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TREATMENT OUTCOMES IN PATIENTS INFECTED WITH MULTIDRUG-RESISTANT TUBERCULOSIS AND IN PATIENTS WITH MULTIDRUG-RESISTANT TUBERCULOSIS CO-INFECTED WITH HUMAN IMMUNODEFICIENCY VIRUS AT BREWELSKLOOF HOSPITAL.

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A thesis submitted in fulfilment of the requirements for the degree of Magister Scientiae in Pharmaceutical Sciences, School of Pharmacy in the Faculty of Natural Sciences of the University of the Western Cape; Bellville. South Africa.

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Treatment outcomes in patients infected with multidrug- resistant tuberculosis and in patients with multidrug –resistant tuberculosis co-infected with human immunodeficiency virus at Brewelskloof Hospital.

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KEYWORDS

Antitubercular agents

Drug interactions

Treatment outcomes

Mycobacterium tuberculosis

Human immunodeficiency virus

Multidrug-resistant tuberculosis

Antiretroviral agents

ABSTRACT

Many studies have reported low cure rates for multidrug-resistant tuberculosis (MDR-TB) patients and MDR-TB patients co-infected with human immunodeficiency virus (HIV). However, little is known about the effect of HIV infection and antiretroviral therapy on the treatment outcomes of MDR-TB in South Africa. Therefore, the objectives of the study are: to find out whether HIV infection and interactions between ARVs and second line anti-TB drugs have an impact on the following MDR-TB treatment outcomes: cure rate and treatment failure at Brewelskloof Hospital. MDR-TB patients were treated for 18-24 months.

The study was designed as a case-control retrospective study comparing MDR-TB treatment outcomes between HIV positive (cases) and HIV negative patients (controls).

Patients were included in the study only if they complied with the following criteria: sensitivity to second line anti-TB drugs, MDR-TB infection, co-infection with HIV (for some of them), male and female patients, completion of treatment between 1 January 2006 and 31 December 2008. Any patients that presented with extreme drug-resistant tuberculosis (XDR-TB) were excluded from the study.

Data were retrospectively collected from each patient's medical records. There were a total of 336 patients of which 242 (72%) were MDR-TB patients and 94 (27.9%) MDR-TB co-infected with HIV patients. Out of the 242 MDR-TB patients, 167 (69.2%) were males and 75 (30.7%) were females. Of the 94 patients with MDR-TB co-infected with HIV, 51 (54.2%) males and 43 (45.7%) females.

Patients with multidrug-resistant tuberculosis co-infected with HIV who qualify for antiretroviral therapy were treated with stavudine, lamivudine and efavirenz while all MDR-TB patients were given kanamycin, ethionamide, ofloxacin, cycloserine and pyrazinamide. The cure rate of MDR-TB in HIV (+) patients and in HIV (-) patients is 34.5% and 30 % respectively. There is no significant difference between both artes (p-value = 0.80). The MDR-TB cure rate in HIV (+) patients taking antiretroviral drugs and in HIV (+) patients without antiretroviral therapy is 35% and 33% respectively. The difference between both rates is not statistically significant. The study shows that 65 (28.0%) patients completed MDR-TB treatment but could not be classified as cured or failure, 29 (12.5%) patients failed, 76 (32.7%) defaulted, 18 (7.7%) were transferred out and 44 (18.9%) died. As far as treatment completed and defaulted is concerned, there is no significant statistical difference between HIV (+) and HIV (-) The number of patients who failed the MDR-TB treatment and who were transferred out is significantly higher in the HIV (-) group than in the HIV (+) group. Finally the number of MDR-TB patients who died is significantly higher in the HIV (+) group).

The median (range) duration of antiretroviral therapy before starting anti-tuberculosis drugs is 10.5 (1-60) months.

According to this study results, the MDR-TB treatment cure rate at Brewelkloof hospital is similar to the cure rate at the national level. The study also shows that HIV infection and antiretroviral drugs do not influence any influence on MDR-TB treatment outcomes.

DEDICATION

Dedicated this study to my mom and dad

and to one of my father in the Lord, who once wrote

"We will never be all Professors

We will never be all Doctors

We will never be all Nurses

We will never be all Pastors

We will never be all Businessmen

We will never be all Careers fellow

We will never be all Traders.

But each one belongs somewhere and finding where you belong is what brings out the full colors" (Oyedepo, 1996).

DECLARATION

I declare that the dissertation titled "*Treatment outcomes in patients infected with Multidrug -resistant Tuberculosis and in patients with Multidrug-resistant Tuberculosis co-infected with human immunodeficiency virus at Brewelskloof Hospital"* is my own work, and that it has not been submitted for any degree or examination in any other university, and that all the sources l have used or quoted have been indicated and acknowledged in my references.

Adewumi Olayinka Anthony August 2012

UWC, Bellville

Signed……

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ABBREVIATIONS AND ACRONYMS

AIDS	Acquired immunodeficiency syndrome.
ALT	Alanine aminotransferase
Anti-TB	Anti-tuberculosis
ART	Antiretroviral therapy
ARV	Antiretroviral
AZT	Zidovudine
BH	Brewelskloof hospital
CD4 CELLS	These are T helper/ inducer lymphocytes with CD4 receptors and are important cells which regulate and control aspects of the immune system. Also called T4 helper cells
CD4	The laboratory test most commonly used to estimate the level of immune-deficiency (CD4 cells) in HIV infection. Cluster of differentiation 4
CSF	Cerebro-spinal fluid
CTX	Co-trimoxazole
CPT	Co-trimoxazole preventative therapy
CXR	Chest x-ray
ddl	Didanosine
DOT	Direct observed treatment
D.O.B	Date of birth
DST	Drug susceptibility testing
ELISA	Enzyme-linked immuno-sorbant assay

EMB	Ethambutol
ETH	Ethionamide
Efavirenz	EFV
FBC	Full blood count
FQN	Fluoroquinoloes
FTC	Emtricitabine
HAART	Highly active anti-retroviral therapy
HBCs	High burden countries
HepBSAg	Hepatitis B surface antigen
HB	Hemoglobin
HIV	Human immunodeficiency virus which causes AIDS
INH	Isoniazid
IUALTD	International union against TB and lung disease
IRIS	Immune reconstitution inflammatory syndrome
Kan	Kanamycin
LFT	Liver function test
LPV/r	Lopinavir/ritonavir
Lamivudine	3TC
MDR-TB	Multidrug-resistant tuberculosis
M.TB	*Mycobacterium tuberculosis*
MDG	Millennium development goal
NDOH	National department of health
NGO	Non-government organization

NRTI	Nucleoside reverse transcriptase inhibitor
NVP	Nevirapine
PI	Protease inhibitor
PF	Pleural fluid
PMTCT	Prevention of mother-to-child transmission
RIF	Rifampicin
SLDS	Second line anti-TB drugs
TDF	Tenofovir
TB	Tuberculosis
TDM	Therapeutic drug monitoring
UNAIDS	Joint United Nations Programme on Acquired Immune Deficiency Syndrome
Vs	versus
VL	Viral load
WCDOH	Western Cape department of health
WCC	White blood counts
WHO	World health organization
XDR-TB	Extensively drug-resistant tuberculosis

CHAPTER ONE

INTRODUCTION

1.1 Background information

Multidrug-resistant tuberculosis is usually the result of irregular adherence to TB treatment and is identified when there is resistance to rifampicin and isoniazid on sputum culture sensitivity testing Department of Health (National, 1993). Multidrug-resistant tuberculosis (MDR-TB) is a public health problem in developing countries (Thelma E et al, 1999) and particularly in South Africa (WHO, 2010). In South Africa, MDR-TB is treated in designated hospitals. In the Western Cape Province, there are four MDR-TB hospitals. These are Brooklyn Chest hospital (BCH) and DP Marais hospital in the cape metropole region, Harrycomay hospital in George and Brewelskloof hospital in Worcester. This study was carried out at the Brewelskloof hospital.

According to the Department of Health (National, 2006), MDR-TB cure rate was between 30-50%. This cure rate is even lower in patients with human immunodeficiency virus (HIV) infection (Nottel HS et al, 1991). There are however, limited data about MDR-TB treatment outcomes and the prevalence of HIV among MDR-TB patients (Sanchez-Padila et al, 2009-2010).

Also, the influence of HIV on treatment outcomes of MDR-TB needs to be investigated. In doing this, the influence of HIV infection on the treatment outcomes of MDR-TB (www.mdpi.com, 2012), one needs to determine whether the interaction between antiretroviral (ART) drug and second-line anti-TB drugs may have an impact on MDR-TB treatment outcomes.

1.2 Motivation for the study

South Africa has an estimated 14,000 incidence cases of MDR-TB every year (WHO, 2010) and 33% of HIV prevalence; Department of Health (Provincial, 2007) and (City of Cape Town, 2009). To date, there has been limited data available on the prevalence of MDR-TB (Helen SC et al, 2009) in South Africa. Investigations are needed in order to understand the main causes of unsuccessful treatment of MDR-TB. Many of these MDR-TB patients are unknown to the health service (Park MM et al 2007), often because they have not been diagnosed and not on treatment Department of Health (City of Cape Town, 2009). Though there was a study in Khayelitsha in 2009, Department of Health (City of Cape Town, 2009) none has been done in the Brewelskloof hospital. There is an urgent need to quantify the extent of the drug-resistant tuberculosis epidemic in these communities in order to advocate for and develop strategies for control (Park MM et al, 2007).

It is against this background that this study seeks to investigate whether HIV infection and drug interactions between ARVs and second line anti-TB drugs (Kawal V et al, 2005) have an impact on the following MDR-TB treatment outcomes: cure rate and treatment failure at Brewelskloof Hospital.

1.3 The aim of the study

The aims of the current study are: (1) to find out the treatment outcomes of MDR-TB; (2) to assess the influence of HIV infection on MDR-TB treatment outcomes and (3) to find out whether the interaction between ARV drugs and second-line anti-TB drugs has any impact on MDR-TB (Goble M et al, 2006) treatment outcomes.

CHAPTER TWO

LITERATURE REVIEW

2.1 The global picture of tuberculosis and human immunodeficiency virus

2.1.1 Incidence of tuberculosis

Incidence of tuberculosis is greatest among those with conditions impairing immunity (Corbett et al; 2003), such as HIV infection. Tuberculosis is the world's greatest infectious killer of women of reproductive age and leading cause of death among those infected with HIV and AIDS globally (www.Maxpedia.org, 2012). There has been an increase in TB (World TB Day, 2008) cases from 6.6 million in 1990 to 8.3 million in 2000. The WHO has declared TB to be a global public health emergency, with an estimated one-third of the world's population being infected with *Mycobacterium tuberculosis* (M.Tb) (www. Microbewiki.Kenyon.edu, 2012).

In 2006, estimated number of active TB carriers is around 20 million worldwide with more than 3 million deaths annually (Zignol et al, 2006). In 2006, it was also discovered that the incidence of TB has been increasing globally in recent years (Lan T.Ho-Pharm, 2010), partially due to the HIV epidemic (Aziz *et al*, 2006). In addition, there has been an increase in the occurrence of strains of Mycobacterium tuberculosis that are resistant to (www.thebody.com, 2012) currently used chemotherapy (Aziz et al, 2006). In 2005, there were an estimated 8.8 million new cases of tuberculosis worldwide (www.biomedcentral.com, 2012); and 9.24 million in 2006 (WHO, 2007). Tuberculosis continues to infect an estimated one-third of the world's population, to cause disease in 8.8 million per year, and to kill 1.6 million of those infected (www.japi.org, 2012). A study by the WHO covering the five regions of the world estimated numbers of TB cases in 2007

to be spread as follows: Asia (55%), Africa (31%), Eastern Mediterranean region (6%), the European Region (5%), and the Region of the Americas (3%) (www.who.int, 2010).

The WHO reported that the resistance includes resistance to isoniazid and rifampicin in combination, followed by the deadly, almost incurable, emergence of MDR-TB (WHO, 2009). The WHO stated that the Asian region is the worst affected, as more than 50% cases of TB occurred in India and China (K. Flouyd. 2012). In 2009, WHO estimated that the incidence cases of TB to be 9.27 million (WHO, 2010).

2.1.2 Prevalence of human immunodeficiency virus and acquired immunodeficiency syndrome

An estimated 34.0 million people were living with HIV as of 2010; 3.4 million of them were children under 15 years, and about 16.8 million were women. Every day, over 7,000 persons became infected with HIV and about 5,000 persons died from AIDS, mostly because of inadequate access to HIV prevention care and treatment services (WHO, 2010). Roughly 17.1 million children under the age of 18 have lost one or both parents to AIDS, and millions more have been affected, with a vastly increased risk of poverty, homelessness, school dropout, discrimination and loss of life opportunities (www.extremeresponse.info, 2012). These hardships include illness and death. Of the estimated 1.8 million people who died of AIDS-related illnesses in 2010, children under 15 years accounted for 250,000 (WHO, 2010).

Human immunodeficiency virus is the main reason for failure to meet TB control targets in high HIV settings. TB is a major cause of death among people living with HIV. Sub-Saharan Africa bears the brunt of the HIV fuelled TB epidemic. The rapidly increasing HIV epidemic in other parts of the world could also increase the number of HIV-related TB cases. In order to control TB in high HIV settings, the Stop TB strategy includes collaborative TB/HIV activities. These

collaborative TB/HIV activities have the objectives of creating the mechanism of collaboration between TB and HIV/AIDS programmes, reducing the burden of TB among people living with HIV and reducing the burden of HIV among TB patients (www.who.int, 2012).

2.1.3 Mortality or morbidity of tuberculosis, human immunodeficiency virus and acquired immunodeficiency syndrome

In 2009 there were 9.4 million new TB cases, including 1.1 million cases among people with HIV; 1.7 million people, (of whom 380,000 were women with HIV-positive) died from TB, an average of 4,700 deaths a day (WHO, 2010). Tuberculosis is one of the three main causes of death among women aged 15 to 44 (www.pulsepakistan.com, 2012). In 2007, WHO indicated there were 9.27 million TB and HIV incident cases, of which an estimated 1.37 million (15%) was HIV positive; 79% of these HIV positive cases were in the Africa Region and 11% were in the South-East Asia Region (www.who.int, 2012). Globally, however, TB incidence is falling slowly, and at least three of six WHO regions are on track to achieve global targets for reducing the number of cases and deaths that have been set for 2015 (WHO, 2010). The statements above have shown that that there is increase in the TB global control.

