDS. In other studies, high rates of MDR-TB transmission were reported in South Africa (Wells *et al.*, 2007) and in the USA among HIV patients in health care settings (Victor *et al.*, 1993). It is worth noting that all the six MDR-TB cases (4 HIV negative) in this study that did not have prior history of treatment with first line anti TB drugs had a history of contact with a household case of MDR-TB. This suggests that MDR-TB can be acquired as a primary infection by an otherwise healthy individual and that MDR-TB transmission is ongoing at household level. Enhancing MDR-TB contact tracing and further studies to determine the magnitude of MDR-TB transmission among close contacts of MDR-TB cases at community level in Kenya are recommended.

Congregation and congestion in health care setting has been documented as a risk factor for tuberculosis (Harries, 2004). In this study hospital admission was not associated with MDR-TB (P= 0.230). This is contrary to several studies that reported nosocomial transmission of MDR-TB (Victor *et al.*, 1993; Fierer., 2006). The

findings are also contrary to a population-based study in Brazil, where a history of hospital admission within two years preceding before MDR-TB diagnosis was significantly associated with an increased risk of MDR-TB (Telles *et al.*, 2005). In this study, although there was an insignificant association between hospital admission and MDR-TB, the number of MDR-TB cases that were reported among the household contacts seems to suggest active transmission. It is therefore recommended that another study designed to specifically address nosocomial MDR-TB transmission be conducted.

Having a DOT observer during TB treatment was protective against MDR-TB. Directly observed treatment for tuberculosis has been shown to have higher compliance and cure rate compared to self-administered therapy (Erhabor *et al.*, 2000; Robert *et al.*, 2004) while poor compliance has been implicated in the development of MDR-TB (Chakaya *et al.*, 2008; Erhabor *et al.*, 2000; Ormerod, 2005). Although the proportion of those on DOT as recorded in the treatment register was higher than what was found during patient interviews, DOT was protective and an independent predictor of MDR-TB. (OR= 0.28; P<0001). These findings are similar to those in a case control study in Hong Kong (Law *et al.*, 2008). DOT prevents development of drug resistance since the treatment supervisor ensures that patient actually swallows the right dose of TB medicines, regularly and for the prescribed period.

The current TB pandemic is driven by the HIV/AIDS pandemic. A HIV positive sero-status has also been found to be significantly associated with MDR-TB in several studies such as a case control study Peru (Campos *et al.*, 2003) and that in

four countries in Europe (Casal *et al.*, 2005), retrospective studies in South Africa, and in the USA (Neville *et al.*, 1994). In all these studies a HIV positive status was consistently associated with an increased risk of MDR-TB. However a descriptive study in India reported a lower association of with HIV (Deivanayagam *et al.*, 2002). In studies conducted in Africa including that in KwaZulu Natal, South African (Gandhi *et al.*, 2006) and the Mozambique study (Nunes *et al.*, 2005) HIV was a major risk factor for MDR-TB. These findings are in tandem with the global MDR-TB surveillance data that suggests convergence of MDR-TB and HIV /AIDS. Some studies indicate that development of rifampicin resistance may be spurred by HIV infection (Burman *et al.*, 2006; Li *et al.*, 2005; LoBue *et al.*, 2005) while in other studies no association was demonstrated between HIV infection and MDR-TB (Espinal *et al.*, 2001).

Multivariate analysis of the current study indicate that a significantly larger proportion of controls were HIV sero-positive compared to cases ($P=0.0253$). In interpreting these results, it is important to consider factors surrounding MDR-TB surveillance, epidemiology and control in Kenya that may considerably differ from the other countries. These factors may favour accelerated MDR-TB disease progression among the HIV infected patients, selective survival of, and transmission of MDR-TB among the HIV sero-negative individuals.

Many African countries including Kenya are said to be underreporting MDR-TB (Amor *et al.*, 2008). Secondly, Kenya has a high prevalence of HIV related TB which is difficult to diagnose due to atypical presentation and lack of appropriate tests to detect smear negative TB that is common among PLHIVs (Mendelson,

2007). MDR-TB diagnosis poses even a greater challenge because it requires specialized laboratories to isolate *M. tuberculosis*. These facilities are not widely available in most resource poor countries (Ormerod, 2005) and therefore not available for routine screening of HIV infected patients for MDR-TB. This leaves a large proportion of patients with HIV who are in fact an MDR-TB high risk group unscreened. Thirdly treatment of smear positive TB cases is usually allowed to proceed and only in the second month when there is evidence of delay of sputum conversion is MDR-TB suspected. This may not be the case in HIV positive TB patients who may present with sputum smear negative pulmonary tuberculosis. HIV infected MDR-TB patients tend to have extremely high mortality (Ormerod, 2005). Infection in these patients' progresses rapidly (within three months) to disease and death may occur within 5 weeks of diagnosis (Drobniewski *et al.*, 2002; Wells *et al.*, 2007; Gandhi *et al.*, 2006) which is a shorter period than it takes to diagnose MDR-TB using traditional culture methods. Therefore many MDR-TB patients may die before they are suspected or diagnosed. In Peru, one half of MDR-TB patients who were HIV positive died in less time than it usually takes to complete susceptibility studies (Fierer, 2006). Findings from the current study also suggest MDR-TB transmission among non-HIV infected individuals, which could partially account for the larger proportion of the HIV sero-negative individuals among the cases.

