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A review of the efficacy of multi-drug resistant tuberculosis regimens used at Charlotte Maxeke Johannesburg academic hospital

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A publishable article submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Medicine in the branch of Internal Medicine.

TITLE:

A REVIEW OF THE EFFICACY OF MULTI-DRUG RESISTANT TUBERCULOSIS REGIMENS USED AT CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

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Johannesburg, 2021

DECLARATION

I, Jared Tulloch, declare that this research report is my own work which is being submitted for the degree Master of Medicine (in the submissable format with my protocol and an extended literature review) in the branch of Internal Medicine, at the University of the Witwatersrand, Johannesburg. It has not been submitted before, for any degree or examination at this or any other university.

…….

…24..Day of…March...2021

This research paper is dedicated to my mother, Barbara Louise Tulloch, who instilled within me the perseverance and fortitude for which I am blessed with today.

In memory of Barbara Louise Tulloch

01 March 1959 – 28 December 2017

ABSTRACT

Background:

South Africa has a high burden of drug resistant Tuberculosis (DR-TB) and the impact of the initiation of a new drug resistant therapy regimen with the availability of bedaquiline and linezolid remains to be seen in this setting.

Objectives:

To describe the drug regimens and outcomes of patients diagnosed with DR-TB treated at Charlotte Maxeke Academic Hospital (CMJAH) TB clinic.

Methods:

The data was collected retrospectively and inserted into a data sheet. The files used were those of patients residing in central Johannesburg with confirmed drug resistant TB that received one of the drug resistant regimens at the specialist clinic over the period 01 January 2015 to 30 June 2019.

Results:

There were 105 individuals who satisfied the research criteria, 75% of the group was HIV positive. 72 patients had rifampicin mono-resistant tuberculosis and 33 had multi-drug resistant tuberculosis. Four regimens were identified: old injectable long course (15%), old injectable short course (4%), new bedaquiline long course (2%) and new bedaquiline short course (24%). The remaining 55% received individualized regimens. The overall cure rate for the period under review was 54% with a mortality rate of 9% and loss to follow up of 16%. In the group that received a bedaquiline based regimen 60% achieved cure, 49% of the injectable group and 62% that received a combination of injectable and bedaquiline therapy during their modified regimen achieved cure.

Conclusion:

The older injectable regimens showed comparable efficacy when cure rate was looked at in isolation. The areas of significant improvement in instituting a drug regimen that has a shorter duration of therapy, fewer side effects and easier administration method will likely reveal itself in years to come.

ACKNOWLEDGEMENTS

I would like to use this opportunity to extend my utmost gratitude to Dr Kapila Hari and Dr Sarah Stacey for their constant support and guidance through the process of this research submission. Their tireless efforts in helping shape my development in scientific research will stand for many years to come.

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ABBREVIATIONS

CMJAH:	Charlotte Maxeke Johannesburg Academic Hospital
TB:	Tuberculosis
Mtb:	*Mycobacterium Tuberculosis*
DR-TB:	Drug resistant tuberculosis
MDR-TB:	Multi-drug resistant tuberculosis
XDR-TB:	Extremely drug resistant tuberculosis
HIV:	Human-immuno deficiency virus
CD4:	Cluster of differentiation 4 molecule
INH:	Isoniazid
Rif:	Rifampicin
TO:	Transfer out
TF:	Treatment failure
WHO:	World Health Organization

CHAPTER 1

1.1 EXTENDED LITERATURE REVIEW

1.1.1 Introduction:

Mycobacterium tuberculosis (*Mtb*) is an organism that has plagued human civilization throughout the course of our human history. Its ability to survive in populations with poor social and living conditions has ensured its particularly devastating impact on these vulnerable groups. While there are numerous estimations of how old the mycobacterium genus is, latest genetic evaluations estimate it to be more than 150 million years old.[1]

Having recently surpassed the Human Immunodeficiency Virus (HIV) as the infectious disease responsible for the most deaths worldwide, there are currently nearly 2 billion people infected with *Mtb.*[2] As such, the target set out by the World Health Organisation(WHO) is for eradication of the disease by the year 2030.[3] In light of this, the growing problem of resistant tuberculosis has become particularly concerning and cannot be disregarded. Fortunately, there have been developments in the treatment of resistant disease in the last fifteen years which have yielded promising results.

1.1.2 History of Tuberculosis:

Through the centuries, tuberculosis (TB) has been described by most of the ancient civilizations by either one pathological presentation or another. Terms such as consumption (pulmonary TB), scrofula/king's evil (cervical lymph adenitis), Pott's disease (anterior wedge compression fracture of the vertebra) were common descriptions of pathology still used colloquially today. The remains of Peruvian and Egyptian mummies from as far back as 2400 BC have been identified with the wedge vertebral deformity classical of *Mtb* of the spine. Deoxyribonucleic acid (DNA) from the tissue of these mummies has also confirmed the presence of TB. The first documented writings on TB were found in India 3300 years ago, and a millennium later in China.[4]

Scientists have estimated, based on the organism's slow mutation rate, that the species' progenitor organism originated from East Africa more than 3 million years ago.[4] The complex of related organisms which is believed to share a common ancestor include *Mycobacterium africanum*, *Mycobacterium bovis* and *Mycobacterium canettii,* which are all present in East

Africa to this day.[1] *M. canettii* has been postulated to be the organism from which *Mtb* primarily originated, based on the research into single nucleotide polymorphisms which has enabled scientists to look at slight genetic differences and evolutionary changes to genomes of this group.[3,4]

Our modern understanding of this disease was facilitated by the work of Rene Theophile Laennec, a 19th century physician, famously known for developing the modern-day stethoscope. He described auscultatory findings associated with tuberculosis and identified the connection between pulmonary TB and extra-pulmonary TB as one distinct entity. He documented the array of pathological lesions noted on autopsy in patients known to be suffering from consumption, identifying areas of caseous material and cavitations of the lung and extra-pulmonary sites thus recognizing their pathological connection. He was then able to correlate these pathological findings with clinical features he discovered using his stethoscope.[4]

Jean-Antoine Villemin was the next physician to make further advances in our understanding of *Mtb*. He did this by performing a study in 1868 where he extracted caseous material from deceased human lungs where the cause of death was attributed to consumption; he then inoculated living rabbits with this material. At autopsy three months later, these rabbits were found to have developed extensive pulmonary tubercular masses.[5] In conducting this experiment he was able to prove the transmissibility of *Mtb* through infected tissue, indicating its infectious nature.

The causative organism was first isolated by Robert Koch in 1882. The isolate was initially examined using methylene blue staining with microscopy.[4] This allowed for objective and diagnostic identification of the aetiology of the disease process by confirming beyond any doubt the cause as an infectious organism which, up until this point in history had been subject to much debate.