2.2 The situation of tuberculosis and HIV – infection on the African continent

2.2.1 Tuberculosis

The African region accounts for more than a quarter of the global TB burden, with an estimated 2.4 million cases and 540,000 deaths annually (www.tbtoolkit.org, 2012). It is also noted that out of the 400,000 people worldwide infected with MDR-TB in 2008, 14,000 died (WHO, 2009). It has been discovered that inadequate treatment of TB has been the cause of MDR-TB (www.whqhbdoc.who.int, 2010).

2.2.2 Human immunodeficiency virus and acquired immunodeficiency syndrome

Human immunodeficiency virus and AIDS are a major public health concern and cause of death in Africa. Africa is home to about 14.5% of the world population. It is estimated to be home to 67% of all people living with HIV and 72% of all AIDS death in 2009 (www. danpritcherd.com, 2012).

According to UNAIDS, some countries has suffered from political factors for not using modern medicine, where leaders have denied the link between HIV and AIDS, favoring alternative medicine (UNAIDS, 2010). Despite the lack of scientific acceptance, AIDS unconfirmed statements has had a negative significant impact, especially in South Africa; where our leader ignored the call for introduction of ARVs in the treatment of HIV patients (UNAIDS, 2010).

African continent also suffered from economic factors of lack of money, natural disasters and conflicts food, shelter or other sources (Online. http://www.villagevoice.com, 2011). A minority of scientists claim that as many as 40% HIV infections in African adults may be caused by unsafe medical practices rather than by sexual activity (Samuel and Fiona, 2009).

There is different regional analysis in HIV/ AIDS in Africa: (1) East-central Africa-Kenya having the highest adult HIV prevalence and Tanzania having the highest death in 2009 (WHO, 2010). (2) West Africa**:** Nigeria having highest adult HIV and AIDS prevalence and death in 2009. The region has generally high levels of infection of both HIV-1 and HIV-2. Nigeria has the second largest number of people with HIV in Africa after South Africa (WHO, 2010). (3) In the mid-1980s**,** Southern Africa HIV and AIDS are virtually unheard of, but it is now the worst-affected region in the world. Of the eleven Southern Africa countries at least seven are estimated to have an infection rate of over 15% (Africa, 2011). Angola presents one of the lowest infection rates of 2.1% (www.Indexmundi, 2012). Most HIV infection found in Southern Africa is HIV-1,

the world's most common HIV infection, which predominates everywhere except West Africa, which is home to HIV-2 (http://www.Wikipedia.org, 2011).

2.2.3 Tuberculosis, human immunodeficiency virus and acquired immunodeficiency syndrome

In a survey of 17 (10%) African countries with tuberculosis, HIV and AIDS including South Africa, there were high burden of TB, HIV and AIDS in African countries. Data indicate resistance to single TB drug treatment; there is also a significant increase in Botswana, where some longitudinal data exist with joint HIV infection (Jones KDJ et al, 2006).

2.3 The situation of tuberculosis and HIV – infection in the sub-Saharan Africa Region

2.3.1 Tuberculosis

Sub-Saharan Africa has been identified as a TB "hotspot"**;** this is not surprising given the poor TB cure rates in many of these countries (WHO, 2010). South Africa has the highest cases of TB in Africa (Kent G, 2010). The WHO in 2010 revealed that the sub-Saharan region accounts for more than a quarter of the global burden of TB with an estimated 2.4 million TB cases and 540,000 TB deaths annually (www.tbtoolkit.org, 2012).

2.3.2 Human immunodeficiency virus and acquired immunodeficiency syndrome

A UNAIDS/WHO study on HIV in sub-Saharan Africa. In 2009, (Dater Anaid et al, 2010) revealed that 68% of all people living with HIV are in sub-Saharan Africa and 3.9% of these were receiving antiretroviral therapy (UNAIDS, 2010). An estimated 22.5 million people were living with HIV in sub-Saharan Africa (www.danadamsgh.com 2011), including minor children (UNAIDS, 2010). The five countries experiencing the worst HIV/AIDS epidemic in sub-Saharan Africa are discussed as followed: Botswana has 320,000 people living with HIV/AIDS (Holtz TH, 2011) (24.8%), Lesotho has 290,000 people living with HIV/AIDS (23.6%), South Africa

has 5,600,000 people living with HIV/AIDS (17.8%), Zimbabwe has 1,200,000 people living with HIV/AIDS (14.3%) and Zambia has 980,000 living with HIV/AIDS (13.5%); the percentages were based on the total population in each country (Online. http://www.village.com, 2011). These figures show that sub-Saharan Africa is the region most affected by TB and HIV (www.stoptb.org, 2009), consequently leading to high morbidity and mortality. These data should be a wake-up call for all African leaders and stakeholders.

2.3.3 Tuberculosis, human immunodeficiency virus and acquired immunodeficiency syndrome

In 2009 data relating to TB and HIV in sub-Saharan Africa showed that there were 1.37 million new cases of TB sub-Saharan among HIV positive people (Jarvis M, 2010). There were 14 million people who are co-infected with TB and HIV globally; around 80% of those who are co-infected live in sub-Sahara Africa (www.aidsmap.com, 2011).

2.4 The South African situation of tuberculosis and HIV-infection

2.4.1 Drug resistance to tuberculosis

In South Africa, TB constitutes more than 80% of all notifiable diseases Department of Health (National, 1990). In 1996 the WHO and NDOH conducted a combined review of the TB Control Program in South Africa. It was reported that drug-resistant TB has become a public health concern because of past TB history, re-infection with drug-resistant strains and inadequate treatment and poor treatment adherence on the part of the patients (WHO, 1996).

Also, there has been an increase in the number of patients who are resistant to first-line anti-TB drugs (www.who.int, 1996). In 2006, the annual tuberculosis incidence rate was estimated to be 628 cases per 100,000 of the population per annum (www.ukpmc.ac.uk, 2012); in a population

of 47.9 million people (WHO, 2008). The WHO estimated that in 2009, 3.3% of all new TB cases were MDR-TB (WHO, 2009).

The observation, however, that tubercule bacillus can survive in cells and tissues of the patient despite the adequate and regular administration of drugs leads one to query reasons other than poor compliance as an explanation for treatment relapses and drug resistance (March et al, 1997). The WHO estimates that there are more than 14,000 MDR-TB incident cases in South Africa each year (www.msf.org.za, 2009).

2.4.2 Human immunodeficiency virus and acquired immunodeficiency syndrome

In 2006, UNAIDS estimated HIV prevalence in South Africa to be 17.8% with household (17.2%) and antenatal (18.3%) statistics (National, 2010). South African National Statistics stated that in 2007 of an estimated total population of 47.9 million people, an estimated 5.7 million people were living with HIV (www.ukpmc.ac.uk, 2012) and, at the end of 2009, the figures included 300,000 children under 15 years old (www.avert.org, 2011). It predicts that South Africa will exceed 6 million people living with HIV by 2015, by which time around 5.4 million South Africans would have died of AIDS (www.griffintheatre.com.au, 2012). The provinces with the highest HIV prevalence were: Kwazulu-Natal (39.5%), Mpumalanga (34.7%), Free State (30.1%) and the North-West (30%). The Northern Cape and Western Cape recorded the lowest prevalence (17.2% and 16.9%) respectively (www.avert.org, 2011).

2.4.3 Tuberculosis, human immunodeficiency virus and acquired immunodeficiency syndrome

Tuberculosis disease synergistically accelerates the progression of HIV infection to AIDS by inciting viral replication in immunologically activated CD4 cells (Tolu Oni, 2009).

The two pandemics of TB and HIV fuel each other and the statistics show the risk of TB after HIV infection. The annual incidence of TB disease doubles within the first year of HIV infection (www.ukpmc.ac.uk, 2012).

The WHO reported that in South Africa, more than half of TB patients tested for HIV are seropositive (www.ukpmc.ac.za, 2011). The City of Cape Town conducted research in 2007 on antenatal HIV prevalence and estimated it to be 30%. The case notification rate for TB was at least 1,500 per 100,000 people per year – among the highest TB incidence rates in the world (www.ukpmc.ac.za, 2011). Siyanyinqoba states that HIV and TB are twin epidemics: nearly 70% of all people with TB are also HIV positive (www.leeds.ac.uk, 2010). It is imperative at this juncture to say that having HIV and contracting TB does not mean a "death sentence" unless the patients refuse to take their prescribed medications. Because of the association between HIV and TB (Karen, 2011) has prompted the South African Government to have Antiretroviral Treatment Guidelines formulated for proper management of adult and adolescents patients.

The combination chemotherapy of five to six drugs assumes a low probability of simultaneous resistance to all drugs in the regimen (www.pubmedcentralnih.gov, 2010). Researchers have reported that, in patients with MDR-TB and in patients with MDR-TB co-infected HIV (www.tfcares.org 2010), and who taking co-trimaxozole preventative therapy (CTP) reduced mortality rates. In patients on ART with low CD4 counts or WHO stages 1, 2, 3 and 4 disease. Observational studies in Malawi, Ivory Coast and South Africa in a retrospective analysis showed 46% reduction in mortality rates of tuberculosis (Mwaungulu, 2004, Dean, 1999 and Hoffman, 2010).

2.5 Overview of multidrug-resistant tuberculosis

Over the past decade, there has been an increase in the appearance of strains of TB that are resistant to anti-TB drugs. Multi-drug resistant TB is caused by TB strains that are resistant to the first line drugs INH and RIF (www.touchbriefing.com, 2010), which are considered to be the most potent drugs against drug-susceptible TB (Iseman, 2002; Sharma and Mohan, 2004). The molecular basis of drug resistance to TB is now largely understood (Garcia De VD et al, 2002). From a microbiological point of view, the resistance is caused by a spontaneous and random mutation in the MTB bacterial chromosome. This leads to amino acids substitutions in their target proteins (for example, beta -subunit of MTB RNA polymerase in case of rifampicin), which result in reduced susceptibility to specific drug, and thus, the drug, becomes ineffective against the mutant bacilli (Blanchard, 1996; Ramaswamy and Muser, 1998).

Drug resistance is categorized into primary, acquired and initial resistance (Kochi et al, 1993; Loddenkemper et al, 2002). Primary resistance is resistance to anti-TB drugs in patients with no history of previous TB treatment, that is, treatment-naïve patients (www.Columbia.edu, 2011). Acquired resistance is resistance to anti-TB drugs from patients with previous treatment (Pinto WP, 2001), which could be one or more treatment. Initial resistance is referred to patients with primary resistance as well as those with undisclosed acquired resistance.

Causes of drug resistance include many factors such as inadequate treatment regimens prescribed, poor drug supply and quality, misuse of (www.eqrosurveillance.org, 2011) anti-TB drugs, poor treatment compliance, treatment default and HIV co-infection (Iseman, 1993; Jain, 1998; Sharma and Mohan, 2004). The HIV-infection epidemic has caused an explosive increase in TB incidence and may be contributing to the increase in MDR-TB infection (www.stoptb.org, 2011). Outbreaks of MDR-TB infection and case-fatality rates reaching 83% among HIV-

infected patients have been reported (www.caprisa.org, 2011). This suggests that the severe immune suppression, which occurs in HIV infected patients, appears to be a predisposing factor for the development of MDR-TB (www.medind.nih.in, 2011).

2.6 The causes of drug resistance

Recent findings on XDR- TB can no longer be considered as occurring in isolated outbreaks as it has been reported in 45 countries from all regions of the world (www.lib.bioinfo.pl, 2011). Human immunodeficiency virus has been associated as independent risk factors for infection with drug-resistant TB (www.lib.bioinfo.pl, 2011). Human immunodeficiency virus patients appear more likely to suffer from primary transmitted resistance as opposed to developing acquired resistance during the course of treatment for TB (www.lib.bioinfo.pl, 2011). New rapid diagnostic offer promise or provide clinically useful first-line drug susceptibility information but require validation in HIV patients and smear negative individuals. Therefore, MDR and XDR TB disproportionately affect HIV patients and results in increased morbidity and mortality (www.lib.bioinfo.pl, 2011). Unfortunately, at least one strain of M. tuberculosis in South Africa had already developed resistance to one or more of those second-line drugs by the time they were introduced. Drug susceptibility tests would have warned prescribers that the standard second-line regimen was unlikely to help the patient but was likely to lead to additional drug-resistance. But these tests were not performed or were not available. Indeed, the reduced efficacy of the regimen allowed the stain to survive and over time, develop resistance to other drugs. So there is need to increase the use of drug resistance surveillance programs to help forestall the development of drug-resistance in M. tuberculosis (www.blackherbals.com, 2011).

Resistance to first-line drugs in most of the pathogens causing these diseases ranges from zero to almost 100%. In some instances, resistance to second and third line agents is seriously

compromising treatment outcomes. Added to this is the significant global burden of resistant hospital-acquired infections, the emerging problems of antiviral resistance and the increasing problems of drug resistance in the neglected parasitic diseases of poor and marginalized populations. This is WHO global strategy for containment of antimicrobial resistance (www. plumbot.com, 2011).

Drug resistance caused by Mycobacterium tuberculosis develops when patients with tuberculosis cannot or do not comply with the medication regimen. A second line of drugs has been used to treat those infected with drug-resistant TB (www.nerve.in, 2011). This second-line medication regimen was adopted in South Africa in 2001 to treat drug-resistant TB. The worldwide emergence of drug-resistant has blunted and may reverse the benefits from the historic rollouts of ARVs (Sheela S, 2009).

Drug resistance is the reduction in effectiveness of a drug such as an antimicrobial or an antineoplastic (www.mejfrm.com, 2007). It can develop naturally, but careless practices in drug supply and use are hastening the problem. The overuse of antibacterial cleaning products in the home may be producing strains of multi-antibiotics-resistant bacteria. The use and misuse of antimicrobials in human medicine and animal husbandary over the last 70 years has lead to the rise in the number and types of micro-organisms resistant to these medicines leading to death, increased suffering and disability and higher healthcare costs. Death from acute respiratory infections, diarrhoeal diseases, measles, AIDS, malaria and tuberculosis accounts for more than 85% of the mortality from infections worldwide (WHO, 2010).

2.7 Terminologies for multidrug-resistant tuberculosis treatment outcomes

The following definitions were recommended by the World Health Organisation (WHO, 2003).

2.7.1 Case of tuberculosis

A patient in whom tuberculosis has been bacteriologically confirmed, or has been diagnosed by a clinician using other methods of diagnosis.