A combination of these factors may have resulted in selective diagnosis of MDR-TB in HIV negative patients and selective survival of HIV negative MDR-TB patients hence the observed association. Further studies to establish the association between HIV and MDR-TB in Kenya should be conducted.

Bacillary load on sputum smear microscopy tends to correlate well with severity of disease. Patients with cavitory pulmonary tuberculosis and lung damage tend to have a high bacillary load (Rieder, 1999) which is associated with an increased risk of random mutations that result in drug resistance (Rieder, 2002). In this study bacillary load of 2+ and more at diagnosis of the last episode of tuberculosis was associated with MDR-TB ($P=0.002$). However this was not an independent predictor of MDR-TB. These findings are comparable to those in a prospective study in India (Pande *et al.*, 2005). High bacillary load has been shown to correlate with the degree of infectiousness (Rieder, 1999) as well as the risk of developing drug resistance.

In Eastern Africa, BCG vaccine is given as part of the routine immunization. The presence of a BCG scar was taken as evidence of BCG vaccination. BCG has been shown to confer varying protective effect ranging from no protection to 80% protection among children (Andersen and Doherty, 2005) and a consistent waning of the protective effect with increase in age to no protection in adults. In Kenya, a case-control study of risk factors for tuberculosis among prisoners, found a protective association between the presence of BCG scar and active tuberculosis (Amwayi *et al.*, 2010). In the current study, the presence of a BCG scar was protective against MDR-TB in the bivariate analysis ($P=0.05$) although it was not an independent predictor of MDR-TB. This seems to contradict the hypothesis that, the use of BCG vaccine in a population over a prolonged period of time selects Beijing genotypes of *M. tuberculosis* that are associated with TB outbreaks and increased risk of MDR-TB (Abebe & Bjune, 2006). The Beijing family genotypes exhibit important pathogenic features such high virulence, multi-drug resistance and exogenous re-infection.

Although few studies have been conducted to determine the effect of BCG vaccination on chronic excretion of *M. tuberculosis*, and MDR-TB, a study to determine transmission of tuberculosis among close contacts of MDR-TB cases, found a protective association that was significant even when age, sex, race, purified protein derivative (PPD) status, and isoniazid prophylaxis were controlled for (Kritski *et al.*, 1996). In a different study BCG was shown to have protection for 50-60 years (Aronson *et al.*, 2004). With the emergence of MDR-TB in many parts of the world, the use of BCG vaccine among adults with prolonged exposure to MDR-TB is recommended in settings where there is high TB prevalence and where BCG has been demonstrated to offer protection in adults (Kritski, 1996; Rieder, 2002).

5.4 Limitations of the Study

Misclassification

Confirmation of diagnosis among the controls was based on sputum smear microscopy and not culture and DST to rule out MDR-TB. This could result in misclassification of control who may have had MDR-TB. However the proportion of MDR-TB among all TB patients in Kenya is small (less than 1%).

The clinical and bacteriological response observed among the controls is assumed to be due to response to treatment with first-line anti-tuberculosis drugs and not the natural healing process of tuberculosis which could in 50% of patients if left untreated over a period of two years. This is unlikely to be the case since all selected controls had sputum conversion at least by the fifth month.

Furthermore this type of misclassification would bias the result towards null findings and therefore not explain any significant difference observed in the study.

Cause effect relationship

In this study it is not possible to establish the cause effect relationship since both exposure and outcome had occurred at the time the study was conducted.

Recall bias

Cases had longer mean duration of illness than the controls. They had also been treated for TB several times without improvement. Therefore they may have been more likely to remember events surrounding their illness than controls. Attempts were made to minimize this problem by ensuring that exposures evaluated were those that do not change rapidly over time. It was assumed that cases were not likely to recall the exposures than controls, thus if recall problems existed then it would affect both groups equally and therefore under estimate the strength of association.

Sampling and representativeness

Being a case control study, selection of the study sites was based on where cases (MDR-TB patients) existed and not a random selection of facilities offering TB care. Hence more facilities were selected in some regions than others. This may limit the representativeness of the findings. However efforts were applied to reach all cases while controls were randomly selected within the selected facilities.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Clinical factors abstracted from the treatment records and patient interviews including previous treatment, DOT and HIV positive serostatus and reported nationality were independent predictors of MDR-TB.
2. Receiving TB treatment under the DOT program was protective against MDR-TB.
3. Previous treatment for tuberculosis was the strongest factor associated with MDR-TB in Kenya.
4. Being of Non Kenyan origin in was a risk factor for MDR-TB.
5. There appears to be a protective association between MDR-TB and the recorded HIV positive sero-status.
6. MDR-TB can be acquired as a primary infection by otherwise healthy individuals.

Based on these findings, both clinical and social factors are associated with having MDR-TB in Kenya. Some of these are can be modified and therefore amenable to public health interventions. Therefore the null hypothesis is rejected.

6.2 Recommendations

This case-control study is the first one to be done on a nationwide scale in Kenya. It provides information that will inform further studies in Kenya. There is need to review the MDR-TB control approaches with the following recommendations.