1.1.3 Epidemiology:

The WHO estimates that 10 million people contracted TB worldwide in 2018, with 1,5 million deaths attributed to the disease in that year. The incidence of drug resistant *Mtb* infections over this period was estimated at 484 000.[3] The majority of cases occurred in men (57%),

while women and children (under 15 years of age) accounted for 32% and 11% of infections respectively.[3]

The current WHO targets for 2030 include a 90% reduction in deaths related to *Mtb* as well as an 80% reduction in the incidence of *Mtb* worldwide.[3]

South Africa has a high burden of TB. The peak incidence of *Mtb* infection in South Africa was recorded in 2008 where 460 000 new infections were noted, while the latest incidence rates according to the 2018 WHO report were 301 000 new *Mtb* infections in that year, with 11 000 multi-drug resistant (MDR)/ rifampicin resistant (RR) cases.[3] In comparison, Kenya, a nation with a similar population (51 million vs 58 million) to South Africa had a total incidence of 150 000 cases with 2300 MDR/RR TB cases for 2018.[3] South Africa, despite having a high disease burden, has responded well to the milestones set out by the WHO for 2020 by decreasing its incidence as well as mortality rate associated with infection. This is largely due to an effective policy of drug roll out which has been made possible by significant financial contributions.[3] The South African budget for tuberculosis for 2018 was $240 million (R3,36 billion). Thirteen per cent of this was funded by international aid organisations and the remainder covered by South Africa's own financial budget.[3]

Susceptibility to infection is wide-ranging amongst different population groups. There are various factors that determine this with low socio-economic circumstances being the classic term used to encompass several factors increasing susceptibility to contracting *Mtb* infection. Low socio-economic conditions have become commonly linked to the massive migration of rural communities into urbanized areas with the industrialization of the world's economy. Migrant workers live in close proximity where transmission of communicable diseases is promoted. A strong correlation has been identified between the progression of latent *Mtb* infection to active infection and the average standard of living of a population group.[6] Smoking and alcohol abuse as well as poor nutrition are well recognized factors increasing susceptibility to *Mtb* infection. It is believed that these issues have been significant in the propagation of this disease as a worldwide public health problem and in particular, developing nations like South Africa.[7] In South Africa, a large amount of the population resides in informal settlements surrounding high density cities. This, with a concomitant high prevalence of HIV of 13.1% within the population, creates the ideal setting for spread of this disease.[8]

Other factors of particular importance to susceptibility can be related to environmental exposures like mining, innate and acquired immune deficiencies as well as immunosuppressive drug therapy. Deficiency in any of the host's immune defenses may increase the risk of active *Mtb* infection, while T lymphocyte deficiency in HIV and TNF-alpha inhibitors used in biologic therapy for various autoimmune conditions are significant predisposing factors.[5] Genetic susceptibility such as variants of the $SLC_{11}A_1$ gene encoding for a specific macrophage membrane protein have been shown to increase susceptibility as well as vitamin D receptor changes and interferon gamma signaling pathways.[5]

An important factor in the epidemiology of any infectious disease is its transmissibility. The two features of importance to this are the reproductive number and the serial interval. The reproductive number can be defined as the average number of secondary cases of infection a primary infection will result in, while the serial interval pertains to the period between disease symptom onset in the primary infected individual until the secondarily infected person develops symptoms.[2]

Mtb has a unique transmission pattern due to the prolonged latent period associated with the disease and the possibility that some individuals who acquire infection never develop symptomatic disease. It is also characterized by a prolonged incubation period due to the variability of the integrity of the host's immune system.[2] Serial interval data measured for *Mtb* ranges from 6 months to 5 years. The reproductive number for *Mtb* ranges from 1 to 4.3, with both these estimates varying significantly between differing worldwide population groups.[2]

1.1.4 Pathogenesis:

Mycobacterium tuberculosis is a slow growing, acid-fast, rod shaped bacillus. It has a highly lipid rich cell wall which makes the traditional gram staining unhelpful when observing it with microscopy and thus acid-fast stains are required for its identification. These lipid fractions in the cell wall allow for survival of the organism inside macrophages.[5]

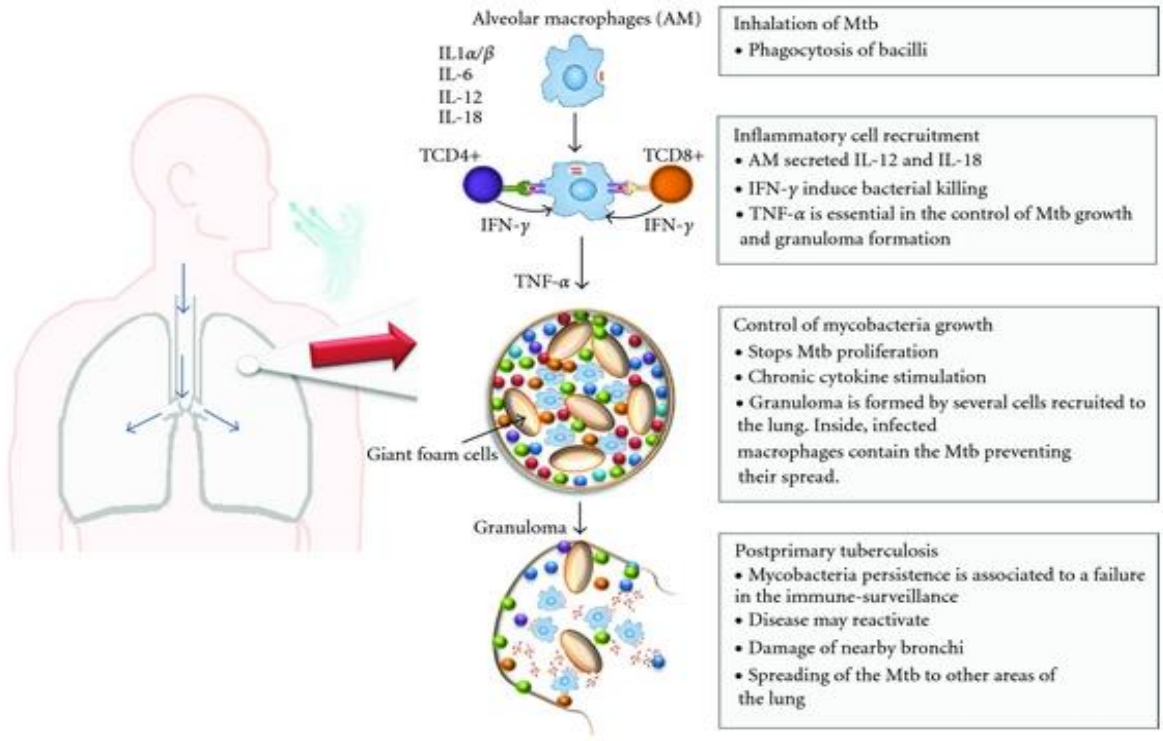

Figure 1. Pathogenesis of tuberculosis, diagrammatic representation of the hosts immune interaction with the *MTB* bacillus[9]