2.7.2 Pulmonary tuberculosis, sputum smear positive

Pulmonary TB, sputum smear positive stands for when two or more initial sputum smear examinations positive for Acid-Fast Bacilli (AFB). It is also when one sputum smear examination positive for AFB plus radiological abnormalities consistent with active pulmonary tuberculosis as determined by a clinician; or when one sputum smear positive for AFB plus sputum culture for M. tuberculosis (www. stoptb.org, 2009).

2.7.3 Pulmonary tuberculosis, sputum smear negative

A case of pulmonary tuberculosis which does not meet the above definitions for smear positive TB, in keeping with good clinical and public health practices, diagnostic criteria should include: at least three sputum specimens negative for AFB; radiographic abnormalities consistent with active pulmonary tuberculosis; no response to a course of broad spectrum antibiotics; and a decision by a clinician to treat with full course of anti-tuberculosis chemotherapy (www. stoptb.org, 2009).

2.7.4 Extra-pulmonary tuberculosis

This is tuberculosis that affects other organs than the lungs, for example, pleural layers, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, meninges and so on. Disease should be based on one culture positive specimen, or histological or strong clinical evidence consistent with active extra-pulmonary tuberculosis, followed by a decision by a clinician to treat with full course of anti-tuberculosis chemotherapy (Abdul Majid W, 2010).

2.8 Terminologies for multidrug-resistant tuberculosis treatment outcomes

The World Health Organisation (WHO, 2003) has classified the treatment outcomes of TB patients into different terms and defined as such (C-S Wang, 2008; Abdul M, 2010).

2.8.1 New

Are patient who has never had treatment for TB, or has taken anti-tuberculosis drugs for less than one month.

2.8.2 Relapse

A patient previously treated for TB who has been declared cured or treatment completed (www.childsurvival.com, 2009).

2.8.3 Treatment failure

A patient who, while on treatment, sputum is smear positive 5 months or later during the course of treatment.

2.8.4 Return after default

These are patients who return with confirmed MDR-TB after interruption of treatment for two months or more.

2.8.5 After failure of first treatment

These are patients who return after the first treatment (Regimen 1) has failed.

2.8.6 After failure of re-treatment

These are patients who return after the re-treatment (Regimen 2) has failed**.**

2.8.7 Transfer-in

Drug-resistant TB patients who have been transferred from another register for treatment of drug-resistant TB, to continue Regimen 4, are referred to as transfer-in patients. The receiving unit should report these patients' treatment outcomes to the transferring unit so that it can report

their outcomes in the context in which they originally started regimen 4 treatment (www.stoptb.org, 2009).

2.8.8 Cure

Patient who sputum smears is negative in the last month of treatment, and on at least one previous occasion.

2.8.9 Treatment completed

Is patient who has completed treatment, but who does not yet meet the criteria to be classified as cured or failures.

2.8.10 Treatment failure

Patient whose sputum smear is positive at five months, or later during treatment**.**

2.8.11 Died

A patient who, has died for any reason(s) during the course of treatment.

2.8.12 Defaulter

Are patients whose treatment was interrupted for 2 consecutive months or more**.**

2.8.13 Transferred/moved out

If a patient is transferred/moved to another facility to continue treatment the date of transfer needs to be entered, as well as where the patient must keep his/her original MDR/ extensive drug-resistant (XDR)-TB Registration Number and the final outcome must be referred back to the original MDR-TB unit.

2.8.14 Treatment success

The sum of patients cured and those who completed treatment.

2.9 Risk factors for tuberculosis

Twin studies in the 1940s showed that susceptibility to TB was heritable. If one of a pair of twins contracted TB, then the other was more likely to get TB if he was identical than if he was not (www.angola.xn, 2007). Diet may also modulate risk. For example, among immigrants in London from the Indian subcontinent, vegetarian Hindu Asians were found to have an 8.5 fold increased risk of tuberculosis, compared to Muslim who ate meat and fish daily. Although a causal link is not proved by this data, this increased risk could be caused by micronutrient deficiencies: possibly iron, vitamin B12 or vitamin D (www.angola.xn, 2007). Diabetes mellitus is also an important risk factor that is growing in importance in developing countries (Strachan et al, 1995-2002). Persons with chronic renal failure and also those on haemodialysis have an increased risk, 10 to 26 times greater than the general population (www. en.Wikipedia.org, 2007). Persons with silicosis have an approximately 30 times greater risk for developing TB (CDC, 2003). Along with overcrowding, poor nutrition may contribute to the strong link observed between tuberculosis and poverty (www.angola.xn, 2007). Further studies have provided more evidence of a link between vitamin D deficiency and an increased risk of contracting tuberculosis (Ustianowski, et al, 2005).

Silica particles irritate the respiratory system, causing immunogenic responses such as phagocytosis, which, as a consequence, results in high lymphatic vessel deposits. Immuno-compromised patients (30 – 40% of AIDS patients in the world also have TB) may have haematologic and reticuloendothelial diseases (www.angola.xn, 2007), such as leukemia and Hodgins' disease, vitamin D deficiency (Nnoaham and Clarke, 2008) and low body weight (Kumar, 2007 and CDC, 2003).

Globally, severe malnutrition common in parts of the developing world causes a large increase in the risk of developing active tuberculosis, due to its damaging effects on the immune system (Schible and Kautmann, 2007 and Lonnroth and Raviglione, 2008). (www.angola.xn, 2007) prisoners, especially in poor countries, are particularly vulnerable to infectious disease such as HIV/AIDS and TB. Therefore, in addition to drug–drug interactions, drug–disease interactions and drug–nutrition/food interactions affecting treatment outcomes in patients, MDR-TB and HIV also affect the TB treatment outcomes (Kim DH et al, 2010).

Other conditions that increase risk include the sharing of needles among intravenous drug users; recent TB infection or a history of inadequately treated TB; chest X-ray suggestive of previous TB, showing fibrotic lesions and nodules; prolonged corticosteroid therapy, and other immunosuppressive therapy. These findings were more recently confirmed by a series of studies in South Africa (Jepson and Banya, 2001; Sepulveda, 1994 and Cobatt, 2010). There are "increasing drug resistance threatens gains of world TB programs, smoking and diabetes are also fuch" (such as HIV which increases risk more than 20-fold, poverty and over-crowding) (Alimuddin, 2011).

2.10 The effects of multidrug-resistant tuberculosis on the human immunodeficiency virus epidemic

The complete link between HIV and MDR-TB is not fully understood. Nevertheless, outbreaks of MDR-TB in HIV-infected patients have been associated with extraordinarily high mortality rate (www.Sahealthinfo.com, 2011). Human immunodeficiency virus patients also tends to have a higher rate of adverse drug reactions, notably peripheral neuropathy, cutaneous reactions, gastrointestinal disturbances, renal toxicity and neuropsychiatric effects (www.Sahealthinfo.com, 2011). Malabsorption of MDR-TB drugs has been reported in patients with HIV-related

enteropathology and dramatic impact of highly active antiretroviral therapy (HAART) on MDR-TB mortality has been reported in co-infected patients (www.Sahealthinfo.com, 2011).

The most potent risk factor for active disease, however, is impaired immunity, the risk increasing to 5 - 10% per year. Although HIV is the most common reason for immune deficiency in South Africa, it should be kept in mind that impaired immunity can also result from malnutrition, congenital syndromes, haematological diseases, endocrine or renal disease and diabetes mellitus. Patients who are receiving immunosuppressive drugs or radiation therapy may also be at increased risk of active disease (www.Sahealthinfo.com, 2011).

2.11 Treatment of multidrug resistant tuberculosis

Treatment for TB uses antibiotics to kill bacteria. Effective TB treatment is difficult, due to the unusual structure and chemical composition of the mycobacterial cell wall, which makes many antibiotics ineffective and hinders the entry of drugs such as kanamycin, Ethionamide, ethambutol, ofloxacin, cycloserine and pyrazinamide (Acharya, 1967; Miglione et al 1966; Acharya and Goldman, 1970 and Brennan et al, 1995). The DOTs strategy of tuberculosis treatment is recommended by the WHO, based on clinical trials done in the 1970s by Tuberculosis Research Centre, Chennai, India. The country in which a person with TB lives can determine what treatment they receive. The reason for this is because MDR-TB is resistant to most first-line medications and the use of second-line anti-tuberculosis medications is necessary to cure the patient. However, the price of these medications is high; thus poor people in the developing world have no or limited access to these treatments (www.anlna.xn, 2007).

The two antibiotics most commonly used are isoniazid and rifampicin. However, instead of the short course of antibiotics typically used to cure other bacterial infections, TB requires much longer periods of treatment (around 6 to 24 months) to entirely eliminate mycobacteria from the

body (www.boerner.net, 2007). Latent TB treatment usually uses a single antibiotic, while active TB disease is best treated with combinations of several antibiotics, to reduce the risk of the bacteria developing antibiotic resistance. People with latent infection are treated to prevent them from progressing to active TB disease later in life.

Drug-resistant tuberculosis is transmitted in the same way as regular TB. Primary resistance occurs in persons infected with a resistant strain of TB. A patient with fully susceptible TB develops secondary resistance (acquired resistance) during TB therapy because of inadequate treatment, not taking the prescribed regimen appropriately, or using low-quality medication (www.boerner.net).

The National TB program recommends first-line anti-tuberculosis treatment which includes rifampicin, isoniazid, ethambutol, pyrazinamide, and streptomycin. In South Africa, the guidelines for MDR-TB treatment have been drawn from the WHO guidelines. There are two regimen approaches, the first one is a standard treatment regimen and the second one is individualized treatment regimen (Weyer, 1999). The standard treatment regimen consists of 6 months intensive phase with five drugs (ofloxacin, kanamycin, ethionamide, cycloserine, and para-aminosalicyclic acid (PAS) (www.Sahealthinfo.com, 2011) followed by a continuous phase of 18-24 months with 4 drugs (ethionamide, ofloxacin, pyrazinamide and cycloserine). The drugs used in the two phases and the dosages of these drugs are given in Table 2.1 and Table 2.2 WHO, 2008; Department of Health, (National, 2006).

Table 2.1: Second line antituberculosis drugs used in the intensive phase (6 months) in South Africa. WHO, 2008; Department of Health, (National, 2006).

Drug	Daily Dosage	
	Average (mg/kg)	Maximum (mg)
Kanamycin	15	1000
Ethionamide	10-20	1000
Pyrazinamide	20-30	1600
Ofloxacin	7.5-15	800
Cycloserine	10-20	1000

Table 2.2: Second line antituberculosis drugs used in the continuation phase (18-24 months) in South Africa

Drug	Daily Dosage	Maximum (mg)
	Average (mg/kg)	
Ethionamide	10-15	1000
Ofloxacin	7.5-15	800
Pyrazinamide	20-30	1500
Drug	Daily Dosage Average (mg/kg	Maximum (mg)
Cycloserine	10-29	1000

Key: mg/kg=milligram/per kilogram

2.12 Classification of drugs for multidrug-resistant tuberculosis treatment in South Africa

The treatment of MDR-TB patients was based on (D. falzon, 2011) drug sensitivity testing of second-line anti-TB drugs. These drugs have bactericidal or bacteriostatic properties. Drugs with moderate bactericidal activity: aminoglycosides, thionamides and, under acid ph conditions, pyrazinamide. Drugs with low bactericidal activity: fluoroquinolones, and drugs with bacteriostatic effect (www.tbrieder.org,) when given at usual dosages in men: ethambutol, cycloserine and para-aminosalicylic acid (PAS).

The ranking of drugs from high to moderate ranking for treatment of MDR-TB is presented in Table 2.3. Drugs should be selected from the higher ranking categories according to bacterial susceptibility. The initial regimen should consist of 5 drugs to which the bacilli has been shown to be susceptible (www.tbrieder.org, 2008). At least 3 of these drugs should not have been administered to the patient previously (that is, 3 months or more). Not more than 1 drug should be chosen from each of these categories in table 2 and all patients should receive an aminoglycoside during the intensive phase of treatment (Weyer et al, 1999).

Apart from the acceptable daily dosages, other criteria should be considered; the effects of the drugs, patient tolerance and acceptability (for example, bulk or flume of drug to be injected, swallowed; taste and pain). This deals with the drugs, formulation, daily dosages, acceptable and main characteristics of antituberculosis drugs available for the treatment of MDR Tuberculosis (www.Sahealthinfo.com, 2011). The drugs are given in Table 2.4 Department of Health, (National, 2006).

Irrespective of whether approach 1 or 2 is followed, certain essential management principles should be adhered to under all circumstances: directly observed therapy throughout the treatment

course is essential. The aim of treatment should be for 18-24 months of treatment always with an initial 6 months of intensive therapy. The continuation period may be shortened provided that 12 months of treatment has been given after sputum conversion demonstrated by 3 consecutive negative monthly cultures. Establishing the HIV status is of clinical importance since HIV sero-positive patients may suffer increased side-effects from antituberculosis drugs. When side effects occur that are not potentially life threatening, every effort should be made to coach patients through with palliation and psychological support. Drugs with known severe side effects should be given in divided doses to improve tolerance. Patients with severe side effects should be treated in hospitals Department of Health, (National, 2009).

Table 2.3: Ranking of drugs from 1 to 5 based on drug susceptibility testing used for treatment of multidrug-resistant tuberculosis in South Africa (WHO, 2008; National Department of Health, 2006).

Rank	Drugs	Average daily dosage	Types of Activity	Peak serum level (μg/ml)
1	Aminoglycosides		Bactericidal	
	(a) Streptomycin	15mg/kg		20-30
	(b) Kanamycin	15mg/kg		5-75
	(c) Amikacin	15mg/kg		10-15
2	Ethionamide	5-10mg/kg	Bactericidal	2-4
3	Pyrazinamide	20-30	Bactericidal	4-8
4	Fluoroquinolones			
	(a) Ofloxacin	7.5-15mg/kg	Weakly bactericidal	7.5-10
	(b) Ciprofloxacin	7.5-15mg/kg	Weakly bactericidal	2.5-5
5	Cycloserine	5-10mg/kg	Bacteriostatic	2-4

The table above shows, for each of the 2nd line anti-TB drugs, the dosage, the antibacterial activity (Cheuk-MT, 2009) and the peak plasma concentrations.