1. There is need to strengthen the access to management of ordinary TB cases through strengthening of DOT and improving recording to ensure that patients with drug susceptible TB do not develop MDR-TB. These should be implemented across all population groups
2. Expansion of MDR-TB surveillance to cover all patients with history of previous treatment for tuberculosis, all patients with infectious tuberculosis (Sputum smear positive TB patients), contacts of known MDR-TB cases and all HIV positive TB patients. HIV positive TB patients should be screened at diagnosis of tuberculosis.
3. Cross-border MDR-TB surveillance should be initiated and routine MDR-TB surveillance strengthened among Non Kenyan especially of Somalia origin.
4. The use of faster MDR-TB diagnostic methods such as Line Probe Assay (LPA) to detect drug resistance within 24 hours should be considered for screening among HIV positive TB patients as they quickly succumb to MDR-TB and among sputum smear positive TB cases that are likely to transmit MDR-TB.
5. Strengthen MDR-TB conduct tracing, screening and documentation.
6. Further studies to investigate the MDR-TB community and nosocomial transmission and to establish the association of HIV and MDR-TB.

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APPENDICES

Appendix 1: Consent Form

Title of Study: Factors associated with Multidrug Resistant Tuberculosis in Kenya

Investigator: Dr. Herman Weyenga

Institutions: Jomo Kenyatta University of Agriculture and Technology and the Ministry of public health and sanitation Division of Leprosy Tuberculosis and Lung Disease.

Sponsor: Ministry of public health and sanitation FELTP

Request: I request you to take part in a research study. The research study aims to determine factors associated with Multidrug resistant Tuberculosis in Kenya. Multidrug resistant tuberculosis is becoming a common problem in Kenya. It is expensive to treat and take long to complete treatment. An understanding of factors associated with the disease may enable us to prevent it, control its spread and prevent development of a more serious form of disease (Extensively drug resistant TB). The study session is expected to last about 30 minutes. During this time, you will be asked some questions about the current and past illness and other practices and experiences your left arm will also be examined for BCG/ TB vaccination scar. The study will not interfere with your current treatment.

Risks and benefits: The study will not pose any risks to you. This study may help to improve our understanding, prevention and treatment of Multidrug resistant TB in future. There will be no costs to you for taking part in this study.

Appendix 2: Questionnaire

Questionnaire

To be administered to patient in patients own, language. Answer as many questions as possible

1. General Information

Questionnaire number	<input type="text"/>
1. Status of patient	
<input type="checkbox"/> Case	<input type="checkbox"/> Control
2. Date of interview dd / mm / yyyy	____ / ____ / _____
3. Interviewer Name	_____
4. Health Facility details	
a) Name of Health Facility _____	Level 2, 3, 4, 5, 6
<input type="checkbox"/> Private	<input type="checkbox"/> Public <input type="checkbox"/> Mission
b) District	_____
c) Province	_____

2. Personal details

1. Patient unique identifier	<input type="text"/>
2. Sex	<input type="checkbox"/> Male <input type="checkbox"/> Female
3. Nationality	_____
5. Where have you been living in the past 1 year?	Country _____ Town or district _____
6. Date of birth dd / mm / yyyy	____ / ____ / _____ Age in years _____ (fill both & check for consistency)
7. Religion	
<input type="checkbox"/> Catholic	<input type="checkbox"/> Protestant <input type="checkbox"/> Muslim <input type="checkbox"/> None
Others specify	_____
8. Marital status	
<input type="checkbox"/> Single	<input type="checkbox"/> Married <input type="checkbox"/> Cohabiting
<input type="checkbox"/> Separated	<input type="checkbox"/> Divorced <input type="checkbox"/> Widowed others specify _____
9. Level of education (highest level of formal education completed)	
<input type="checkbox"/> None	<input type="checkbox"/> Primary <input type="checkbox"/> Secondary <input type="checkbox"/> Tertiary
Others specify	_____

10. Current Employment

Salaried employment Self employed Not employed

Casual employment Young in school Specify _____

11. Have you changed your employment since illness?

Yes *if no go to question 13*

12. if yes what was your employment prior to current illness

Salaried employment Self employed Not employed

Casual employment Specify _____

13. What means of transport were you using to travel to the hospital where you were treated for the last TB episode *(Report what the patient is currently using if he/she has never been treated for TB)*

On foot Motorcycle Personal car

Bicycle Matatu

How long does it take to get to hospital _____(Minutes)

Estimated distance _____Kms

4. Clinical information

History of current TB episode

1. When were you diagnosed to have the current episode of TB? dd/mm/yyyy ____/____/____ Duration of illness to the present date _____ month

2. How was the TB diagnosed? (check the TB register)

<input type="checkbox"/> Clinical examination	<input type="checkbox"/> Sputum microscopic examination
<input type="checkbox"/> X ray	<input type="checkbox"/> Don't Know

3. What type of TB did you have? (confirm from the TB facility register)

<input type="checkbox"/> New Sputum smears positive PTB	<input type="checkbox"/> Year negative relapse
<input type="checkbox"/> New Sputum smear negative PTB	<input type="checkbox"/> Year positive relapse
<input type="checkbox"/> Extra-pulmonary TB	<input type="checkbox"/> Treatment Failure Others Specify _____

4. For those with sputum smear positive TB check the register for bacillary load on AFB microscopy at month 0?

<input type="checkbox"/> +	<input type="checkbox"/> ++	<input type="checkbox"/> +++	<input type="checkbox"/> Not available
----------------------------	-----------------------------	------------------------------	--

5. For how long were you supposed to take TB drugs during the last episode of ordinary TB?

<input type="checkbox"/> Six months	<input type="checkbox"/> Eight months
-------------------------------------	---------------------------------------