Its method of transmission is by aerosolization from an infected individual's respiratory tract to another person.[10] The bacilli arrive in the alveoli of the new host where it makes first contact with the host's alveolar macrophages. The macrophages phagocytose the bacilli however, the lipid fractions in the cell wall of the organism ensure that replication continues within the macrophages. It is the hosts hypersensitivity in response to the presence of the *Mtb* bacillus that causes the tissue destruction associated with this disease.[10]

Primary Tuberculosis

The accumulation of phagocytosed bacilli by respiratory macrophages with activation of the hosts immune system forms the primary lung lesion in *Mtb*, the Ghon focus. This lesion is classically found adjacent to the pleura in the upper part of the lower lobe or the lower part of the upper lung lobe where ventilation is maximal.[10] The bacilli disseminate via the lymphatic system to the tracheo-bronchial lymph nodes where further caseating granulomatous formation occurs. This stage of the disease is described as the Ghon complex. *Mtb* can also disseminate haemotogenously, this usually occurs at the time of primary infection, particularly in the immune-compromised host where highly active lesions seed to

the circulatory system disseminating to distant organ systems including the liver, spleen, meninges as well as the urogenital system.[10]

Secondary Tuberculosis

This occurs in a host that has been previously exposed to *Mtb* and develops active disease sometime after the initial inoculation. This can either be triggered by re-infection of *Mtb* or when a previously suppressed *Mtb* complex re-activates at a time of immunological impairment in the host.

The lesions in secondary tuberculosis occur in the apical and posterior segments of the upper lobes of the lungs. The bacilli travel to these regions during the initial phase of tuberculous bacteraemia and disease favours the upper apices for their highly ventilated properties.[10]

There are thus three possible outcomes following new infection of a host; the bacillus can be destroyed by the host's immune response resulting in immediate eradication, this occurs when the bacillary inoculum load is low and the receiving hosts immunity is competent[(10,11)], the bacilli can be contained by the formation of a granuloma in which the organism can remain dormant for many years, which is known as latent disease, or it may progress to active disease, particularly in hosts with impaired immunity.[5] While initial infection with the bacillus progresses to clinical illness in only 10% of patients within the first 2 years, the majority develop latent infection with the bacilli remaining quiescent until the immunological environment is conducive for active disease.[10]

Granuloma formation occurs as a result of macrophage, giant cell, T cell, B cell and fibroblast aggregation around the bacilli. The bacilli are ingested by the macrophages and a resultant cytokine immune activation is triggered resulting in the release of interferon gamma (IFN-γ), tumour necrosis factor alpha (TNF-α), interleukin-12, interleukin-1 and interleukin-6 in particular. The *Mtb* is largely contained by the granuloma however active bacilli remain inside some of the inactivated macrophages resulting in dormant disease. TNF-α is a pro-inflammatory cytokine that works in unison with IFN-γ to produce reactive nitrogen intermediates (RNI's) in enforcing their bacteriostatic effects on the bacilli. It is also responsible for signaling of immune cells to the site of infection.[5,11] TNF-α's integral role has

been confirmed with numerous studies showing the increase in incidence of tuberculosis infection in patients treated with TNF-α inhibitory drugs.[5]

The subsequent role of dendritic cells, lymphocytes and macrophages is in the containment of latent disease by ensuring that the bacilli are held within the granuloma preventing further systemic dissemination. The macrophage cytoplasmic wall is actively lysed by the replicating TB bacilli resulting in the accumulation of these lysed macrophages in the central zone of a granuloma.[11]

1.1.5 Diagnostic tools:

Traditional testing for *Mtb* was based on microscopy and culture of the bacillus. Microscopy is still favored in many resource limited settings as it is relatively affordable, requires minimal infrastructure and results are rapidly available. The limitations of microscopy are that identification of the acid-fast bacilli requires an experienced technician, with sensitivity rates varying significantly across different institutions. Auramine staining remains the standard technique used for smear preparation in South Africa, yet it lacks sensitivity which makes eradication of *Mtb* in developing regions difficult. Its sensitivity for a single specimen is 22-43% with even lower rates of detection in the paediatric population and those with concomitant HIV. Numerous micro-organisms stain acid fast however non-tuberculous mycobacteria appear as short, straight bacilli as opposed to *Mtb* which appear long, curved and somewhat beaded.[12]

The current and preferred method for the identification of *Mtb* as well as MDR-TB is the Cepheid Xpert MTB/Rif (Xpert MTB/RIF), which is a polymerase chain reaction (PCR)-based technology that identifies *Mtb* and resistance to rifampicin.

Line probe assay(LPA) is the gold standard for resistance testing. Specimens are cultured and assays are performed on these samples in order to detect resistance to rifampicin, isoniazid, fluoroquinolones and second line injectable antimicrobials.

A line probe assay involves the extraction of DNA from a substrate. Previously a cultured sample was required, current technology is now able to extract DNA from microscopy identified fluid samples, as well as tissue.[13] Thereafter PCR amplification is performed on the resistance-determining region of the genetic sequence. Once the genetic sequence has been amplified, the PCR products are labelled and undergo hybridization where oligonucleotide

probes are immobilized onto a strip. The hybrid probes are then labelled, captured and interrogated for *Mtb* identification followed by the presence of mutations for resistance along the known nucleotide sequencing regions. This method is termed reverse hybridization.[13,14]

Tuberculin skin testing or Mantoux skin test is an old test used to identify the presence of latent *Mtb* in an individual. This is based on assessing the immune response to a purified protein derivative of tuberculosis indicating previous exposure. The test lacks specificity because the antigens injected can be seen in non-tuberculous mycobacterial infections as well as in the Bacillus-Calmette-Guerin (BCG) vaccine. The test also lacks sensitivity, particularly in patients who are HIV positive with cluster of differentiation-4(Cd_4) count of less than 200(cells per mm^3) due to their immune systems inability to respond adequately to the introduction of a pathological antigenic protein.[14]

The interferon gamma release assays (IGRA) for which there are two types currently available, QuantiFERON TB gold and TSPOT.T, assess the quantity of interferon-gamma release by T-lymphocytes upon exposure to a tuberculous protein antigen and is used to test for latent *Mtb*. This test maintains the Mantoux tests sensitivity while improving the specificity by removing BCG vaccination and non-tuberculous mycobacterium as confounders, as the protein derivative introduced is specific for *Mtb*.[14] The test is popular in developed countries where the prevalence of *Mtb* is low due to its high specificity. In comparison, regions like Southern Africa and Asia where the prevalence of *Mtb* is high, its value is less appreciated.