2.13 Pharmacology of the drugs used for the treatment of multidrug-resistant tuberculosis in South Africa

The treatment of MDR-TB involves second line drugs which include an aminoglycoside (amikacin or **kanamycin**) (Mirsaeidt SM, 2005), a fluoroquinolone (**ofloxacin** or ciprofloxacin), a glycopeptides (capreomycin), a thiomide (**ethionamide** or prothionamide), cycloserine, ethambutol, para-aminosalicylic acid (PAS) and pyrazinamide (Tomioka, 2000; WHO, 2003; Perri and Bonora, 2004). Ethambutol and pyrazinamide are still used in patients where resistance to ethambutol and pyrazinamide is not confirmed.

2.13.1 Pyrazinamide

Pyrazinamide is active only against M. tuberculosis including M. bovis. And among the genus, mycobacteria are naturally resistant to pyrazinamide. The drug is largely bacteriostatic, but can be bacteriocidal as and when tuberculosis bacterium actively replicates itself. It was recognized early on that pyrazinamide acts only in an acid environment. The active derivative of pyrazinamide is pyrazinoic acid, which is preferentially accumulated in an acidic pH. Pyrazinamide as such is not active against intracellular growing M. tuberculosis. Only the accumulation of pyrazinoic acid through the action of the amidase pyrazinamidase by susceptible M. tuberculosis leads to its intracellular bactericidal action. The presence of both the pyrazinamidase and pyrazinamide-transport-system in M. tuberculosis has been postulated as prerequisites for drug susceptibility.

Pyrazinamide plays a unique role in shortening the therapy from previously 9–12 months to 6 months, (Zhaung and Mitchson, 2003). It kills a population of semi-dormant tubercle bacilli in acidic pH environments that are not killed by other TB drugs (Mitchison 1995). Pyrazinamide is a paradoxical and unconventional drug (Zhang and Mitchison 2003). Despite its remarkable

sterilizing activity *in vivo (Grosset 1978).* pyrazinamide is not active against *Mycobacterium tuberculosis* under 'normal' culture conditions near neutral pH. Pyrazinamide is only active against *M. tuberculosis* at acid pH (Mc Dermott and Tompsett, 1954), an environment that is produced during active inflammation, and its activity is closely related to the acidity of the medium (Salfinger and Heifets 1988); Even at acid pH (e.g. pH 5.6), pyrazinamide kills *M. tuberculosis* slowly and incompletely with no more than 76% of the bacterial population being killed by 1000 mg/L pyrazinamide (Heifets and Lindholm-L et al, 1990); which is 10–20 times higher than the already high minimum inhibitory concentration (MIC) of 50–100 mg/L (Zhang and Mitchison 2003). It has been shown that the acid pH requirement of pyrazinamide action is the result of increased accumulation of pyrazinoic acid (POA), the active form of pyrazinamide, in the tubercle bacilli at acid pH but not at neutral pH.

After oral intake of 1500 mg of pyrazinamide, a peak level of 25 to 30 mg/L (Salfinger et al, 1988) is achieved after one to one and a half hours. Pyrazinamide is well absorbed orally. Pyrazinamide has one of the best penetrations into cerebrospinal fluid among the anti-tuberculosis medications. It crosses inflamed meninges and is an essential part of the treatment of tuberculous meningitis. Pyrazinamide is metabolized by the liver and the metabolic products are excreted by the kidneys. About four per cent of pyrazinamide is excreted unchanged in urine, and about 30% excreted as pyrazinoic acid. Pyrazinamide metabolism is only slightly influenced by ingestion of antacids, but, with a fatty meal, Tmax (drug peak time) is delayed and Cmax (maximum concentration of pyrazinamide) (www.atcl.ru, 2011) slightly lowered, though these effects are unlikely to bear clinical relevance. Absorption of pyrazinamide is not influenced by food intake. It has dosage of 25–39 mg/kg daily (www.atcl.ru, 2011). It has side effects by having two major adverse drug events of pyrazinamide are hepatotoxicity and interference with

the metabolism of purine. The latter leads to decreased excretion and to accumulation of uric acid, occasionally accompanied by gout-like arthralgia. The arthralgia can be distressing to patients, but is never harmful. The most dangerous side effect of pyrazinamide is hepatotoxicity, which is dose related. The present reduced recommended dose has significantly brought down the incidence of the side effect, hepatitis. In the standard four-drug regimen (isoniazid, rifampicin, pyrazinamide, and ethambutol), pyrazinamide is the most common cause of drug-induced hepatitis. Other side effects include nausea and vomiting, anorexia, sideroblastic anemia, skin rash, urticaria, pruritus and hyperuricaemia (www.atcl.ru, 2011).

The MICs of pyrazinamide (PZA) were determined for Mycobacterium tuberculosis cultivated under different pH conditions in 7H12 liquid medium. Mycobacterial growth was monitored by the radiometric method (BACTEC system; Johnston Laboratories, Inc., Towson, Md.). It has been predicted eightfold difference between the MICs determined at pH 5.5 and those determined at pH 5.95. The highest MICs for 21 susceptible strains were 50.0 micrograms/ml at pH 5.5 and 400 micrograms/ml at pH 5.95. This eightfold difference enabled us to predict MICs at pH 5.5 from the values observed at pH 5.95. The use of 7H12 broth at pH 5.95 simplified the radiometric PZA susceptibility test by avoiding the addition of acid solutions in the course of cultivation, which was required when the test was performed at pH 5.5. An additional benefit of using pH 5.95 instead of pH 5.5 was that all tested strains grew at pH 5.95, while some of them, especially PZA-resistant strains, did not grow at pH 5.5 (Salfinger et al, 1988).

2.13.2 Ethambutol

Ethambutol is a bacteriostatic, antimycobacterial drug prescribed to treat tuberculosis (Yan dapally et al 2008). It is usually given in combination with other tuberculosis drugs, such as isoniazid, rifampicin and pyrazinamide (www.absoluteastronomy.com, 2008). Ethambutol is

bacteriostatic against actively growing TB bacilli. It works by obstructing the formation of cell wall. Mycolic acids attach to the 5'-hydroxyl groups of D-arabinose residues of arabinogalactan and form mycolyl-arabinogalactan-peptidoglycan complex in the cell wall. It disrupts arabinogalactan synthesis by inhibiting the enzyme arabinosyl transferase. Disruption of the arabinogalactan synthesis inhibits the formation of this complex and leads to increased permeability of the cell wall.

It is well-absorbed from GIT (gastrointestinal tract) with protein binding 20 to 30%. It has half-life of 3-4 hrs ("E", 2007) (increased in impaired renal function). It is well distributed, except to CSF, where 10-50% penetrates the inflamed meninges' prolonged in renal failure. Up to 25% is metabolized in the liver. It is well absorbed from the GIT and well distributed in body tissues and fluids, 50% is excreted unchanged in urine (www.Vandvdevelopments). Elimination is mainly by excretion of unchanged drug in the urine (H. Mcilleron, 2009). Ethambutol is used during the intensive phase of standard retreatment regimen. In South Africa, the rate of acquired resistance to ethambutol is high, which has led to replacing with cycloserine. It has adverse effects like optic neuritis (Lim SA, 2006).

The MIC were determined between MICs of ethambutol for both Mycobacterium avium and Mycobacterium tuberculosis strains by using broth dilution (7H12 broth, radiometric method) and agar dilution (7H11 agar) methods. It has been discovered that MIC's to be much lower in liquid than in solid medium. The broth-determined MICs for susceptible M. tuberculosis and most of the M. avium strains were comparable to the levels in blood of patients, being lower than the peak levels. It has been proposed that the MICs, determined radiometrically in 7H12 broth, be considered as tentative criteria for susceptibility testing of M. avium isolates in future clinical trials. The use of these values instead of critical concentrations should also be considered as an

alternative to the conventional susceptibility testing method in chemotherapy of tuberculosis. Ethambutol produced bactericidal effects against both M. tuberculosis and M. avium, and the MIC/MBC ratios were in the same range for both species when MICs and MBCs were tested in 7H12 broth by conventional sampling and plating (www.bionersonline.com, 2010).

2.13.3 Kanamycin

Kanamycin belongs to a group of drugs known as aminoglycosides which includes streptomycin, amikacin and so on. It has bactericidal activity against M. tuberculosis (Ho et al, 1997). Kanamycin is one of the second-line anti-TB agents (www. lrsitbrdruc.in, 2009) used and are clinically efficacious (Katherine MC et al, 2009). The potential for pharmacokinetic drug interactions is low, although additive toxicities may occur. The 16S rRNA from *Escherichia coli* is well studied among the rRNA subunits, and in particular, the interactions of various aminoglycoside antibiotics with the 16S rRNA and their effects on the process of translation of mRNA into polypeptide have been scrutinized (Noller, 1991).

Similar rRNA structures exist in other organisms, such as yeast and *Tetrahymena* (LP. Kotra, 2000). Treatment of rRNA with an aminoglycoside protects several nucleic bases in rRNA from chemical modification, implying that these molecules possess high affinities for certain sites in rRNA. This mode of binding was likened by (Noller, 1991) to that of enzyme inhibitors, which usually bind to the active sites of enzymes and interfere with their activities. Different classes of aminoglycoside antibiotics bind to different sites on the rRNA, depending on the structural complementarity between the two. For example, neomycin, paranomycin, gentamicin, and kanamycin are believed to bind to the A-site on the 16S rRNA in *E. coli* in a similar fashion and were shown to protect bases A1408 and G1494 in chemical foot printing experiments (www, intl-aac.usm.org, 2011). Four bases, A1408, A1492, A1493, and G1494, in the rRNA A-site

interact with tRNA, although with different affinities. The binding of the aforementioned aminoglycosides to the A-site in the decoding region (i.e., the site of codon and anticodon recognition) interferes with the accurate recognition of cognate tRNA by rRNA during translation (Noller, 1991). These interactions are also thought to interfere with the translocation of tRNA from the A-site to the peptidyl-tRNA site (P-site).

Aminoglycosides achieve high concentrations in bone, pleural, synovial and peritoneal fluids and show poor CSF penetration (except amikacin in children with meningitis (Katherine MC et al, 2009)). It is widely used in this study because of less toxicity when compare with other group. Kanamycin should not be administered with NRTI and tenofovir because it causes renal dysfunction. Aminoglycosides are ototoxic (Brummet et al, 1989).

TEM-1 β-lactamase is a highly efficient enzyme that is involved in bacterial resistance against β-lactam antibiotics such as penicillin. It is also a robust scaffold protein which can be engineered by molecular-evolution techniques to bind a variety of targets. One such β-lactamase variant (BlaKr) has been constructed to bind kanamycin (kan) and other aminoglycoside antibiotics, which are neither substrates nor ligands of native β-lactamases. In addition to recognizing kan, BlaKr activity is up-regulated by its binding via an activation mechanism which is not yet understood at the molecular level. In order to fill this gap, determination of the structure of the BlaKr-kan complex was embarked upon. A crystallization condition for BlaKr-kan was identified using high-throughput screening, and crystal growth was further optimized using streak-seeding and hanging-drop methods. The crystals belonged to the orthorhombic space group (Karen Van dewater et al, 2011) P2 (1)2(1)2(1), with unit-cell parameters a = 47.01, b = 72.33, c = 74.62 Å, and diffracted to 1.67 Å resolution using synchrotron radiation. The X-ray structure of BlaKr with its ligand kanamycin should provide the molecular-level details

necessary for understanding the activation mechanism of the engineered enzyme (Karen et al, 2011).

Kanamycin is given intravenously or intramuscularly at a dose of 15mg/kg per min in two or three divided doses, with a dose reduction in renal impairment. It is also excreted rapidly by glomerular filtration with a serum half-life of about 4hr. Therapeutic drug monitoring may be useful, aiming for Cmax of 15-30μg/ml and Ctrough (Katherine MC et al, 2009) (area under the momentum curve from zero to infinity) of less than 10μg/ml (A. Tampuz, 2008). Previous studies have demonstrated that exposure to sublethal levels of kanamycin antibiotic increased the capsular polysaccharide production by *Escherichia coli* B23 cells. The capsular polysaccharides were associated with resistance against antibiotics. This study attempted to investigate whether an interaction exists between the antibiotic and either isolated capsular polysaccharide or isolated soluble polysaccharide to assess a direct relationship between antibiotic insensitivity and capsular polysaccharides. Three types of polysaccharides including capsular polysaccharides obtained from *Escherichia coli* B23 grown with or without kanamycin pre-treatment and soluble capsular polysaccharide secreted by *Escherichia coli* K1-K12 hybrid EV36 were mixed with kanamycin and these samples were used for the MIC assay, kanamycin sensitivity test and the Kirby-Bauer (KB) assay.

It was found that the addition of polysaccharides did not have a significant effect on either the growth of cells or the MIC of kanamycin while all three types of polysaccharides provided protection against low concentrations of kanamycin observed in the kanamycin sensitivity tests. In the KB assay, filter disks containing 20 μg/ml and 10 μg/ml of kanamycin near the filter disks of the capsular polysaccharide from cells with kanamycin pre-treatment showed smaller and inward distorted shaped inhibition zones, while the disks of the same kanamycin concentrations

near the disks of the capsular polysaccharide from cells grown without kanamycin pre-treatment had normal zones. The difference in the degree of protection observed against exposure to low concentrations of kanamycin between the two types of capsular polysaccharides could not be determined (Park et al, 2007).

2.13.4 Ethionamide

Ethionamide belongs to a group of drugs known as thioamides. Ethionamide is administered orally with a bioavailability of over 90% (Katherine MC et al, 2009). It is started at 250mg daily. The dose is titrated up to 15-20mg/kg per day (maximum 1 g daily) if gastric irritation permits. It is usually given once daily but divided doses can lessen gastrointestinal symptoms. It reaches high concentration throughout the body including in CSF; and 10-30% is protein bound (Katherine MC et al, 2009).

The side effects associated with tuberculosis therapy bring with them the risk of noncompliance and subsequent drug resistance. Increasing the therapeutic index of antituberculosis drugs should thus improve treatment effectiveness. Several antituberculosis compounds require *in situ* metabolic activation to become inhibitory. Various thiocarbamide-containing drugs, including ethionamide, are activated by the mycobacterial monooxygenase EthA, the production of which is controlled by the transcriptional repressor EthR. Here we identify drug-like inhibitors of EthR that boost the bioactivation of ethionamide. Compounds designed and screened for their capacity to inhibit EthR-DNA interaction were co-crystallized with EthR. We exploited the three-dimensional structures of the complexes for the synthesis of improved analogs that boosted the ethionamide potency in culture more than tenfold. In *Mycobacterium tuberculosis*–infected mice, one of these analogs, BDM31343, enabled a substantially reduced dose of ethionamide to lessen the mycobacterial load as efficiently as the conventional higher-dose treatment. This provides

proof of concept that inhibiting EthR improves the therapeutic index of thiocarbamide derivatives, which should prompt reconsideration of their use as first-line drugs (www. lib.bioinfo.pl, 1984).