Other specify _____

6. Was your TB treatment period changed from what you had been informed at the beginning of treatment

<input type="checkbox"/> Yes	<input type="checkbox"/> No
------------------------------	-----------------------------

What were the reasons for the change _____

7. Were you injected as part of TB treatment

<input type="checkbox"/> Yes	No <input type="checkbox"/>
------------------------------	-----------------------------

8. If yes for how long? _____ months

9. What messages concerning TB were you given before or during treatment for the current episode of TB?

10. Did you have someone to observe you while taking your TB medicine

<input type="checkbox"/> Yes	<input type="checkbox"/> No
------------------------------	-----------------------------

Confirm DOTS status in treatment register after interviewing the patient

<input type="checkbox"/> DOTS done	<input type="checkbox"/> DOTS not done
------------------------------------	--

11. If yes in 10 above who was it?

Health care worker Family member

Community member Workmate Others specify _____

Other specify _____

12. How available was the person to observe you while taking treatment

Daily Once in 2 days Once in 3 days Once in 4 days

Once a week Once in 2 weeks once a month Other specify _____

13. Were you for any reason during treatment for the current TB episode unable to take your TB medicine or go for injections

Yes No Cannot remember *if no go to question 16*

If yes, What were the reasons _____

14. If yes in (13) above, for how long were you unable to take your medicine

dates from dd/mm/yyyy ____/____/____ to ____/____/____ estimate the period

less than 1 week 1 Week 2 weeks 3 weeks

4Weeks 2 months 3 months others specify _____

15. Did you continue taking the same medicines you had been taking when you resumed treatment?

Yes No

16. Was sputum sent to Nairobi or KEMRI for drug resistance testing?

Yes No *If No go to question 17*

For those tested, what is drug resistance pattern? (Check the District MDRTB register/ lab request forms)

Drug	Rifampicin	Isoniazid	Ethambutol	Streptomycin	Not Available
Sensitive					
Resistant					

Indicate if MDR-TB Non MDR-TB XDR-TB

For M/X DRTB are you on treatment? Yes No

17. Were you admitted in hospital at any time within two years prior to the onset of the current illness?

Yes No

18. Did you come in contact with someone with TB or a chronic cough prior to the onset of the current illness?

Yes No Don't Know

If yes was it MDR-TB? Yes No Don't know

19. Have you ever received treatment (Isoniazid Preventive Therapy) to protect you from developing TB Disease

Yes No Don't Know

If yes for what reasons and how long? Reason _____ duration _____ months

20. Do you have diabetes

Yes No

21. Did you have any chronic lung condition prior to diagnosis of the TB?

Yes No Don't Know

If yes, what was the condition

Asthma Other chronic lung condition

22. Were you tested for HIV during TB treatment?

Yes No

23. If yes in 22 above, what were the results

HIV+ HIV-

24. Confirm HIV testing from TB register

HIV+ HIV- Not Tested *If HIV negative or not tested go to page 6*

25. If HIV positive are you on antiretroviral drugs

Yes No

If on ART, when was it started relative to TB diagnosis? Before After

Indicate the difference in period of time _____

26. Was CD4 count done?

Yes No If yes, what was the number when last done? _____

Previous Medical history

1. Have you ever been treated with anti TB drugs before the current treatment

Yes No

If No, in 1 above go to question 8

2. If yes, in 1 above how many separate episodes of TB did you have _____

What were the dates, length of treatment and treatment outcomes of each of the episodes?

(Ask the patient what he/she was told the treatment outcome was for each of the treatments)

a) Date _____ length treatment _____ outcome _____

b) Date _____ length treatment _____ outcome _____

c) Date _____ length treatment _____ outcome _____

d) Date _____ length treatment _____ outcome _____

e) Date _____ length treatment _____ outcome _____

f) Date _____ length treatment _____ outcome _____

g) Date _____ length treatment _____ outcome _____

h) Date _____ length treatment _____ outcome _____

3. Were you explained to on how to take the TB medicine during each of the TB treatment?

Yes No

4. Were you for any reason unable to take TB medicine while undergoing any of the previous TB treatment?

Yes No

5. If yes for how long were you unable to take the medicine?

dates from dd/mm/yyyy _____/_____/_____ to _____/_____/_____

Less than 1 Week 1 Week 2 weeks 3 weeks

4 Weeks 2 months 3 months Other specify _____

6. Did you have someone to observe you when taking your medicine during the previous episode of ordinary TB?

Yes No

7. If yes in 6 above how available was the person to observe you while taking treatment

Daily Once in 2 days Once in 3 days Once in 4 Days

Once a week Once in 2 weeks Once a month Others specify _____

8. Did you receive the BCG (TB) vaccine during your childhood or at any other time?

Yes No Don't know

I would like to examine your left forearm. *Examination for BCG scar*

Present Absent

Behavioral and Social Factors

1. Have you ever smoked cigarettes? Yes No *If no go to question 6*

2. If yes when did you start (year) _____

3. Are you currently smoking? Yes No

If no when did you stop? Date dd/mm/yyyy ____/____/____

4. Duration of smoking _____

5. How many sticks do you or were you smoking per day _____

6. Have ever lived with someone who smokes Yes No
If yes for how long did you live with the person during his/ her active smoking _____