1.1.6 Drug development:

The discovery of penicillin by Alexander Fleming in 1928 had an impact on the successful treatment of bacteria for the first time in our history. Further antimicrobial therapy followed and by 1944 streptomycin and para-aminosalicylic acid were discovered.[15] These drugs showed great promise in the treatment of *Mtb* with notable treatment successes for the first time in the disease's history. While the medical community now had drugs with clear activity against the bacillus, it was only when antimicrobial drugs were combined that cure rates became significant. The formulation of the triple regimen involving streptomycin, para-aminosalicylic acid and isoniazid in 1952 brought about a robust regimen with cure rates of

90 to 95%. The triple therapy regimen was however cumbersome, requiring 24 months to reach cure.[16]

Para-aminosalicylic acid was later replaced by ethambutol in 1960, a bacteriostatic drug which affects cell wall synthesis. The benefits of this were an improved side effect profile and shortened duration of treatment to 18 months. In the 1970s, the introduction of rifampicin, a potent bactericidal agent further reduced treatment duration to nine months. Initially discovered in 1952, pyrazinamide was added to the regimen in the 1970's which resulted in a 95% eradication rate after 6 months of therapy.[16]

Treatment options have remained relatively unchanged for drug-sensitive *Mtb* over the last few decades, with the current recommended regimen including rifampicin, isoniazid, ethambutol and pyrazinamide.[17]

The mechanism of action of each drug in the first line treatment of *Mtb* is as follows:

Rifampicin:

Rifampicin's mechanism of action involves the inhibition of messenger ribonucleic acid (mRNA) elongation via its binding to the beta sub-unit of ribonucleic acid (RNA) polymerase, resulting in bactericidal action. Its most common side effect is a cholestatic hepatitis which is usually transient in nature.[13,18]

Isoniazid:

Isoniazid acts by inhibiting mycolic acid synthesis of the bacillus. Mycolic acid is a crucial constituent of the bacterial cell wall.[13] The active form of isoniazid targets mycolic acid synthesis of the bacillus via a nicotinamide adenine dehydrogenase (NADH) dependent protein reductase pathway encoded for by the *inhA* gene The two most common and significant side effects associated with this drug are peripheral neuropathy and hepatotoxicity.[13,18]

Pyrazinamide:

Pyrazinamide is a prodrug which is catalyzed by bacterial enzymes to its active form intracellularly. It is seen to be crucial in the treatment of tuberculosis as it has excellent absorption and is widely distributed in the body. It is most effective in the acidic environment of macrophages, reaching those areas of tuberculosis-induced lung destruction

where many bacilli avoid eradication. Its mechanism of action is not fully understood but it is thought to deplete the bacillus' energy stores by inhibition of translation and inhibition of the synthesis of pantothenate and coenzyme A (CoA). It is considered to be the most hepatotoxic drug of the regimen.[13,18]

Ethambutol:

Ethambutol's action is also directed at the cell wall. It interferes with the synthesis of an important component of the wall, arabinogalactan. It does this by inhibiting the arabinosyltransferase enzyme which is encoded for by the *embB* gene. The *embB* gene has been identified as the site for resistance to this drug. The most important side effect encountered with this drug is retrobulbar neuritis which is largely reversible when the drug is ceased. It has poor central nervous system penetration.[13,18]

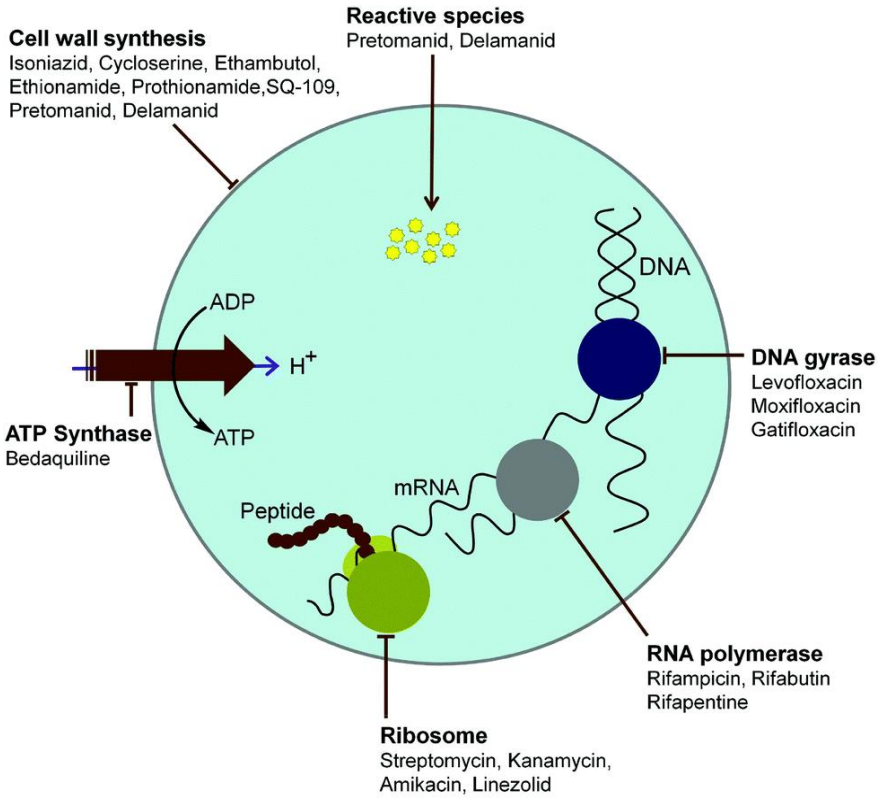

Figure 2 Diagrammatic representation of pharmacological sites of action of anti-tuberculous drugs.[19]

1.1.7 Drug Resistance:

Drug resistance occurs by means of mutations to the bacillus' genome. The bacillus can evade destruction by incurring deletions, insertions and changes to single nucleotide polymorphisms which encode for areas of traditional drug targeting and areas of enzymatic activation of the drugs.[13]

Isoniazid resistance most commonly occurs due to a mutation to the *katG* gene, which is a gene that produces a catalytic enzyme crucial for the conversion of isoniazid (a prodrug) into its active form. This is known as the catalase-peroxidase (*katG*) S315T mutation. The NADH dependent protein reductase pathway encoded for by the *inhA* gene, responsible for mycolic acid synthesis, is the second site of mutation; *inhA* gene expression.[13]

Mutations to the gene encoding for RNA polymerase, *rpoB* are the main mutations responsible for resistance to rifampicin.[13]

Tuberculosis resistance patterns can be defined as follows:[20]

- Mono-resistance: resistance to one anti-tuberculous drug in the standard TB regimen
- Poly-resistance: resistance to two drugs in the standard regimen that does not include both rifampicin and isoniazid
- Multi-drug resistance (MDR): resistance to both isoniazid and rifampicin
- Extensive drug resistance (XDR): resistance as defined by MDR plus resistance to any fluoroquinolone plus any one of the injectables which include kanamycin, amikacin and capreomycin

1.1.8 Drug Resistant Tuberculosis Treatment:

Treating drug resistant *Mtb* (DR-TB) has been extremely difficult due to the lack of second line treatment options available, and once second line regimens were developed, their adverse side effect profile and prolonged duration of therapy lasting 18 to 24 months led to poor compliance in this patient group. These factors contributed to the poor outcomes associated with drug resistant *Mtb*.