Ethionamide is metabolized in the liver to seven metabolites, some of which are biologically active. Metabolism occurs by sulphoxidation, desulphuration and deamination, followed by methylation (Jenner et al, 1984, Berning et al, 1998). The involvement of CYP450 enzymes means that drug interactions are likely. Less than 1% is excreted unchanged by the kidneys.

Therapeutic drug monitoring is recommended in hepatic impairment. A high incidence of hepatotoxicity has been reported when Ethionamide has been used with other hepatotoxicity drugs such as rifampicin (Katherine MC et al, 2009). Blood tests were taken during treatment to detect side effects of ethionamide and rifampicin on the liver of the patients. Caution should therefore be exercised if co-prescribed antiretroviral which have been strongly associated with abnormal liver function, including the NNRTIs efavirenz and nevirapine (Bruck et al, 2008). Blood tests were also taken for toxicity of drugs on the patients.

Ethionamide can cause depression, anxiety and psychosis. It may therefore not be advisable to start efavirenz at the same time, since these side effects are commonly experienced in the first weeks of efavirenz therapy (Katherine MC et al, 2009). Blood tests were also taken for toxicity. A psychotic reaction has been reported with the combination of ethionamide and excess alcohol (Lansdown et al, 1967). It has been reported that patients experience antabuse when alcohol is taken with alcohol. Patients should therefore be counseled to avoid excess alcohol. Neurotoxic effects can be prevented by giving pyridoxine 200mg daily.

2.13.5 Ofloxacin

Ofloxacin belongs to a group of drugs known as fluoroquinoles (FQNs). There are broad-spectrum antibiotics and tuberculocidal. It demonstrated the activity of fluoroquinoles (FQNs) within human macrophage, where many tubercle bacilli reside (Katherine MC et al, 2009). Their mechanism of action is inhibition of bacterial topo-isomerase IV and DNA gyrase enzymes required for bacterial DNA replication, transcription, repair and recombination. There is no substantial loss from first-pass metabolism. Oral bioavailability of 98% and with a peak serum concentration reached within 1-2 hours (Ziganshina et al, 2005).

The absorption of all FQN is reduced by buffered drugs including older formulations of didanosine, and they should be taken 2 hr before or 6 hr after any buffered drugs. They can also be administered intravenously. Binding to plasma proteins is moderate 20-50% (Katherine MC, 2009). It is included because of its high protein-binding but not high enough to have significant interactions with protein binding of other drugs.

Levofloxacin is the optical S- (-) isomer of ofloxacin and is twice as potent in vitro (Katherine MC et al, 2009). Ofloxacin is widely distributed in body fluids, and penetrates CSF better than levofloxacin. Dose reduction is required in renal failure and caution should be exercised when co-prescribed drugs associated with renal dysfunction including tenofovir. Only 4-8% of the dose is recovered from the feces (Katherine MC et al, 2009). Many in *vitro* studies reported that ofloxacin has relatively potent activity against MTB and other species of mycobacteria for example, Mycobacterium kansasii and Mycobacterium avium complex (Vacher et al, 1999). Ofloxacin is active against drug resistant Mycobacterium tuberculosis with 2μg/ml as a minimum inhibitory concentration for 90% (MIC90) of tested stains, other studies demonstrated that activity of fluoroquinoles including ofloxacin within human macrophage, where many

tubercule bacilli reside (Wise and Honeybourne, 1999, Ruiz-Serrano et al, 2000). In addition, the antituberculosis effects of low, non-bactericidal concentration of ofloxacin within the macrophage is enhanced by the presence of PZA, which is important for the use of these agents in TB preventative combination regimens (Jacobs, 1999. Berning, 2001).

2.13.6 Cycloserine / Terizidone

Cycloserine or terizadone is obtained by combining two molecules of cycloserine and one molecule of terephtalaldehyde and is a broad spectrum antibiotic which greatly improved the disadvantages associated with Cycloserine. Terizidone has potent and extended antimycobacterial activities, and exerts remarkable effects against not only strains causing pulmonary tuberculosis or urinary tract infections but also strains which have become resistant to existing antimycobacterial drugs (www. japi.org, 2009).

Its mode of action is similar to cycloserine that is, it acts by inhibiting cell wall synthesis by competitively inhibiting two enzymes, L-alanine racemase and D-alanine ligase, thereby impairing peptidoglycan formation necessary for bacterial cell wall synthesis. Although, being broad spectrum, the molecule in principle active against other bacteria as well, terizidone is not recommended for use in the treatment of infections other than tuberculosis (www, japi.org, 2009). Terizadone is readily and almost completely absorbed from the GIT. Plasma protein binding is less than 20%. It is widely distributed throughout body fluids including CSF (Katherine MC et al, 2009).

It is probably advisable not to start efavirenz concurrently because CNS side effects may be worsened by concomitant use of psychoactive drugs or alcohol.

It is readily absorbed widely distributed to tissues and fluids including CSF (H. Mcillron, 2009). Cycloserine is bacteriostatic in the usual dosage. It is a valuable companion drug to prevent

resistance to other second-line drugs, since it does not share cross-resistance with other active TB drugs. It has a high incidence of side effects. Terizidone is obtained by combining two molecules of cycloserine and one molecule of terephtalaldehyde ans is a broad spectrum antibiotics which greatly improved the disadvantages associated with cycloserine. Its mode of action is similar to cycloserine that is, it acts by inhibiting cell wall synthesis by competitively inhibiting two enzymes, L-alanine racemase and D- alanine ligase, thereby impairing peptidoglycan formation necessary for bacterial cell wall synthesis (www. japi.org, 2009).

The MICs of Terizidone for susceptible strains are 4-130 mg/ml. Terizidone is completely and rapidly absorbed after oral administration. Maximum concentration in blood are achieved in 2 to 4 hours. It was noted that the blood concentration of terizidone was higher at all time intervals than the concentration attained in the blood after the same doses of Cycloserine. Excretion in urine is quicker in the young ones. Its concentration in the urine after 30 hr administration sufficiently exceeded its minimum inhibitory concentration. This justifies its use in the treatment of urogenital TB. It was found that the increase in the dose does not cause a proportional increase in the concentration of the drug in the blood. It is well distributed in all body fluids and tissues.

The half-life of terizidone was significantly greater than that of cycloserine with doses of 250 mg and 500 mg. Also, it was significantly higher in the elderly than the young patients. The molecule does not have cumulative toxicity and hence better tolerability (www.japi.org, 2009).

2.13.7 Para-aminosalicyclic acid

Para-aminosalicyclic acid (also known as aminosalicyclic acid) is bacteriostatic and active against M. tuberculosis. Other mycobacteria are usually resistant. It prevents plate biosynthesis in sensitive organisms. It has a relatively weak action compared with other anti-TB drugs and resistance may develop quickly if it is used alone, however it is still sometimes used in multi-drug resistant tuberculosis in combination with other anti-TB drugs (Mathas D, 2009). It is readily absorbed (>90%); peak plasma concentration after oral dosing: 1-4 hours. Time to peak, serum: 6 hours. It diffuses into most body tissues and fluids (including breast milk) except CSF (unless meninges inflamed). Plasma protein binding: sodium salt: 15%; acid: 50-70%. It is metabolised both intestinally and hepatically (>50%) mainly by acetylation. It is excreted via urine: ≥80% dose within 24 hr (50% as acetylated metabolite). Half-life: approx 1 hr (reduced in renal impairment) (www.mims.com/USA, 2012).

The MICs of all the clinical strains and spontaneous mutants were determined by the agar dilution method and BacT/Alert 3D system (bioMérieux, France). Briefly, samples (10^7 CFU/ml; diluted 1:100; 100 µl plated) were plated simultaneously on 7H11 plates containing 0, 16, 32, 64, and 128 µg/ml of PAS (D. Mathas, 2009), and the colony formation was tabulated. Likewise, MICs were determined using the BacT/Alert 3D system as recommended by the supplier. Finally, bacillary growth was monitored spectrophotometrically (optical density at 600 nm) every 72 hours for 32 consecutive days in triplicate. Growth curves were determined for four to six strains per group, including clinical isolates (n = 6), spontaneous mutants with an early stop codon in the *thyA* gene (n = 2) or with other mutations within the *thyA* gene (n = 6), and PAS^r isolates including wild-type genes in the folate and pyrimidine biosynthesis pathway (n = 6).

Growth curves were done on 7H9 broth in the presence of 0, 16, 32, 64, and 128 μg/ml of PAS. (www. ncbi.nlm.gov, 2011).

Para-aminosalicyclic acid does not penetrate CSF unless the meninges are inflamed, in which case CSF concentration reach 10-50% of plasma concentrations (Katherine MC et al, 2008). It completely blocks absorption of B12 and can induce a malabsorption syndrome (Katherine MC et al, 2009). It is one of the side effects of PAS, yet is being used.

Para-aminosalicyclic acid is rapidly acetylated in the liver and then excreted by glomerular filtration (Katherine MC et al, 2009). It is 80% excreted into urine, with 50% excreted in the inactive acetylated form. Approximately 50-70% of PAS is protein bound and the plasma half-life is 45-60 min (Katherine MC et al, 2009). No interactions with antiretroviral agents have been described.

2.14 The South African antiretroviral treatment guidelines

The specific objectives for using ART is to prioritise ARVs for patients with CD4 counts < 200 cells/mm3 or with severe HIV disease irrespective of CD4 count. The use of ARVs is for patients co-infected with TB/HIV. The use of ARVs is also for pregnant women with CD4 ≤ 350 cells/mm3 for lifelong ART and CD4 > 350 cells/mm3 for prophylaxis. The use of stavudine must be reduced; to enable nurses to initiate ARVs for treatment and prevention and to enable primary health care (PHC) facilities to initiate, manage, monitor and refer patients for (Lawrence L, 2011) further management. The drugs used are given in Table 2.4 and Table 2.5 Department of Health (National, 2010).

However, there is an amendment to the South African Antiretroviral Treatment Guidelines 2010. The Western Cape Department of Health (WCDOH) has started giving ARVs to all HIV positive patients, with CD4 ≤ 350cells/ml Department of Health (Provincial, 2011).

2.15 Pharmacology of first-line antiretroviral drugs

The first-line antiretroviral drugs that were used during the course of study are: (1) Stavudine. (2) Lamivudine. (3) Efavirenz. However, Stavudine has been replaced with Tenofovir.

2.15.1 Stavudine

It has been reported that stavudine has high renal toxicity and more side effects than tenofovir; while tenofovir is associated with fewer side –effects compared to AZT (Bygrave H et al, 2011). Stavudine is a nucleoside analogue of thymidine, is phosphorylated by cellular kinases to the active metabolite Stavudine triphosphate. Stavudine triophosphate inhibits the activity of HIV-1 reverse transcriptase (RT) by competing with the natural substrate thymidine triophosphate (Ki=0.0083 to 0.032μm) and by causing DNA chain termination following its incorporation into viral DNA (www. packageinserts.bms.com, 2011). Stavudine triophophate inhibits cellular DNA polymerases β and γ and markedly reduces the synthesis of mitochondrial DNA. The cell culture antiviral activity of Stavudine was measured in peripheral blood mononuclear cells, and lymphoblastoid cell lines. The concentration of drug necessary to inhibit HIV-1 replication by 50% (EC50) ranged from 0.009 to 4μM against laboratory and clinical isolated of HIV-1. In cell culture, Stavudine exhibited additive to antagonistic activity in combination with zidovudine. Stavudine in combination with either abacavir, Didanosine, tenofirvir, or zalcitabine exhibited additive to synergistic anti-HIV-1 activity (www. packageinserts.bms.com, 2011). The relationships between cell culture susceptibility to stavudine have been selected in cell culture (strain-specific) and were also obtained from patients treated with stavudine. Phenotypic analysis of HIV-1 isolates from 61 patients receiving prolonged (6-29 months) stavudine monotherapy showed that post-therapy isolates from patients exhibited EC50 values more the 4-fold (range 7-16 fold) higher than average pre-treatment susceptibility of baseline isolates. The genetic basis

for Stavudine susceptibility changes has not been identified. Cross-resistance among HIV-1 reverse transcriptase inhibitors has been observed. Several studies have demonstrated that prolonged Stavudine treatment can select and or maintain mutations associated with zidovudine resistance (www. packageinserts.bms.com, 2011). The pharmacokinetics of Stavudine has been evaluated in HIV-infected adult and pediatric patients. Peak plasma concentration (Cmax) and area under the plasma concentration-time curve (AUC) increased in proportion to dose after both single and multiple doses ranging from 0.03 to 4 mg/kg. There was no significant accumulation of Stavudine with repeated administration every 6, 8, or 12 hours. Following oral administration, Stavudine is rapidly absorbed, with peak plasma concentrations occurring within 1 hour after dosing. The systemic exposure to Stavudine is the same following administration as capsules or solution. Binding of Stavudine distributes equally between red blood cells and plasma. Metabolism plays a limited role in the clearance of stavudine (www.squibb.com, 2011). Unchanged Stavudine was the major drug-related component circulating in plasma after an 80mg dose of 14C-stavudine, while metabolites constituted minor components of the circulating radioactivity. Minor metabolites include oxidized Stavudine, glucuronide conjugates of Stavudine and its oxidized metabolite, and an N-acetylcysteine conjugate of the ribose after glycosidic cleavage, suggesting that thymine is also a metabolite of Stavudine.

Following an ingestion of 80 mg radioactive, the result showed 73.7% of urine and 62% of feces respectively. The mean elimination half-life is approximately 2.3 hours following single doses. Mean renal clearance of the parent compound is approximately 272ml/min, accounting for approximately 67% of the apparent oral clearance. In HIV-infected patients, renal elimination of unchanged drug accounts for about 40% of the overall (www.packageinserts.bms.com, 2011).

Stavudine does not inhibit major cytochrome P450 isoforms CYP1A2, CYP2C9, CY2C19, CYP2D6, and CYP3A4; therefore, it is unlikely that clinically significant drug-interactions will occur with drugs metabolized through pathways. Stavudine is not protein-bound; it is not expected to affect the pharmacokinetics protein-bound drugs.