7. Any history of alcohol intake?
 Yes No If yes when did you start (Year/ month) _____

Are you currently taking alcohol? Yes No
If no when did you stop (Year/month) _____
Duration of alcohol intake _____

How frequent is or was the alcohol intake?
 Daily, Weekly Monthly, Others specify _____

What type of alcohol were you or do you take? _____

How much were you/do you take per day _____ glasses

8. Have you ever used any substance for recreation (e.g. chewable tobacco, Miraa / Khat, injectable drugs etc)
 Yes No

If yes, which one (specify) _____ Is it current? Yes No

9. Do you live with your family?
 Yes No

10. How many people do you live with _____

11. What type of house do you live in?
 Temporary Semi permanent house Permanent house

12. Who owns the house
 Rental Personal Accommodated

If rental, how much rent do you pay per month ? _____ shillings

13. How many rooms are there in the house? _____

14. How many windows are there in the room you use _____

15. Do you open it/ them. Yes No If yes how many hours per day is window open
 Less than an hour 1 hour 2hours 6 hours 8 hours
 10 hours 12 hours 24 hours others specify _____

16. Do you share the room with anyone else?

Yes

No

(If no go to question 18)

17. If yes in 15 above, what is the age of those you share the house with? Indicate their Age breakdown

1st ___ 2nd ___ 3rd ___ 4th ___ 5th ___ 6th ___ 7th ___ 8th ___ 9th ___ 10th ___

18. Have you ever been imprisoned or detained in police custody

Yes

No

If yes which one and for how long? Prison/ custody _____ Duration _____ months

19. Have you ever been in a situation where you did not have a house to live in?

Yes

No

20. Have you ever stayed in a refugee camp or internally displaced persons camp?

Yes

No

If yes which one and how for how long? Camp _____ Duration _____ Months

21. Have you ever travelled outside of the country?

Yes

If yes which one and _____ for how long _____ months

22. How many meals were you typically having per day prior to developing current episode of TB

1

2

3

4

5

Others specify _____

23. How Did this number of meals influence how you swallowed your TB drugs

24. Could you describe what you do while coughing? _____

Decide if patient practices cough hygiene

Yes

No

25. Have you disclosed the form of TB you have to any one?

Yes

No

If yes to whom have you disclosed? _____

If no what are the reasons for non disclosure? _____

26. What do you recommend to the TB control program regarding TB care?

Appendix 3: Grading of Sputum Smear for AFB

International Union against Tuberculosis and Lung Disease recommended grading of sputum smear microscopy results

Number of Acid Fast Bacilli counted	Recording and Reporting
No AFB in at least 100 fields 0/negative	Negative
1 to 9 AFB in 100 fields* Actual AFB counts	Scanty
10 to 99 AFB in 100 fields	+
1 to 10 AFB per fields in at least 50 fields	++
> 10 AFB per field in at least 20 fields	+++

Appendix 4: Ministry of Science and Technology Study Authorization

REPUBLIC OF KENYA



NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Telegrams: "SCIENCETECH", Nairobi
Telephone: 254-020-241349, 2213102
254-020-310571, 2213123
Fax: 254-020-2213215, 318245, 318249
When replying please quote

P. O. Box 30623-00100
NAIROBI-KENYA
Website: www.ncst.go.ke

Our Ref:

NCST/5/002/R/762/6

Date:

9th November, 2009

Herman Owuor Weyenga
Jomo Kenyatta University
of Agriculture and Technology
P. O. Box 6200-00200
NAIROBI

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on "*A case study on factors associated with multidrug resistant tuberculosis Kenya*" I am pleased to inform you that you have been authorized to undertake your research in *all Districts in all Provinces in Kenya* for a period ending *31st July 2012*.

You are advised to report to *The District Commissioners and The District Education Officers in all the Districts that you will visit* before embarking on your research project.

Upon completion of your research project, you are expected to submit two copies of your research report/thesis to our office.

A handwritten signature in black ink, appearing to read 'S. A. Abdulrazak'.

PROF. S. A. ABDULRAZAK Ph.D, MBS
SECRETARY

Appendix 5: Study Authorization by DLTLD



The Republic of Kenya

Ministry of Public Health & Sanitation

Division of Leprosy, Tuberculosis and Lung Disease (DLTLD)

New NASCOP Building – Kenyatta National Hospital
P. O. BOX 20781 NAIROBI

Tel (254) 2-713198/721890 Fax (254) 2-713198 Email – info@nltp.co.ke

Ref No: NLTP/ ADM/1/10 (17)

21st July, 2009

TO
DR HERMAN O WEYENGA
P.O 14003 -0100
NAIROBI

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on, "*Factors Associated With Multidrug Resistant Tuberculosis in Kenya*"

I am pleased to inform you that you have been authorized to carry out research at TB diagnostic and treatment sites in the country for a period ending 30th July 2010.

You are advised to provide a copy of this letter to PTLCs, DTLCs and officers in charge of Health facility to facilitate access to patient and their records.

On completion of the research, you are expected to present a copy of the research report to this office.

Yours Faithfully

A handwritten signature in black ink, appearing to be 'JS'.

Dr. Joseph Sitienei
HEAD DLTLD

DIVISION OF LEPROSY,
TUBERCULOSIS & LUNG DISEASE

Appendix 6: Kenyatta National Hospitals ERC Study Approval



KENYATTA NATIONAL HOSPITAL

Hospital Rd. along, Ngong Rd.

P.O. Box 20723, Nairobi.