Scientists have been attempting to develop new drugs and regimens in a race to curb the problem of drug resistant tuberculosis. The Damien Foundation set out to study whether a shorter fluoroquinolone-based regimen could be used to promote compliance to therapy

and thereby improve eradication rates. The prospective trial conducted in Bangladesh was a success. Gatifloxacin was used with a higher dose of isoniazid as compared to standard regimens along with ethambutol, pyrazinamide, clofazimine, kanamycin and prothionamide. The targeted duration of therapy was 9 months with 87.9% of the sample group achieving relapse-free cure within that period.[19] This was termed the "Bangladesh Regimen" which began to be used widely throughout the rest of the world with further success.

The Standard Treatment Regimen of Anti-Tuberculous Drugs for patients with MDR-TB (STREAM) phase one trial was a multi-centre trial conducted to confirm the efficacy of a shortened DR-TB regimen when compared alongside the WHO advised regimen of 2011 which advised DR-TB therapy for 20 months. The shortened regimen, which was modeled on the Bangladesh regimen, was proven to be non-inferior to the long regimen.[21]

The discovery of diarylquinolines as well as the repurposing of drugs like linezolid have begun to show promise in the eradication of drug resistant tuberculosis. The second phase of the STREAM trial currently ongoing aims to compare the shortened injectable based regimen from phase one of the trial with a completely oral, shortened regimen which includes bedaquiline and linezolid.[21]

With the introduction of the new treatment regimen for the management of multidrug resistant tuberculosis, bedaquiline, a diarylquinoline has become central in the bactericidal armamentarium. The excitement surrounding the new regimen is largely based upon the expected improvement in treatment compliance rates as a result of decreased duration of therapy as well as the regimen consisting solely of oral antibacterials.

Bedaquiline's mechanism of action involves inhibition of mycobacterial ATP synthase to exert its bactericidal effect. The drug is active against dormant bacilli which is a critical feature of any anti-tuberculous therapy as relapse is common via dormant bacilli reactivation. Bedaquiline has shown some promise in studies already conducted in which the time to clearance of the mycobacterium was significantly decreased in comparison to previous regimens used to treat drug resistant tuberculosis.[22]

The first multi-stage trial done for a diarylquinoline was the TMC207 trial. TMC207 was the initial name for bedaquiline. The trial compared patients with multi-drug resistant tuberculosis in two arms The first group received standard, five drug second line therapy

plus placebo, while the second group received the standard second line therapy plus bedaquiline 400mg daily for two weeks followed by 200mg three times per week for six weeks.[21] The outcomes of the trial showed a shortening in the duration of time to conversion of sputum culture to negativity, a higher number of culture negative cases after week 8 of treatment and an acceptable side-effect profile.[21]

There were 2 deaths noted in the non-bedaquiline group and 10 deaths in the bedaquiline group however, of the 10 deaths there were no deaths directly related to drug toxicity. 5 of those patients died of overwhelming tuberculosis after defaulting therapy during the trial, 3 died from other comorbid conditions, 1 died in a motor vehicle accident and another who was excluded from the study after being found with Extended drug resistant TB also demised.[21] The cardiac QT interval was not increased in any of the patients who died and no death was attributed to sudden cardiac arrest or arrhythmia. This finding of increased mortality was not reproduced in a subsequent large, multi-center study done in South Africa. The study published in Lancet Respiratory Medicine Journal by Schnippel and colleagues found that bedaquiline added to a second line regimen was associated with a reduction in all-cause mortality when compared with the standard second line regimen in multi-drug resistant tuberculosis, rifampicin resistant tuberculosis and extremely drug resistant tuberculosis.[22]

The landscape for future drug resistant regimens looks promising. In the last decade, the transition from an injectable to oral regimen, as well as a significant shortening of duration of treatment gives hope that we are likely to see improved adherence rates and subsequent cure rates. However, much work is yet to be done if we are to achieve the target set out by the WHO for the eradication of TB by 2030.

RESEARCH PROTOCOL

1.2.1 Aim:

To describe the drug regimens and outcomes of patients diagnosed with DR-TB treated at Charlotte Maxeke Academic Hospital (CMJAH) TB clinic.

1.2.2 Study Objectives:

1. Record the drug regimens prescribed to patients diagnosed with INH mono-resistant TB, Rifampicin-resistant TB and MDR-TB between 01 January 2015 and 30 June 2019.
2. Assess match between regimens prescribed and recommended regimens. This will vary over the period of the review
3. Record the outcomes of all patients in terms of
    a. Time to culture conversion
    b. Cure
    c. Treatment completion
    d. Default
    e. Failure
    f. Death
    g. Treatment interruptions
    h. Loss to follow up

Compare outcomes between the following regimens as per South African National Tuberculosis Guidelines 2018:

I: Old long course with injectables (18-20 months):

Moxifloxacin(fluoroquinolone)+Pyrazinamide+Ethionamide+Terizodone+Kanamycin (Second line injectable group)

II: Modified short course with injectables (9-11 months):

Intensive phase (4 -6 months):

Moxifloxacin+Kanamycin+High dose INH+Ethionamide+Clofazamine+Pyrazinamide+Ethambutol

Continuation phase (5 months):

Moxifloxacin+Clofazamine+Pyrazinamide+Ethambutol

III: New long regimen, injectable-free:

Intensive phase (6-8 months):

Linezolid+Bedaquiline+Levofloxacin+Clofazamine+Terizidone

Continuation phase (12 months):

Bedaquiline+Levofloxacin+Clofazamine+Terizidone

IV: New short regimen, injectable-free:

Intensive phase (4-6 months):

Bedaquiline(24weeks)+Linezolid(8weeks)+Levofloxacin+Clofazamine+Pyrazinamide+ High dose Isoniazid+Ethambutol

Continuation phase (5months):

Levofloxacin+Clofazamine+Pyrazinamide+High dose INH+Ethambutol

V: INH Mono-resistant regimen:

High dose isoniazid+Rifampicin+Pyrazinamide+Ethambutol+Levofloxacin for 6 months

VI: Rifampicin Mono-resistant regimen:

As per short injectable-free regimen.

In order to avoid HIV co-infection as a confounding factor; HIV status, CD4 count, therapy detail and response to antiretroviral therapy will be noted.