However, fatal lactic acidosis has been occurred in patients treated with zerit® combination with other antiretroviral agents. If motor weakness develops, zerit® should be discontinued. Zerit® therapy has also been associated with peripheral sensory neuropathy, which can be severe, is dose related. And occurs more frequently in patients being treated with other drugs that have been associated with neuropathy (including Didanosine), in patients with advanced HIV infection, or in patients who have previously experienced peripheral neuropathy (www.squibb.com, 2011). It has been suggested that stavudine should be stop if any of these signs of an allergic reaction occurs such as: hives, difficulty breathing; swelling of face, lips, tongue or throat. Other serious side effects includes liver damage- nausea, stomach pain, low fever, loss of appetite, dark urine, clay-colored stools, jaundice (yellowing of the skin or eyes); lactic acidosis-muscle pain or weakness, numb or cold feeling in arms and legs, trouble breathing, nausea with stavudine (www. Kids.sutterhealth.org, 2011).

2.15.2 Lamivudine

Lamivudine is an analogue of cytidine. It can inhibit both types (1 and 2) of HIV reverse transcriptase and also the reverse transcriptase of hepatitis B. It is phosphorylated to active metabolites that compete for incorporation into viral DNA. They inhibit the HIV reverse transcriptase enzyme competitively and act as a chain terminator of DNA synthesis. The lack of a 3'-OH group in the incorporated nucleoside analogue prevents the formation of the 5' to 3' phosphodiester linkage essential for DNA chain elongation, and therefore, the viral DNA growth

is terminated. Lamivudine is administered orally, and it is rapidly absorbed with a bio-availability of over 80% (www.Vand developments, 2011).

Some research suggests that lamivudine can cross the blood-brain barrier. Lamivudine is often given in combination with zidovudine, with which it is highly synergistic. Lamivudine treatment has been shown to restore zidovudine sensitivity of previously resistant HIV. Lamivudine showed no evidence of carcinogenicity or mutagenicity in *vivo* studies in mice and rats at doses from 10 to 58 times those used in humans (www.Vandvdevelopments, 2011). Lamivudine tablets, capsules, and oral solution dosage forms are bioequivalent. Protein binding is less than 36%. Approximately 5% is recovered in the urine as a transsulfoxide metabolite and 70% is excreted unchanged in the urine. Active tubular secretion appears to play a role in the clearance. The most common side effects are nausea, diarrhoea and headache. Peripheral neuropathy, myalgias, pancreatitis, and lactic acidosis occur infrequently. In drug interactions with Zalcitabine, lamivudine and zalcitabine may result in mutual inhibition of intracellular Phosphorylation (www.antimicrobe.org/drugpopup/Lamivudine.pdf, 2011). Stop using lamivudine and get emergency medical help if you have any of these signs of an allergic reaction: hives; difficulty breathing; swelling of your face, lips, tongue, or throat. Other serious side effects: liver damage - nausea, stomach pain, low fever, loss of appetite, dark urine, clay-colored stools, jaundice (yellowing of the skin or eyes); lactic acidosis - muscle pain or weakness, numb or cold feeling in your arms and legs, trouble breathing, nausea with vomiting, and fast or uneven heart rate; pancreatitis - severe pain in your upper stomach spreading to your back, nausea and vomiting, fast heart rate; peripheral neuropathy - numbness, tingling, or pain in your hands or feet; easy bruising or bleeding, unusual weakness, pale skin; white patches or

sores inside your mouth or on your lips; fever, chills, body aches, flu symptoms; or any other signs of new infection. Less serious side effects may include: cough; sleep problems (insomnia), strange dreams; nausea, vomiting, diarrhea; joint or muscle pain; dizziness, headache, tired feeling; or changes in the shape or location of body fat (especially in your arms, legs, face, neck, breasts, and trunk). (www. Kids.sufferhealth.org, 2011).

2.15.3 Efavirenz®

Efavirenz® is a selective non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immunodeficiency virus type 1 HIV-1. Efavirenz ® diffuses into the cell where it binds adjacent to the active site of reverse transcriptase. This produces a conformational change in the enzyme and inhibits its function. Efavirenz ® is a noncompetitive inhibitor of HIV-1 reverse transcriptase (RT) with respect to template, primer or nucleoside triphosphates, with a small component of competitive inhibition. HIV-2 RT and human cellular DNA polymerases alpha, beta, gamma and delta are not inhibited by concentrations of efavirenz® (www.home.intekon.com, 2011).

Peak efavirenz® plasma concentrations of 1, 6 –9, 1 microM were attained by 5 hours following single oral doses of 100 mg to 1600 mg administered to uninfected volunteers. Dose-related increases in Cmax and AUC were seen for doses up to 1600 mg; the increases were less than proportional suggesting diminished absorption at higher doses. Steady state plasma concentrations are reached in 6 –7 days. Efavirenz ® is very highly bound (approximately 99,5 –99,75%) to human plasma proteins, predominantly albumin. In HIV-1 infected patients who received efavirenz® 200 to 600 mg once daily for at least one month, cerebrospinal fluid concentrations ranged from 0,26 to 1,19% (mean 0,69%) of the corresponding plasma

concentration. This proportion is approximately three-fold higher than the nonprotein-bound (free) fraction of efavirenz in plasma. Efavirenz ® is principally metabolised by the cytochrome P450 system to hydroxylated metabolites with subsequent glucuronidation of these hydroxylated metabolites. These metabolites are inactive against HIV-1. CYP3A4 and CYP2B6 are the major isozymes responsible for efavirenz® metabolism. Efavirenz ® has been shown to induce P450 enzymes, resulting in the induction of its own metabolism. Efavirenz ® has a long terminal half-life of 52 to 76 hours after single doses, and 40 –55 hours after multiple doses. Approximately 14 –34% of a radiolabelled dose of efavirenz was recovered in the urine and 16–61% was recovered in faeces, mainly in the form of metabolites.

In drug interactions, aspen efavirenz ® is an inducer of CYP3A4. Other compounds that are substrates of CYP3A4 may have decreased plasma concentrations when co-administered with aspen efavirenz ®. When indinavir (800 mg every 8 hours) was given with aspen efavirenz ® (200 mg every 24 hours), the indinavir AUC and Ctrough were decreased by approximately 31% and 16% respectively, as a result of enzyme induction. Therefore, the dose of indinavir should be increased from 800 mg to 1000 mg every 8 hours when aspen efavirenz ® and indinavir are co-administered. No adjustment of the dose of aspen efavirenz ® is necessary when given with indinavir. When aspen efavirenz ® 600 mg (given once daily at bedtime) and ritonavir 500 mg (given every 12 hours) were studied in infected volunteers, the combination was not well tolerated and was associated with a higher frequency of adverse clinical experiences (for example, dizziness, nausea, paraesthesia and elevated liver enzymes occurred). When saquinavir (1,200 mg given 3 times a day, soft capsule formulation) was given with aspen efavirenz ® the saquinavir AUC and Cmax were decreased by 62% and 50% respectively. Use of aspen efavirenz ® in combination

with saquinavir as the sole PI is not recommended. Rifampicin reduced aspen efavirenz AUC by 26% and Cmax by 20% in 12 uninfected volunteers. The dose of efavirenz® must be increased to 800 mg/day when taken with rifampicin. No dose adjustment of rifampicin is recommended when given with aspen efavirenz®. The clinical significance of these change in clarithromycin plasma levels is not known. Co-administration of aspen efavirenz ® with methadone, in HIV-infected IV drug users, resulted in decreased plasma levels of methadone and signs of opiate withdrawal (www.adcock.co.za, 2011).

Patients on aspen efavirenz ® should not concomitantly use products containing St. John's Wort (Hypericum perforatum) since it may be expected to result in reduced plasma concentrations of aspen efavirenz ®. This effect is due to an induction of CYP3A4 and may result in loss of therapeutic effect and development of resistance. Aspen efavirenz ® does not bind to cannabinoid receptors (www.home.intekon.com, 2011).

The common side effects include abnormal dreams; diarrhea; dizziness; drowsiness; headache; nausea; tiredness; trouble concentrating; trouble sleeping; upset stomach; vomiting.

The following side effects are founds with patients when using efavirenz which include: Severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); burning, numbness, or tingling; change in personality; confusion; cough; delusions; fainting; fever, chills, or persistent sore throat; hallucinations; irregular heartbeat; memory loss; mental, mood, or behavior changes (for example, abnormal thoughts, agitation, aggression, anxiety, depression, nervousness, paranoia); mouth sores; rash with or without fever; red, swollen, blistered, or peeling skin; seizures; severe or persistent tiredness or weakness; severe stomach pain; shortness of breath; suicidal thoughts or behaviors; symptoms of

liver problems (for example, dark urine, loss of appetite, pale stools, yellowing of the skin or eyes); vision changes (www.drugs.com/sfx/efavirenz. 2011).

2.16 Antituberculosis agents side effects

Antiretrovirals (ARVs) are important for the treatment of HIV (+) patients and second-line anti-TB drugs are used for MDR- TB in patients. The restoration of immune function is necessary for a successful therapeutic response (Gandhi et al, 2010). Studies show that there were drug interactions between anti-TB and HIV drugs. These drugs have additive toxicities of second-line anti-TB drugs (SLDs) and ARV therapy on the patients. The cumulative toxicities of these drugs include peripheral neuropathy with nucleoside reverse transcriptase inhibitors and aminoglycosides, neuropsychiatric toxicity with efavirenz and cycloserine and gastrointestinal intolerance with many ARV and SLDs. The drugs are given in Table 2.4 (Fried land, 2007 and WHO, 2006). The effects drug interactions in this study show poor MDR-TB treatment outcomes.

Table 2.4: Antituberculosis agents' side effects

(Friedland, 2007 and WHO, 2006).

Diseases	Drugs interactions
Hepatitis	Interactions with following drugs: Nevirapine, ritonavir- boosted protease, Isoniazid, rifampicin and Pyrazinamide show hypersensitivity reaction as side effects
Gastrointestinal distress	All antiretroviral with Ethionamide, Fluoroquinolones, Para-aminosalicyclic acid causes gastrointestinal intolerance but less common with Lamivudine and emtricitabine.
Nephrotoxicity	Tenofovir (renal tubular dysfunction, idinavir-ritonavir, streptomycin-Aminoglycosides (nephrotoxic TB agents carry particular risk of potentiating lactic acidosis common to the NRTIs used in antiretroviral roll-out regimens).
Neuropsychiatric disorder	Efavirenz **(**insomnia, drowsiness, vivid dreams**),** cycloserine **(**headaches, tremor, seizure), terizadone (mainly headache and seizures)
Peripheral Neuropathy	The side effects of the following: stavudine, Didanosine, cycloserine, streptomycin, aminoglycosides, isoniazid causes nephrotoxicity.

Key:

TB= Tuberculosis; NRTIs= Nucleoside reverse transcriptase inhibitors.

2.17 Interaction between antituberculosis and antiretroviral drugs

Multidrug-resistant tuberculosis treatment is complicated and frequently associated with treatment failure, relapse and high incidences of adverse drug reactions, which lead to prolonged illness, disability and death as a consequence (Cohn, 1995; Flament-Saillour et al, 1999). Treatment of MDR-TB in patients co-infected with HIV (Gerald F, 2007) is associated with poor outcomes and high mortality rates of 72-89% (Cohn, 1995). Deaths during treatment caused by MDR-TB infection or by other HIV-related diseases are more frequent, particularly, in the advanced HIV-infection stages (www.stoptb.com 2003). This could be due to IRIS before the commencement of ARVs. It could also be caused by drug interaction between ARVs and anti-TB drugs. However, starting treatment early may increase the survival time in HIV patient co-infected with MDR-TB (Martins B, 2007). By contrast, HIV-negative patients with MDR-TB (Jacobs RF, 1996) have good response to appropriate MDR-TB chemotherapy regimens with 64% outcomes of completing the course of therapy and no relapse (Telzak et al, 1995; Kwon et al. 2008).

Immuno-compromised patients are at a high risk of drug (CE. French, 2008)-drug interactions during their TB treatment. This is due to lowered immune system. Outbreaks of multidrug-resistant tuberculosis among HIV-infected patients have been documented since the 1980s. Recently, an outbreak of highly-lethal multidrug-resistant tuberculosis was discovered in South Africa, primarily involving HIV-infected patients (www. pubichealth.cacountry.gov, 2008). The link between this outbreak and drug interactions was due to inadequate TB treatment and poor drug sensitivity test in these patients. Prompt initiation of antiretroviral therapy may be one way to decrease the alarmingly high death rate among HIV-infected patients with multidrug-resistant tuberculosis. By doing so, would reduce morbidity and mortality.

Most of the "second-line drugs" (fluoroquinolone antibiotics, ethionamide, cycloserine, kanamycin, amikacin, capreomycin, para-amino salicylate) were developed and approved nearly 40 years ago, prior to the development of modern laboratory techniques to determine pathways of drug metabolism. Furthermore, there are no published studies of possible drug-drug interactions (Weyer, 2005); between second-line antituberculosis drugs and antiretroviral drugs. Based on the existing, albeit incomplete, knowledge of the metabolism of the second-line drugs, only ethionamide has a significant possibility of an interaction with antiretroviral drugs (www.cdc.gov, 2003). Ethionamide is thought to be metabolized by the CYP450 system, though it is not known which of the CYP isozymes are responsible. However, there are drug interactions with other second line anti-TB drugs (www.cdph.ca.gov, 2003).

Stavudine ® and ethionamide causes peripheral neuropathy. Tenofovir and protease inhibitors (Lopinavir-ritonavir) causes increased tenofovir plasma levels. Efavirenz ® and protease inhibitors cause decreased efavirenz plasma levels. Ethambutol and pyrazinamide causes potential elevation of urate by pyrazinamide. Amikacin causes increased risk of oto- or nephrontoxicity. Ofloxacin inhibit hepatic microsomal enzymes. Ethionamide and cycloserine and terizadone causes increased risk of CNS (central nervous system) toxicity. Zidovudine has no drug interaction (South African Medicines Formulary, 2010). In conclusion, the proper management of MDR-TB and HIV patients needs to be understood for (Kent S, 2010) better treatment outcomes. Multidrug-resistant tuberculosis treatment is complicated and (Charles DW, 2007) frequently associated with treatment failure, relapse and high incidences of adverse drug reactions, which lead to prolonged illness, disability and death as a consequence. Some hypothesis reports suggest adverse effects of HIV, like diabetes mellitus on the treatment outcome of TB patients with an increased rate of failures, deaths, and defaults (www.japi.org,

2009). Prompt initiation of antiretroviral therapy may be one way to decrease the alarmingly high death rate among HIV-infected patients with multidrug-resistant tuberculosis (www. publichealth.cacountry.gov, 2008).