Tel: 726300-9

Fax: 725272

Telegrams: MEDSUP, Nairobi.

Email: KNHplan@Ken.Healthnet.org

29th September 2009

Ref: KNH/UON-ERC/ A/314

Herman O. Weyenga
P O BOX 14003-0100
NAIROBI

Dear Herman

RESEARCH PROPOSAL: "A CASE CONTROL STUDY ON FACTORS ASSOCIATED WITH MDRTB IN KENYA"
(P245/8/2009)

This is to inform you that the Kenyatta National Hospital/UON Ethics and Research Committee has reviewed and **approved** your above revised research proposal for the period 29th September 2009 - 28th September, 2010.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimen must also be obtained from KNH-ERC for each batch.

On behalf of the Committee, I wish you fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of database that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

DR. L. MUCHIRI
AG. SECRETARY, KNH/UON-ERC

c.c. The Chairperson, KNH/UON-ERC
The Deputy Director CS, KNH

Supervisors: Dr. Juliette Ongus, Dept. of Medical Lab. Sciences, JKUAT
Dr. Joseph Oundo, Centers for Disease Control, Kenya
Dr. Jared Omollo, Ministry of Public Health & Sanitation
Dr. Joseph Sitienei, Ministry of Public Health & Sanitation

Appendix 7: Unconditional Logistic Regression model

Step 1

All variables with $P \leq 0.1$ were entered into the model with the tabulated results.

Term	AOR	95% C.I.		Coefficient	S. E.	Z-statistic	P-Value
1. BCG scar Absent (Yes/No)	2.1427	0.6844	6.7088	0.7621	0.5823	1.3087	0.1906
2. Bacillary load ≥ 2 + (Yes/No)	0.9186	0.3665	2.3025	-0.0849	0.4688	-0.1810	0.8563
3. DOT (Yes/No)	<u>0.2228</u>	<u>0.0853</u>	<u>0.5821</u>	-1.5016	0.4901	-3.0639	<u>0.0022</u>
4. Given TB Message (Yes/No)	0.6429	0.2389	1.7299	-0.4417	0.5050	-0.8747	0.3818
5. No education (Yes/No)	0.7584	0.0697	8.2469	-0.2766	1.2176	-0.2272	0.8203
6. Non- Kenyan (Yes/No)	<u>5.0217</u>	<u>1.0251</u>	<u>24.5991</u>	1.6138	0.8107	1.9906	<u>0.0465</u>
7. Positive HIV status (Yes/No)	0.4207	0.1588	1.1147	-0.8657	0.4971	-1.7415	0.0816
8. Previous TB treatment (Yes/No)	<u>60.4082</u>	<u>19.7771</u>	<u>184.5140</u>	4.1011	0.5697	7.1987	<u>0.0000</u>
9. Travelling <3 KM to hospital (Yes/No)	0.4830	0.1872	1.2460	-0.7277	0.4835	-1.5050	0.1323
10. Unfavourable outcome (Yes/No)	3.6189	0.8448	15.5027	1.2862	0.7423	1.7328	0.0831

Appendix 7: Unconditional Logistic Regression model building process (continued)

Step 2

“Bacillary load $\geq 2+$ ” was removed from the model since it has the highest P- value.

The remaining variables were re-entered into the model with the results tabulated below.

Term	AOR	95% C.I.	Coefficient	S. E.	Z-Statistic	P-Value	
1. BCG scar Absent (Yes/No)	2.1082	0.6816	6.5208	0.7459	0.5761	1.2947	0.1954
2. DOT (Yes/No)	<u>0.2234</u>	<u>0.0855</u>	<u>0.5836</u>	-1.4988	0.4900	-3.0589	<u>0.0022</u>
3. Given TB Message (Yes/No)	0.6411	0.2386	1.7222	-0.4446	0.5042	-0.8818	0.3779
4. No education (Yes/No)	0.7769	0.0739	8.1647	-0.2525	1.2002	-0.2104	0.8334
5. Non Kenyan (Yes/No)	<u>5.0390</u>	<u>1.0305</u>	<u>24.6411</u>	1.6172	0.8098	1.9970	<u>0.0458</u>
6. Positive HIV status (Yes/No)	0.4267	0.1632	1.1158	-0.8517	0.4905	-1.7365	0.0825
7. Previous TB treatment (Yes/No)	<u>60.6727</u>	<u>19.8700</u>	<u>185.2627</u>	4.1055	0.5695	7.2084	<u>0.0000</u>
8. Travelling <3 KM (Yes/No)	0.4835	0.1876	1.2460	-0.7267	0.4830	-1.5045	0.1324
9. Unfavourable outcome (Yes/No)	3.5946	0.8424	15.3385	1.2794	0.7403	1.7283	0.0839

Appendix 7: Unconditional Logistic Regression model building process (continued)

Step 2

“Bacillary load $\geq 2+$ ” was removed from the model since it has the highest P- value.

The remaining variables were re-entered into the model with the results tabulated below.