1.2.3 Study Design:

Single centre retrospective review of out-patient files obtained from the CMJAH TB clinic.

1.2.4 Study Population and Sample:

The study population will comprise patients referred to CMJAH TB clinic with a confirmed diagnosis of DR-TB.

The sample will include all adult patients with a confirmed diagnosis of DR-TB seen between 01 January 2015 and 30 June 2019 for whom records are available.

1.2.5 Inclusion criteria:

Patients entered into one of the above listed regimens from 01 January 2015 to 30 June 2019.

Patients older than 16 years of age.

1.2.6 Exclusion criteria:

Patients who had previously been treated for MDR Tuberculosis.

Patients pregnant during the period of their treatment.

Patients younger than 16 years of age.

Individualized drug regimens not listed above.

1.2.7 Methods:

The data will be retrospectively collected and inserted into a data sheet. The files used will be those that received one of the drug resistant TB regimens at the specialist clinic over the above stated period.

1.2.8 Variables:

Primary parameters to be recorded will include: the initiation date and regimen type received by the patient and the date at which the second sputum produced by the patient reveals no growth on a culture medium. As secondary variables, information regarding HIV status, initiation date and response to antiretroviral therapy (VL and CD4 count) and treatment interruption/default will be included. Defaulting of TB treatment and adverse outcomes resulting in regimen changes will be included for completeness of the data set.

1.2.9 Data collection:

The time period for the patient to record two consecutive negative cultures will be captured and an average period in months will be obtained for each regimen. The number of mortalities in the data set will be assigned to each regimen. HIV status, CD4 count, adverse

treatment events and number of treatment defaulters will be represented for the entire data set.

In the event of data missing for primary outcomes, this was reported within the results as "missing'. Where secondary outcomes and variables were unavailable, these were omitted.

The outcomes will be compared to those done by the international community who have reviewed Bedaquiline outcomes already against traditional MDR TB regimens. REDCap will be the statistical application used to collate the data set.

1.2.10 Ethics:

The files used will be assigned study numbers in order to preserve confidentiality of the patient's medical data. The assignation will be conducted at random by myself alone. There will be no association between the study number and the patients personal details including hospital number, date of birth or family name.

1.2.11 Timing:

	Protocol Preparation	Literature Review	Protocol Asessment	Ethics Application	Data Collection	Write-up	Submission
Jan-Mar '19	X	X	X				
Apr-May '19	X	X	X	X			
June-Oct '19				X	X		
Nov-Mar '20					X	X	
April-July'20						X	
Aug-Nov '20						X	X

1.2.12 Costing Analysis:

Internet usage	R500-00
Stationary	R250-00
Photocopying, printing, binding	R3000-00
TOTAL	**R3750-00**

The total costs will be covered by the researcher.

1.2.13 References:

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CHAPTER 2 SUBMISSABLE ARTICLE

Title: A review of the efficacy of multi-drug resistant tuberculosis regimens used at Charlotte Maxeke Johannesburg Academic Hospital, South Africa

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Authors' Contributions: Jared Tulloch was the principal investigator and primary author of this research manuscript and protocol. Dr Kapila Hari and Dr Sarah Stacey assisted with the design of the study, provided guidance with reporting on the data obtained and final editing of the manuscript.

Disclosure: No conflict of interest to report.

2.1 Abstract

Background:

South Africa has a high burden of drug resistant Tuberculosis (DR-TB) and the impact of the initiation of a new drug resistant therapy regimen with the availability of bedaquiline and linezolid remains to be seen in this setting.

Objectives:

To describe the drug regimens and outcomes of patients diagnosed with DR-TB treated at Charlotte Maxeke Academic Hospital (CMJAH) TB clinic.

Methods:

The data was collected retrospectively and inserted into a data sheet. The files used were those of patients that received one of the drug resistant TB regimens at the specialist clinic over the period 01 January 2015 to 30 June 2019.

Results:

There were 105 individuals who satisfied the research criteria, 75% of the group was HIV positive. 72 patients had rifampicin mono-resistant tuberculosis and 33 had multi-drug resistant tuberculosis. Four regimens were identified: old injectable long course (15%), old injectable short course (4%), new bedaquiline long course (2%) and new bedaquiline short course (24%). The remaining 55% received individualized regimens. The overall cure rate for the period under review was 54% with a mortality rate of 9% and loss to follow up of 16%. In the group that received a bedaquiline based regimen 60% achieved cure, 49% of the injectable group and 62% that received a combination of injectable and bedaquiline therapy during their modified regimen achieved cure.

Conclusion:

The older injectable regimens showed comparable efficacy when cure rate was looked at in isolation. The areas of significant improvement in instituting a drug regimen that has a shorter duration of therapy, fewer side effects and easier administration method will likely reveal itself in years to come however this study begins to outline the improvement in treatment compliance expected.

2.2 INTRODUCTION:

Mycobacterium Tuberculosis (*Mtb*) has recently become the infectious disease with the highest mortality rate in the world.[1] The WHO estimates that 10 million people contracted TB worldwide in 2018, with 1,5 million deaths attributed to the disease in the same year.[1] The high mortality is despite many efforts from the international community to eradicate the pandemic.

The epidemiological factors associated with this disease characterize its resistance to eradication. Poverty, treatment non-compliance along with drug resistant *Mtb* are the key propagators of this global pandemic. People moving to cities to seek employment, lack of

access to adequate nutrition as well as concomitant Human Immuno-deficiency Virus (HIV) infection have created the perfect environment for tuberculosis acquisition and transmission and this allows it to thrive in economically vulnerable groups.[1]

Of the 10 million new infections that occurred in 2018, 484 000 were estimated to be drug resistant.[1] South Africa is a high disease burden nation with 301 000 new infections and 11 000 drug resistant [MDR)/ rifampicin resistant (RR)] *Mtb* cases reported during this period.[1]

South Africa officially rolled out a new regimen for the management of multidrug resistant tuberculosis in 2018.[2] The regimen which included bedaquiline and linezolid as part of the drug armament caused much excitement.[2] Bedaquiline, a diarylquinoline has become central in the bactericidal armamentarium. The excitement surrounding the new regimen is largely based upon the expected improvement in treatment compliance rates as a result of a decreased duration of therapy and the use of oral antibacterials only.

The new regimens' impact on the long-term target of eradicating *Mtb* will likely unfold in the coming years. However encouraging outcomes from similar regimens have already been noted from large trials in terms of time to culture conversion, decreased mortality as well as treatment success rates.[3–5] A close review of the health facilities involved in treating drug resistant *Mtb* in the South African setting will give local insight into the effectiveness of the regimen.

2.3 METHOD

A single centre retrospective review of out-patient files obtained from the Charlotte Maxeke Johannesburg Academic Hospitals (CMJAH) MDR-TB clinic was conducted. The study population comprised all patients referred to CMJAH MDR-TB clinic with a confirmed diagnosis of drug resistant TB.