2.18 Experimental hypothesis

1 There is a difference in the cure rates of MDR-TB between HIV (-) and HIV (+) patients at Brewelskloof hospital.

2. The cure rate among HIV (+) patients will be affected by the use of ART drugs.

2.19 Null hypotheses

1. There is no difference between the cure rate of MDR-TB for HIV (+) patients and HIV (-) patients at Brewelskloof hospital.

2. The cure rate among HIV (+) patients will not be affected by the use of ART drugs.

2.20 Research questions

1. What are the treatment outcomes of MDR-TB patients and patients co-infected with MDR-TB and HIV?

2. What are the reasons for the low cure rate of MDR-TB in patients who are HIV negative?

3. Does HIV infection influence MDR-TB treatment outcomes?

4. Do antiretroviral drugs have any impact on MDR-TB treatment outcomes among HIV (+) patients?

2.21 Objectives of the study

The objectives of the study are:

1. To find out whether HIV infection has an impact on the following treatment outcomes in MDR-TB patients: cure rate and treatment failure at Brewelskloof hospital.

2. To investigate any drug interactions between ARVs and second line anti-TB drugs which could have an impact on the following MDR-TB treatment outcomes : cure rate and treatment failure at Brewelskloof hospital.

CHAPTER THREE

METHODS

3.1 Study design

The study was designed as a retrospective case control study comparing MDR-TB treatment outcomes in HIV – positive patients (experimental group) and HIV-negative patients (control group).

3.2 Study site

The study was conducted at Brewelskloof hospital (BH) in Cape Winelands East and Overberg District of Worcester, Western Cape province; South Africa. Brewelskloof Hospital is one of the South African hospitals specialized in the management of MDR-TB. Patients are referred from state and private hospitals, general practitioners and primary health care clinics. Brewelskloof Hospital serves approximately 1.3 million people and receives (ukpmc.ac.uk) approximately 100 referrals per month.

Brewelskloof hospital covers areas like Ceres, Matjiefontein, Touws River, De Doorns, Robertson, Montagu, part of Stellenbosch, Swellendam, Heidberg, Riviersonderend, Caledon, Grabouw, Kleinmond, Hermanus, Bredasdorp, Klein Bay and Part of Paarl (see Figure 1).

Figure1. Map of the Western Cape Province

3.3 Inclusion criteria

A patient was included in the study only if he/she complied with all the following criteria:

1. MDR-TB sensitive to second line anti-TB drugs.

2. MDR-TB sensitive to second line anti-TB drugs and co-infected with HIV.

4. Completion of treatment between 1 January 2006 and 31 December 2008.

3.4 Exclusion criteria

Any case of XDR-TB was excluded in this study**.**

3.5 Antituberculosis medications used at Brewelskloof hospital

The drugs used to treat MDR-TB patients at BH involve a combination of the following first and second-line drugs: ethambutol, pyrazinamide, kanamycin, ofloxacin, ethionamide and cycloserine. Ethambutol and pyrazinamide are still used in patients where resistance to ethambutol and pyrazinamide is not confirmed.

3.6 Data collection

Data was collected from TB registers and clinical records of all MDR-TB and MDR-TB patients co-infected with HIV who finished their treatment at BH, from 1 January 2006 to 31 December 2008. All data collected was entered into a database using Excel (Microsoft Office 2003). Data collection form was used to collect data from registers (see appendix III). Information about the patients includes patients demographisc, age distribution, patient biological profile, and number of patients per anti-TB drugs, drug interaction between ARVs and anti-TB drugs; and duration of ARVs therapy before the start of MDR-TB treatment outcomes.

3.7 Validity and reliability of data collected

The patients' folder and TB register was made available by the sister-in charge for data collection. The patient's information officer was around to check for completeness and accuracy of information on the database of the hospital. Data for this study is available on the hospital data base.

3.8. Treatment outcomes used

Standard WHO definitions for TB disease classification, registration and treatment outcomes categories were applied. Treatment outcomes included cured or treatment completed failure, default, transfer-in and death.

3.9 Statistical analysis

All quantitative variables were analysed using medians as a measure of location and either range or interquartile range as measure of spread. Nominal variables were descriptively analysed using frequency distributions indicating the absolute and relative frequencies. Data was displayed graphically using histograms, bar charts or box and whisker plots where applicable. Differences in quantitative variables between the HIV (+) and HIV (-) groups were analysed using a Mann-Whitney U test. The primary analysis was to determine whether there is a difference in cure rate between the HIV (+) and HIV (-) groups. For this comparison the Pearson's chi-square test was used and the Fisher's Exact test used as small expected frequencies were evidenced. A significance level of 5% was applied throughout.

3.10 Ethical considerations

Ethical approval was obtained from the Ethics committee of the University of the Western Cape for the study (Ethics registration number 09/3/18, Appendix I).

The permission to conduct the study at Brewelskloof Hospital was granted by the Western Cape Department of Health (Ref: 19/18/RP87/2009, Appendix II).

The study was done in accordance with Helsinki Declaration and confidentiality was observed.

3.11 Dissemination of research results

This study results will be disseminated through the following:

a. Presentation at department, School of Pharmacy, University of the Western Cape

b. Conference presentations at the national and international level

c. Publication in scientific journal

d. Thesis for masters degree at the University of the Western Cape

CHAPTER FOUR

RESULTS

4.1: Patient demographics

As indicated in table 4.1, 336 patients started MDR-TB treatment between the 1st of January 2004 and 31st December 2006 at Brewelskloof hospital. These patients included 242 (72%) MDR-TB patients and 94 (27.9%) MDR-TB patients co-infected with HIV. The 242 patients with MDR-TB, include 167 (71.0%) male and 75 (30.9%) female. The 94 patients with MDR-TB co-infected with HIV include 51 (54.2%) male and 43 (45.7%) female.

Table 4.1: Patients' demographics data per year

Years	Patients with MDR-TB Number /year/gender (%)			Patients with MDR-TB + HIV Number/year/gender (%)		
	Male	Female	Total (M+F)	Male	Female	Total (M+F)
2006	44 (74.5)	15(25.4)	59	14(58.3)	10(41.6)	24
2007	56 (72.7)	21(27.2)	77	20 (54.0)	17 (45.9)	37
2008	67 (63.2)	39(36.7)	106	17 (51.5)	16(48.4)	33
Total	167(71.3)	75(30.7)	242	51(54.2)	43(45.7)	94

Key: M= Male; F= Female; MDR-TB= Multidrug-resistant tuberculosis; MDR-TB + HIV= Multidrug-resistant tuberculosis co-infected with HIV.

4.2: Age distribution

The patients are categorized into groups of 10 years intervals.

Table 4.2: Age distribution

Age	Patients with MDR-TB			Patients with MDR-TB & HIV		
	Number/ gender / (%) / n			Number/ gender / (%) / n		
	Male	Female	Total (M+F)	Male	Female	Total (M+F)
0-14	10(66.6)	5(33.3)	15	3(50)	3(50)	6
15-24	22(68.7)	10(31.2)	32	5(45.4)	6(54.4)	11
25-35	44(65.6)	23(34.3)	67	13(59)	9(40.9)	22
35-45	48(65.7)	25(43.2)	73	18(51.4)	17(48.5)	35
45-55	30(78.9)	8(21)	38	6(54.5)	5(45.4)	11
55 and older	13(76.4)	4(23.5)	17	6(66.6)	3(33.3)	9
Total	167(71.0)	75(30.7)	242	51(54.2)	43(45.7)	94
Total (M+F)	242(72)			94(27.9)		

Key for table 4.2: M= Male; F= Female; MDR-TB= Multidrug-resistant tuberculosis; MDR-TB + HIV= Multidrug-resistant tuberculosis co-infected with human immunodeficiency virus; %= percentage; N= total number of patients.

As indicated in table 4.2, MDR-TB and MDR-TB plus HIV affect more male than female patients and more than 50% of patients in both HIV (+) and HIV (-) groups are between 25 and 45 years of age.

4.3: Renal and liver function:

Renal and liver function test are not included in the TB register.

4.4 Influence of HIV infection on MDR-TB cure rate

Table 4.4 shows the number of patients who were not cured and those who were cure in both HIV (-) and HIV (+) groups. Out of 94 MDR-TB patients co-infected with HIV, 32 (34%) were cured and 72 (76.5%) were not cured. In the group of MDR-TB patients without HIV infection, the cure rate is 30%. The difference between both cure rates is not statistically significant (p-value = 0.45)

Table 4.4: Influence of HIV infection on MDR-TB cure rate

	HIV (-)	HIV (+)	Total
Not cured	170 70 %	62 65.95 %	232
Cured	72 **30 %**	32 **34.05 %**	104
Total	242	94	336

As shown in the above table, MDR-TB cure rate 34% in HIV (+) patients with and without antiretroviral therapy. The difference between both cure rates in not statistically significant (p-value =0.45).

4.5: Influence of antiretroviral therapy on MDR-TB cure rate

Out of 94 HIV (+) patients, 54 patients were on ARVs. The cure rate is 35% in the group of HIV (+) positive patients on ARVs and 33% in the group without ARVs.

Table 4.5: Influence of antiretroviral therapy on MDR-TB the cure rate

ARVs	No	Yes	Total
Not cured	27 67%	35 64%	62
Cured	13 **33%**	19 **35%**	32
Total	40	54	94

The difference between both cure rates is not statistically significant (p- value = 0.79)

4.6: Number of patients per antituberculosis drugs

This table details the anti-TB drugs that were used by the patients during the treatment period.

Table 4.6: Number of patients per antituberculosis drugs

Drug	Patients treated for MDR-TB = 242			Patients treated for MDR-TB co-infected with HIV = 94		
	Number/ gender / (%) / n			Number/ gender / (%) / n		
	Male	Female	Total (M+F)	Male	Female	Total (M+F)
Pyrazinamide	167(69.2)	75(30.7)	242	51(54.2)	43(45.7)	94
Ethambutol	167(69.2)	75(30.7)	242	51(54.2)	43(45.7)	94
Kanamycin	167(69.2)	75(30.7)	242	51(54.2)	43(45.7)	94
Ofloxacin	167(69.2)	75(30.7)	242	51(54.2)	43(45.7)	94
Ethionamide	167(69.2)	75(30.7)	242	51(54.2)	43(45.7)	94
Cycloserine	167(69.2)	75(30.7)	242	51(54.2)	43(45.7)	94
CTX prophylaxis	0	0	0	58(61.7)	36(38.2)	94

Key to table 4.6: CTX= Co-trimoxazole; MDR-TB= Multidrug-resistant tuberculosis; MDR-TB + HIV= Multidrug-resistant tuberculosis co-infected with human immunodeficiency virus; %= percentage. M=male; F=female. N= total number of patients

All MDR-TB patients, HIV infected or not, were treated with pyrazinamide, ethambutol, kanamycin, ofloxacin, ethionamide and cycloserine.

Out of 94 HIV - positive patients recorded, 58(61.7%) male and 35 (38.2%) female patients were given CTX prophylaxis. Furthermore, all 94 HIV positive patients were receiving ARVs as shown in table 4.6.

4.7: MDR-TB treatment failures

As indicated in table 4.7, MDR-TB treatment failed in 232 (166 HIV – negative plus 66 HIV-positive) patients out of 346 involved in the study. Furthermore, table 4.7 shows that 65 (28.0%) patients completed MDR-TB treatment but could not be classified as cured or failure, 29 (12.5%) patients failed, 76 (32.7%) defaulted, 18 (7.7%) were transferred out and 44 (18.9%) died. As far as treatment completed and defaulted is concerned, there is no significant statistical difference between HIV (+) and HIV (-) The number of patients who failed the MDR-TB treatment and who were transferred out is significantly higher in the HIV (-) group than in the HIV (+) group. Finally the number of MDR-TB patients who died is significantly higher in the HIV (+) group).

Table 4.7: Treatment failures

MDR-TB treatment outcomes	HIV (-) MDR-TB patients			HIV (+) MDR-TB patients			Total
	Male	Female	Total	Male	Female	Total	Total
Treatment completed	26	23	49 (29.5%)	10	6	16 (24.2)	65(28.0%)
Failure	19	5	24 (14.4%)	4	1	5 (7.5%)	29(12.5%)
Defaulted	34	18	52 (31.3%)	14	10	24 36.3%)	76(32.7%)
Transfer out	11	2	13 (7.83)	3	2	5 (7.5%)	18(7.7%)
Died	25	3	28 (16.8%)	10	6	16 (24.2%)	44(18.9%)
Total	115	51	166 (71.5%)	41	25	66 (28.4%)	232

Key to table 4.7: MDR-TB= Multidrug-resistant tuberculosis; MDR-TB + HIV= Multidrug-resistant tuberculosis co-infected with human immunodeficiency virus; %= percentage.

4.8: Duration of antiretroviral therapy before the start of multidrug-resistant tuberculosis treatment

As shown in table 4.8, the antiretroviral drugs used in HIV (+) patients include stavudine, lamivudine and efavirenz. The antiretroviral treatment was given to 54 (57.4%) MDR-TB patients co-infected with HIV.

According to the results shown in (table 4.9), the median (range) duration of antiretroviral therapy before starting anti-tuberculosis drugs is 10.5 (1-60) months in patients who are taking ARVs before the start of MDR-TB treatment.

Table 4.8: Duration of antiretroviral therapy before the start of multidrug-resistant tuberculosis treatment

Total number of Patients	Total number of Months	Drugs used
4	1	d4T, 3TC and EFV
6	2	d4T, 3TC and EFV
4	3	d4T, 3TC and EFV
2	4	d4T, 3TC and EFV
2	5	d4T, 3TC and EFV
1	6	d4T, 3TC and EFV
1	7	d4T, 3TC and EFV
2	8	d4T, 3TC and EFV
1	10	d4T, 3TC and EFV
1	11	d4T, 3TC and EFV
2	12	d4T, 3TC and EFV
1	13	d4T, 3TC and EFV
1	14	d4T, 3TC and EFV
2	15	d4T, 3TC and EFV
9	24	d4T, 3TC AND EFV
3	30	d4T, 3TC and EFV
1	36	d4T, 3TC and EFV
1	60	d4T, 3TC and EFV
Median (range)	10.5 (1-60)	

Key for table 4.9: ART= Antiretroviral treatment; MDR-TB= Multidrug-resistant tuberculosis; MDR-TB + HIV= Multidrug-resistant tuberculosis co-infected with human immunodeficiency virus; d4T= stavudine ®; 3TC= lamivudine; EFV= efavirenz.