Term	AOR	95% C.I.	Coefficient	S. E.	Z-		
					Statistic	P-Value	
1. BCG scar Absent (Yes/No)	2.0553	0.6839	6.1765	0.7204	0.5614	1.2833	0.1994
2. DOT (Yes/No)	<u>0.2208</u>	<u>0.0850</u>	<u>0.5738</u>	-1.5104	0.4872	-3.1003	<u>0.0019</u>
3. Given TB Message (Yes/No)	0.6496	0.2443	1.7275	-0.4313	0.4990	-0.8644	0.3874
4. Non Kenyan (Yes/No)	<u>4.7637</u>	<u>1.0744</u>	<u>21.1222</u>	1.5610	0.7599	2.0543	<u>0.0399</u>
5. Positive HIV status (Yes/No)	0.4304	0.1651	1.1215	-0.8431	0.4887	-1.7253	0.0845
6. Previous TB treatment (Yes/No)	<u>61.265</u>	<u>20.1111</u>	<u>186.6340</u>	4.1152	0.5683	7.2407	<u>0.0000</u>
7. Travelling <3 KM (Yes/No)	0.4873	0.1899	1.2507	-0.7188	0.4809	-1.4948	0.1350
8. Unfavourable outcome (Yes/No)	3.5164	0.8357	14.7959	1.2574	0.7331	1.7151	0.0863

Appendix 7: Unconditional Logistic Regression model building process (continued)

Step 3

“Given TB Message” was removed from the model since it has the highest P- value.

The remaining variables were re-entered into the model with the results tabulated below.

Term	AOR	95%	C.I.	Coefficient	S. E.	Z-	
						Statistic	P-Value
1. BCG scar Absent (Yes/No)	1.9476	0.6573	5.7706	0.6666	0.5542	1.2028	0.2291
2. DOT (Yes/No)	<u>0.2233</u>	<u>0.0865</u>	<u>0.5764</u>	-1.4993	0.4838	-3.0987	<u>0.0019</u>
3. Non Kenyan (Yes/No)	<u>5.6079</u>	<u>1.3252</u>	<u>23.7313</u>	1.7242	0.7360	2.3425	<u>0.0192</u>
4. Positive HIV status (Yes/No)	0.4280	0.1650	1.1106	-0.8486	0.4865	-1.7443	0.0811
5. Previous TB treatment (Yes/No)	<u>64.4892</u>	<u>21.2921</u>	<u>195.3240</u>	4.1665	0.5654	7.3691	<u>0.0000</u>
6. Travelling <3 KM (Yes/No)	0.4441	0.1772	1.1133	-0.8116	0.4689	-1.7311	0.0834
7. Unfavourable outcome (Yes/No)	3.4496	0.8350	14.2506	1.2382	0.7238	1.7108	0.0871

Appendix 7: Unconditional Logistic Regression model building process (continued)

Step 3

“BCG scar absent” was removed from the model since it has the highest P- value. The remaining variables were re-entered into the model with the results tabulated below.

Term	AOR	95%	C.I.	Coefficient	S. E.	Z-	
						Statistic	P-Value
1. DOT (Yes/No)	<u>0.2152</u>	<u>0.0833</u>	<u>0.5555</u>	-1.5364	0.4839	-3.1748	<u>0.0015</u>
2. Non Kenyan (Yes/No)	<u>6.9636</u>	<u>1.7362</u>	<u>27.9294</u>	1.9407	0.7087	2.7385	<u>0.0062</u>
3. Positive HIV status (Yes/No)	0.4148	0.1604	1.0725	-0.8799	0.4846	-1.8156	0.0694
4. Previous TB treatment (Yes/No)	<u>61.336</u>	<u>20.7762</u>	<u>181.0786</u>	4.1164	0.5523	7.4526	<u>0.0000</u>
5. Travelling <3 KM (Yes/No)	0.4285	0.1721	1.0672	-0.8474	0.4655	-1.8202	0.0687
6. Unfavourable outcome (Yes/No)	3.3221	0.8069	13.6770	1.2006	0.7220	1.6629	0.0963

Appendix 7: Unconditional Logistic Regression model building process (continued)

Step 4

“Unfavourable outcome” was removed from the model since it has the highest P-value.

The remaining variables were re-entered into the model with the results tabulated below.

Term	AOR	95% C.I.	Coefficient	S. E.	Z-Statistic	P-Value	
DOT (Yes/No)	<u>0.2171</u>	<u>0.0850</u>	<u>0.5544</u>	-1.5274	0.4784	-3.1929	<u>0.0014</u>
Non Kenyan (Yes/No)	<u>6.3879</u>	<u>1.6221</u>	<u>25.1553</u>	1.8544	0.6993	2.6517	<u>0.0080</u>
Positive HIV status (Yes/No)	<u>0.3577</u>	<u>0.1398</u>	<u>0.9156</u>	-1.0279	0.4795	-2.1440	<u>0.0320</u>
Previous TB treatment (Yes/No)	<u>80.2555</u>	<u>27.9044</u>	<u>230.8217</u>	4.3852	0.5390	8.1358	<u>0.0000</u>
Travelling <3 KM (Yes/No)	0.4696	0.1938	1.1379	-0.7559	0.4516	-1.6739	0.0941

Appendix 7: Unconditional Logistic Regression model building process (continued)

Step 5

“Unfavourable outcome” was removed from the model since it has the highest P-value.