Patients are routinely diagnosed with *Mtb* at their local clinic or secondary hospital, most commonly by the Cepheid Xpert ® MTB/RIF PCR assay, which simultaneously identifies resistance to rifampicin. If a patient is found to have rifampicin resistant TB a further sputum is collected for extended resistance testing and the patient is referred to CMJAH's TB clinic for initiation of treatment. The clinic has approximately 20 patients per week for follow-up. Patients are booked monthly for review until their treatment is complete and then

monitored for a further 2 years after completion of therapy to ensure there is no recurrence of disease.

Permission for data extraction was obtained from the CMJAH hospital executive committee as well as the University of the Witwatersrand Human Research Ethics Committee (medical) with the certificate no.M190866.

Adult patients with a confirmed diagnosis of rifampicin mono-resistant, isoniazid mono-resistant or MDR TB seen between 01 January 2015 and 30 June 2019 were included in the study. Patients with a prior diagnosis of MDR TB, pregnant patients and those with extremely drug resistant TB (XDR TB) were excluded.

The data was retrospectively collected from the out-patient files stored at the clinic and inserted into a data sheet for collation using REDCap software.

The outcomes were compared to those obtained by the international community which has already reviewed bedaquiline outcomes against traditional MDR TB regimens.[3–5]

2.4 VARIABLES

Primary parameters recorded included: initiation date and regimen type received by the patient, time to culture conversion, adverse drug reactions recorded during the patient's treatment and whether cure was achieved.

As secondary variables, information regarding the patient's age, sex and HIV status were included. CD4 count and HIV viral load were added in HIV positive patients in order to ensure that these were not confounding factors.

Defaulting of treatment and adverse outcomes resulting in regimen changes were included for completeness of the data set.

2.5 DATA MANAGEMENT AND ANALYSIS

Completed data collection forms were captured into a REDCap database. Data from the REDCap database were exported into Stata® 14.2 for analysis. Descriptive statistics were used to describe demographic and clinical profiles of the patients registered for DR-TB during the defined period. Median and interquartile ranges were used for continuous variables while frequencies and percentages were used for categorical data. Demographic

variables analysed included age and gender while clinical variables included method of TB diagnosis, drug resistant TB profile, presence of mutations, HIV status as well as CD4 count and viral load levels for those who were HIV positive. Data analysis methods used for analysis of outcomes are described below according to the study objectives.

Objective 1:

Record the drug regimens prescribed to patients diagnosed with INH mono-resistant TB, Rifampicin-resistant TB and MDR-TB between 01 January 2015 and 30 June 2019.

Objective 2:

Assess match between regimens prescribed and recommended regimens.

Frequencies and proportions were used to describe number of patients who were prescribed any of the four standardized regimens according to their drug resistance profile at diagnosis. The number and proportion of the patients who received individualised regimens were also determined and presented by DR profile.

Objective 3:

Determine the outcomes of all patients in terms of time to culture conversion, cure, treatment completion, treatment failure, death, treatment interruptions and loss to follow up.

TB treatment outcomes were determined as frequencies and proportions (percentages). Each outcome was determined as the number of patients who had the outcome of interest as a proportion of all patients who started treatment for DR-TB. Outcomes of DR-TB treatment were described by regimens prescribed. Factors associated with a favourable outcome (defined as cure) were determined using univariable and multivariable logistic regression. Variables which had a p-value of <0.2 in univariable analysis were included the multivariable model.

Objective 4:

Compare outcomes between the following regimens as per South African National Tuberculosis Guidelines 2018

Outcomes of DR-TB treatment were determined for the following regimens of DR-TB treatment as frequencies and proportions.

- Old long course with injectables (18-20 months)
- Modified short course with injectables (9-11 months)
- New long regimen, injectable-free
- New short regimen, injectable-free

2.6 RESULTS

There were a total of 105 individuals who satisfied our study criteria over the period of 01 January 2015 to 30 June 2019. Basic demographics were outlined in table 1 where a median patient age was 35 years. a significant proportion of the study group had concomitant HIV infection(75%) and the majority were being treated for TB diagnosed on sputum specimens, with Rifampicin mono-resistant TB being the predominant form as noted in figure 1 (67.6%). A notable proportion of the patients received an individualised regimen (55%). Table 3 notes that there were 9 recorded deaths within the group while 57 patients achieved cure. Figure 4 highlights kanamycin as the drug with the highest association to adverse events, with 28 recorded adverse events. 49% of patients that received an injectable regimen achieved cure, while the bedaquiline based regimen and those who received both injectable and bedaquiline had cure rates of 60 and 62% respectively.

1. Demographic and clinical profile of patients treated for DR TB

Table 1: Demographic and clinical characteristics of enrolled patients

Characteristic	**N (%)**
Age (median, IQR)	35 (30- 42)
Age <35 years	47 (44.8)
Males	51 (49)
Females	54 (51)
Specimen used for TB diagnosis	
Sputum	96 (91)
Histology	1 (1)
Aspirate	8 (8)
HIV positive	79 (75)
CD4 category*	
<50 cells/ml	17 (22)
50- 200 cells/ml	33 (42)
>200 cells/ml	29 (37)
Viral Load category*	
≤1000 copies/ml	27 (34)
>1000 copies/ml	52 (66)