CHAPTER FIVE

DISCUSSION

The study was designed as a retrospective case control study comparing the treatment outcomes of MDR-TB between two groups of patients including HIV – positive patients (experimental group) and HIV-negative patients (control group) both of them infected with MDR-TB.

It was hypothesized that there is a difference in MDR-TB cure rate between HIV – positive and HIV- negative patients and that antiretroviral drugs could influence the MDR-TB treatment outcomes.

The demographic characteristics (gender and age) of patients involved in this study are well distributed into HIV (+) and HIV (-) patients. The sample size is big enough to confirm the study hypotheses.

5.1 Age distribution

Firstly, the result in table 4.2 shows that in Brewelskloof hospital, MDR-TB is common with working class population (25-55 years), who are sexually active; when compared with statistics from the United States of America, that show that TB is mainly a disease of 55 years and older or of the immunocompromised (Kumar et al, 2007; WHO, 2006).

In the study, there were differences in study population which includes 0-14, 15-24, 25-35, 45-55, and older. These differences are unlikely to reverse the conclusions of this study; as compared to similar study with bias and excluded population: the excluded group had a larger number of older patients (≥ 50 years) (Pillai et al, 1997).

5.2 Number of patients per antituberculosis drugs.

In South Africa, prophylaxis co-trimoxazole preventative therapy (CPT) is for prophylaxis and treatment of Pneumocystis jirovecii formerly P. carinii infections. Co-trimoxazole has been made available in all the clinics and hospitals for use on all TB and MDR-TB patients co-infected with HIV. Similar to anti-retroviral treatment, the current National HIV/AIDS policy allows for the provision of CPT to all HIV positive TB patients with WHO stage 3 of TB or 4 HIV, WHO stage 1 with CD4+ T-lymphocyte <200 cells/mm^3, or WHO stage 2 with total lymphocyte count <1200 cells/mm^3 Department of Health (National, 2010).

This study is similar to other studies done in Malawi, Ivory Coast, and South Africa which demonstrated a 46% mortality reduction for HIV-infected TB patients taking CPT prophylaxis (Mwaungulu et al, 2004; Dean et al, 1999 and Hoffman et al, 2010).

In addition, all MDR-TB patients with HIV infected or not, were treated with pyrazinamide, ethambutol, kanamycin, ofloxacin, ethionamide and cycloserine. The treatment of MDR-TB is usually continued for -24 months rather than 12-18 months for drug-sensitive drug (Katherine et al 2008). In this study, the MDR-TB co-infected with HIV cure rate are 32.1%; which is within the range when compared with multidrug resistant tuberculosis cure rate are 30 – 50% in patients without HIV infection Department of Health (National, 2006).

There were 69.2% males and 30.2% females that were treated second-line anti-TB drugs and 54.2% males and 44.7% females that were treated with ART. The result shows that there were more males than females in both groups. This is similar to other study conducted at Vietnam where there were more males than females with 79% males and 21% females (Thuy et al, 2007)

5.3 Drug interactions between antiretroviral and antituberculosis drugs

There are 94 MDR-TB co-infected with HIV-positive, who received ARVs and 242 MDR-TB without HIV infection. The results show MDR-TB treatment outcomes as having 32.1% cured rate and 12.7% failures rate. This could mean that the failure was due to drug-drug interactions and not from HIV infections; because all MDR-TB co-infected with HIV received co-trimoxazole prophylaxis (27.9%) and MDR-TB without co-trimoxazole (72%). It has a statistical significance P- value of less than 0.001.

Immuno-compromised patients are at a high risk of drug-drug interactions during their TB treatment. This study shows that was drug-drug interactions between ART and anti-TB drugs where we 32.1% cured rate, treatment completed rate 26.7%, 12.7% failure rate, 33.3% defaulted rate, 7.9% transfer-out and 19.2% death rate. However, there are no published studies of possible drug-drug interactions between second-line antituberculosis drugs and antiretroviral drugs (Weyer, 2005). In addition, anti-TB drugs poor absorption has been reported in HIV-infected patients with association to HIV-enteropathy; and poor absorption of anti-TB drugs resulted in drug sub-therapeutic serum concentration levels with high treatment failure, relapse, and acquired drug resistance (Patel et al; 1995; Peloquin et al; 1996; Weiner et al; 2005). It has been reported that anti-TB drugs serum (peak) concentrations were lower in TB patients co-infected with HIV and having CD4 cell count <200 cell/mm³ (Peloquin et al; 1996). Also, that the patients that died could be as result of renal or kidney problems and not from HIV because all MDR-TB co-infected with HIV is on co-trimoxazole prophylaxis.

There was relationship between MDR-TB (HIV negative) and (HIV positive) and the differences between HIV negative and HIV positive are in the used of ARVs. In addition, there are drug-

drug interactions between ART and anti-TB drugs. The only anti-TB drug that has drug interaction with ART is ethionamide which is thought to be metabolized by the CYP450 system, though it is not known which of the CYP isozymes are responsible (Burman et al, 1999).

In this study, there was high failure and defaulted rate which is similar to what Cohn and Flament-Saillour et al reported in MDR tuberculosis treatment that is complicated and frequently associated with treatment failure, relapse and high incidences of adverse drug reactions, which lead to prolonged illness, disability and death as a consequence (Cohn, 1995; Flament-Saillour et al, 1999). In addition, this study showed mortality rate of 19.2% when compared with similar study with mortality rates of 72-89% (Cohn, 1995). Deaths during treatment caused by MDR-TB infection or by other HIV-related diseases are more frequent, particularly, in the advanced HIV-infection stages (Cohn, 1995).

5.4 Duration of antiretroviral therapy before the start of multidrug-resistant tuberculosis treatment

We have already demonstrated that human immunodeficiency virus infection does not influence MDR-TB treatment outcomes, since no difference in the treatment outcomes was found between the group of patients MDR-TB co-infected with HIV and the group of MDR-TB patients without HIV - infection. Furthermore, since there is no difference between the treatment outcomes within the group of MDR-TB patients co-infected with HIV and taking ARVs and the group of MDR-TB patients with HIV – infection but not receiving ARVs. We can state that antiretroviral therapy does not have any effect on the MDR-TB treatment outcomes.

Finally, does the duration of antiretroviral therapy have any impact on the MDR-TB treatment outcomes? The answer to this question can be found in table 4.8. In that table the median (range)

duration of antiretroviral treatment before MDR-TB therapy is 10.5 (1-60) months. This duration is long enough to show any influence antiretroviral therapy might have on MDR-TB treatment outcomes if any. Therefore, we can conclude that, neither the fact of being on ARVs nor the duration of antiretroviral therapy does influence MDR-TB treatment outcomes.

5.5 Were objectives of the study achieved?

Yes the objectives of the study were achieved. Firstly, the study found that HIV infection does not have an impact on the cure rate and the treatment failure at Brewelskloof hospital. Secondly, no drug interactions were found between antiretroviral drugs and second-line antituberculosis agents. Thirdly, the fact of taking ARVs drugs does not influence the above mentioned MDR-TB treatment outcomes.

5.6 Limitations of the study

This was a retrospective study. Therefore, data collection was based on the available clinical records and on the data available in the tuberculosis register. The CD4 counts and viral load were not included in the TB register. This is an important limitation since we do not know how the biological profile of each patient was.

Renal and liver function test were not included in the TB register as well. Therefore, this study could not investigate the influence of liver and renal diseases on the MDR-TB treatment outcomes.

CHAPTER SIX

6 CONCLUSIONS and RECOMMENDATIONS

6.1 Conclusions

This retrospective study was conducted at Brewelskloof hospital in order to investigate the MDR-TB treatment outcomes in patients with MDR-TB and in patients with MDR-TB co-infected with HIV infection who started their treatment between January 2004 and December 2006.

Firstly, the study demonstrated that there is no difference in the MDR-TB cure and failure rates in HIV (-) and HIV (+) patients. Secondly, the MDR-TB cure rate at Brewelskloof between January 2004 and December 2006 was similar to the MDR-TB cure rate at the national level. Thirdly, according to this study results, antiretroviral therapy does not affect the MDR-TB treatment outcomes.

If HIV infection and antiretroviral drugs do not influence the MDR-TB cure rate, which other factors could keep it as low as 32% at Brewelskloof hospital?

6.2 Recommendations

Further studies involving a higher number of patients with similar virological profile are needed in order to confirm that HIV does not have any impact on MDR-TB treatment outcomes.

Finally, studies focusing on the real causes of factors such as relapse, defaulting and failure leading to death need to be undertaken. These studies would also have to find out whether the drugs currently used for the treatment of MDR-TB are indeed effective.

Last but not the least, the actual mechanism by which resistance to antituberculosis occurs needs to be clarified

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APPENDICES

Appendix I : Ethics approval certificate

OFFICE OF THE DEAN
DEPARTMENT OF RESEARCH DEVELOPMENT

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8 June 2009

To Whom It May Concern

I hereby certify that the Senate Research Committee of the University of the Western Cape has approved the methodology and the ethics of the following research project by: Prof P Mugabo (School of Pharmacy)

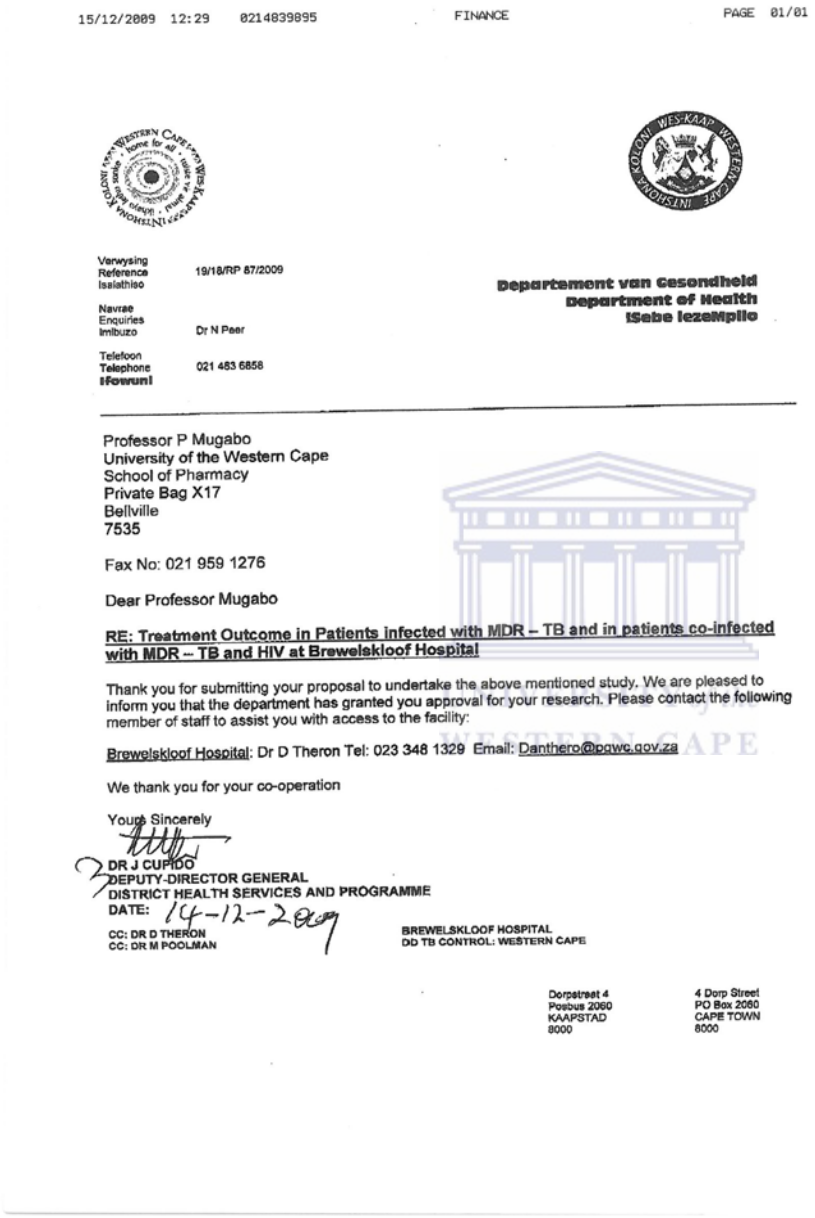
Research Project: Treatment outcome in patients infected with MDR-TB and patients co-infected with MDR-TB and HIV at Brooklyn Chest Hospital, Cape Town

Registration no: 09/3/18

Peter Syster
Research Development
University of the Western Cape

Appendix II

Permission to conduct the study at Brewelskloof Hospital



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Verwysing Reference Isalathiso: 19/18/RP 87/2009

Navrae Enquiries Imibuzo: Dr N Paar

Telefoon Telephone IFowuni: 021 483 6858

Departement van Gesondheid
Department of Health
iSebe lezeMpilo

Professor P Mugabo
University of the Western Cape
School of Pharmacy
Private Bag X17
Bellville
7535

Fax No: 021 959 1276

Dear Professor Mugabo

RE: Treatment Outcome in Patients infected with MDR – TB and in patients co-infected with MDR – TB and HIV at Brewelskloof Hospital

Thank you for submitting your proposal to undertake the above mentioned study. We are pleased to inform you that the department has granted you approval for your research. Please contact the following member of staff to assist you with access to the facility:

Brewelskloof Hospital: Dr D Theron Tel: 023 348 1329 Email: Danthero@pgwc.gov.za

We thank you for your co-operation

Yours Sincerely

DR J CUPIDO
DEPUTY-DIRECTOR GENERAL
DISTRICT HEALTH SERVICES AND PROGRAMME
DATE: 14-12-2009

CC: DR D THERON
CC: DR M POOLMAN

BREWELSKLOOF HOSPITAL
DD TB CONTROL: WESTERN CAPE

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KAAPSTAD
8000

4 Dorp Street
PO Box 2060
CAPE TOWN
8000

Appendix III

The data collection tool

TREATMENT OUTCOME IN PATIENT WITH MDR-TB AND IN PATIENTS CO-INFECTED WITH MDR-TB AND HIV

REG DATE	CLINIC	SUB DISTR	NAME IN FULL		DOB	AGE	GENDER	ADDRESS	DATA COLLECTION PATIENTS MDR-TB CA				
									Primary MDR – TB				
									MDR TB	MDR previously treated with Regimen 1			
										Failure	Defaulted	Relapse After Cure	F
	-												