The remaining variables were re-entered into the model to produce the Final best Fit Model’ below

Term	AOR	95% C.I.		Z-			
				Coefficient	S. E.	Statistic	P-Value
DOT (Yes/No)	<u>0.2310</u>	<u>0.0923</u>	<u>0.5783</u>	-1.4653	0.4682	-3.1298	<u>0.0017</u>
Non Kenyan (Yes/No)	<u>5.5151</u>	<u>1.3944</u>	<u>21.8133</u>	1.7075	0.7016	2.4338	<u>0.0149</u>
Positive HIV status (Yes/No)	<u>0.3416</u>	<u>0.1333</u>	<u>0.8756</u>	-1.0741	0.4802	-2.2366	<u>0.0253</u>
Previous TB treatment (Yes/No)	<u>85.0237</u>	<u>29.7063</u>	<u>243.3497</u>	4.4429	0.5365	8.2809	<u>0.0000</u>

Appendix 8: Glossary

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

Relapse: A patient previously treated for TB who has been declared cured or treatment completed, and is diagnosed to be bacteriologically positive (smear or culture) TB.

Failure: A new smear-positive patient who is smear positive at 5 months or later after starting treatment.

Return after default (RAD): A patient who returns to treatment, positive bacteriologically, following interruption of treatment for 2 months or more.

Cured: A patient who is sputum-smear negative in the last month of treatment and on at least one previous occasion.

Treatment completed: A patient who has completed treatment but who does not meet the criteria for 'cure' or 'failure'.

Chronic: A patient with TB who is sputum positive at the end of a standard re-treatment regimen with essential anti-TB drugs.

Died: A patient who dies for any reason during the course of treatment.

Default: A patient whose treatment was interrupted for two consecutive months or more.

Transferred out: A patient who has been transferred to another recording and reporting unit and for whom the treatment outcome is unknown.

Category I: New cases of acid-fast bacillus (AFB) smear-positive pulmonary TB and other newly diagnosed sputum-negative or extra pulmonary seriously ill patients with severe TB forms.

Category II: Patients who have received anti-TB treatment for more than one month in the past (and therefore are at increased risk of having multi-drug resistant disease). These include smear-positive relapses, smear-positive failure cases, and smear-positive patients being treated after default. Category II also includes smear-negative pulmonary and extra pulmonary cases due to failure and relapse (infrequent and exceptional).

Category III: New cases of AFB smear-negative pulmonary and extra-pulmonary TB who are not seriously ill.

Appendix 9: Description of symbols used in the Fleiss formula for sample size calculation and their corresponding value

Description of Symbols in the formula	Value
Z - Score for two-tailed test based on α level ($z_{\alpha/2}$)...	1.96
Z - Score for one-tailed test based on β level ($z_{1-\beta}$).....	0.84
Ratio of controls: cases (r).....	2:1
Proportion of cases with exposure (P_1).....	23%
Proportion of controls with exposure (P_2).....	9%
(Unpublished data from MDR-TB surveillance system evaluation)	
$1 - P_1$ (q_1)	77%
$1 - P_2$ (q_2).....	91%
Number of cases (n_1).....	77
Number of controls (n_2).....	154
Total sample size ($n_1 + n_2$)	<u>231</u>

Appendix 10: Cases and Controls by facility and Province

Province/ facility	Cases		Tracked
	Enrolled No (%)	Controls (No)	MDR-TB (No)
Nairobi Province	41(54%)	82	76
1. Bahati Health Centre	0	2	
2. Casino Health Centre	0	2	
3. Coptic hospital	0	2	
4. Eastleigh Health Centre		4	
5. Huruma Health Centre		8	
6. St Mary's Hospital		3	
7. Kenyatta National hospital	20	0	
8. Kibera Health Centre	1	6	
9. Mbagathi District hospital		16	
10. MSF Blue house(Mathari)	19	24	
11. Mwiki Health Centre		4	
12. Rhodes Health Centre	1	6	
13. Riruta Health Centre	0	5	
Nyanza Province	16 (61%)	32	26
14. Akala Health Centre	1	2	
15. Chulaimbo Health centre	1	2	
16. Homabay District Hospital	12	24	
17. Segal Cottage Hospital	1	2	
18. Siaya District Hospital	1	2	

Province/ facility	Cases		Tracked
	Enrolled	Controls	MDR-TB
	No (%)	(No)	(No)
Coast Province	10(33%)	20	30
19. Bamburi Health centre	0	2	
20. Diana Health Centre		1	
21. Ganjoni Health Centre	5	6	
22. Kilindini Health Centre		2	
23. Kisauni Health Centre	2	1	
24. Kizingo Health Centre	0	2	
25. Magongo Health Centre		1	
26. Mgongeni Health Centre		1	
27. Mvita Health Centre		1	
28. Portreitz District Hospital	2	2	
29. Tudor Health Centre	1	1	
Western Province	5(71%)	10	7
30. Busia District Hospital	1	2	
31. Hamisi District Hospital	1	2	
32. Kakamega Provincial Hospital	1	2	
33. Khunyangu District Hospital	1	2	
34. Matayos Health Centre	1	2	
North eastern Province	4 (57%)	8	7
35. Ifo (Daadab Refugee Camp)	3	6	
36. IOM (Daadab Refugee Camp)	1	2	

Province/ facility	Cases		Tracked
	Enrolled	Controls	MDR-TB
	No (%)	(No)	(No)
Rift Valley Province	3(15%)	6	20
37. Kitale District Hospital	1	2	
38. Kolongolo Health Centre	1	2	
39. Nakuru Provincial Hospital	1	2	
Central Province	1(20%)	2	5
40. Kiambu District Hospital	1	2	
Eastern Province	1(7%)	2	15
41. Embu Provincial Hospital	1	2	