*Denominator is HIV positive patients

Percentages rounded to the nearest whole number

2. *Drug resistance profile of patients treated for DR TB at start of treatment*

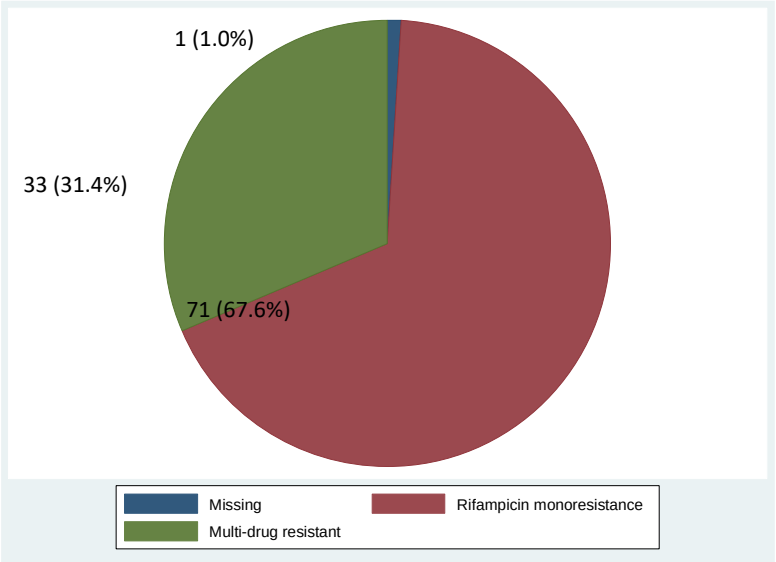

Figure 1: Profile of drug resistance at start of DR TB treatment

3. *Prevalence of different type of mutations at baseline*

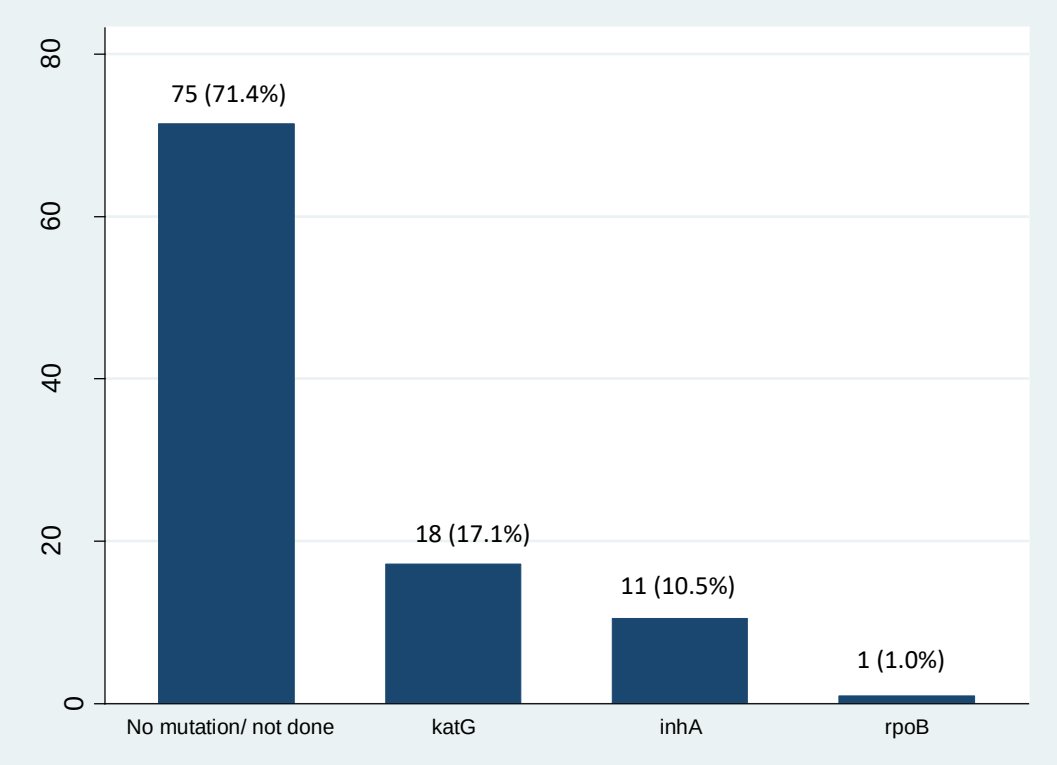

Figure 2: Distribution of resistance mutation at start of treatment

4. Drug resistance and the regimens prescribed

Of the 105 patients, 47 received standardized regimens while 58 received individualised regimens. Of the 47 who received the standardized regimens, 31 had Rifampicin monoresistance while 16 had MDR-TB.

Table 2: Drug regimens received by drug resistance status

Drug resistance pattern	Standardized regimens	N (% who received regimen)
Rifampicin mono-resistance N=72	Old long course with injectables (18-20m)	10 (14.1)
	Modified short course with injectables (9-11m)	2 (2.8)
	New long regimen, injectable-free	1 (1.4)
	New short regimen, injectable-free	18 (25.4)
	Individualised regimen	41 (57.8)
Multi-drug resistance N=33	Old long course with injectables (18-20m)	6 (18.2)
	Modified short course with injectables (9-11m)	2 (6.1)
	New long regimen, injectable-free	1 (3.0)
	New short regimen, injectable-free	7 (21.1)
	Individualised regimen	17 (51.5)

5. Outcomes of drug resistance treatment

Table 3: Description of outcomes of DR TB treatment among patients enrolled

Type of outcome	N(%)
Culture conversion	
< 4 months	71 (67.2)
4- <6 months	14 (13.3)
6- <12	5 (4.8)
12- <16	1 (1.0)
>=16	5 (4.8)
Missing	9 (8.6)
Drug related adverse event	
No	54 (51.4)
Yes	36 (34.3)
Missing	15 (14.3)
Adverse event grade (N=36)	
Grade 1	20 (55.6)
Grade 2	13 (36.1)
Grade 3	2 (5.6)
Grade 4	0
Missing	1 (2.8)
Outcomes	
Died	9 (8.6)
*LTFU**	12 (11.4)
Transfer out	11 (10.5)
Treatment failure	11 (10.5)
Cure	57 (54.3)
Missing	5 (4.8)

Grade 1-Mild; mild discomfort, no medical intervention needed.

Grade 2-Moderate; minimal supportive medical care required.

Grade 3-Severe; marked limitation to activity, medical therapy required with possible hospital admission.

Grade 4-Life threatening; marked limitation to activity with hospital admission, significant medical intervention required.[6]

**LTFU- Loss to follow-up.*

6. Factors associated with cure (favourable outcome)

Overall, of the 105 enrolled, 57 (54.3%) had documented cure and were considered to have had a favourable outcome.

Table 4: Univariable and multivariable analysis of factors associated with a favourable outcome (cure) among enrolled patients (N=105)

Variable	n (%) Favourable outcome	Univariable OR (95% CI)	p-value	Multivariable OR (95% CI)	p-value
Age <35 years					
No	35/58 (60.3)	1		1	
Yes	22/47 (46.8)	0.58 (0.27- 1.26)	0.168	0.58 (0.24- 1.40)	0.228
Gender					
Female	32/57 (59.3)	1			
Male	25/51 (49.0)	0.66 (0.31- 1.43)	0.293		
HIV positive					
No	16/26 (61.5)	1			
Yes	41/79 (51.9)	0.67 (0.27- 1.67)	0.393		
Drug resistance profile					
Rif resistance	40/71 (55.6)	1			
MDR TB	17/33 (51.5)	0.82 (0.36- 1.89)	0.646		
Standard regimen					
No	28/58 (48.3)	1		1	
Yes	29/47 (61.7)	1.73 (0.79- 3.77)	0.171	1.29 (0.50- 3.32)	0.599
Severe adverse events					
No	51/88 (58.0)	1		1	
Yes	6/17 (35.3)	0.40 (0.13- 1.17)	0.093	0.42 (0.11- 1.53)	0.189
Culture conversion < 4m					
No	8/34 (23.5)	1		1	
Yes	49/71 (69.0)	7.24 (2.83- 18.5)	<0.001	6.78 (2.61- 17.64)	<0.001

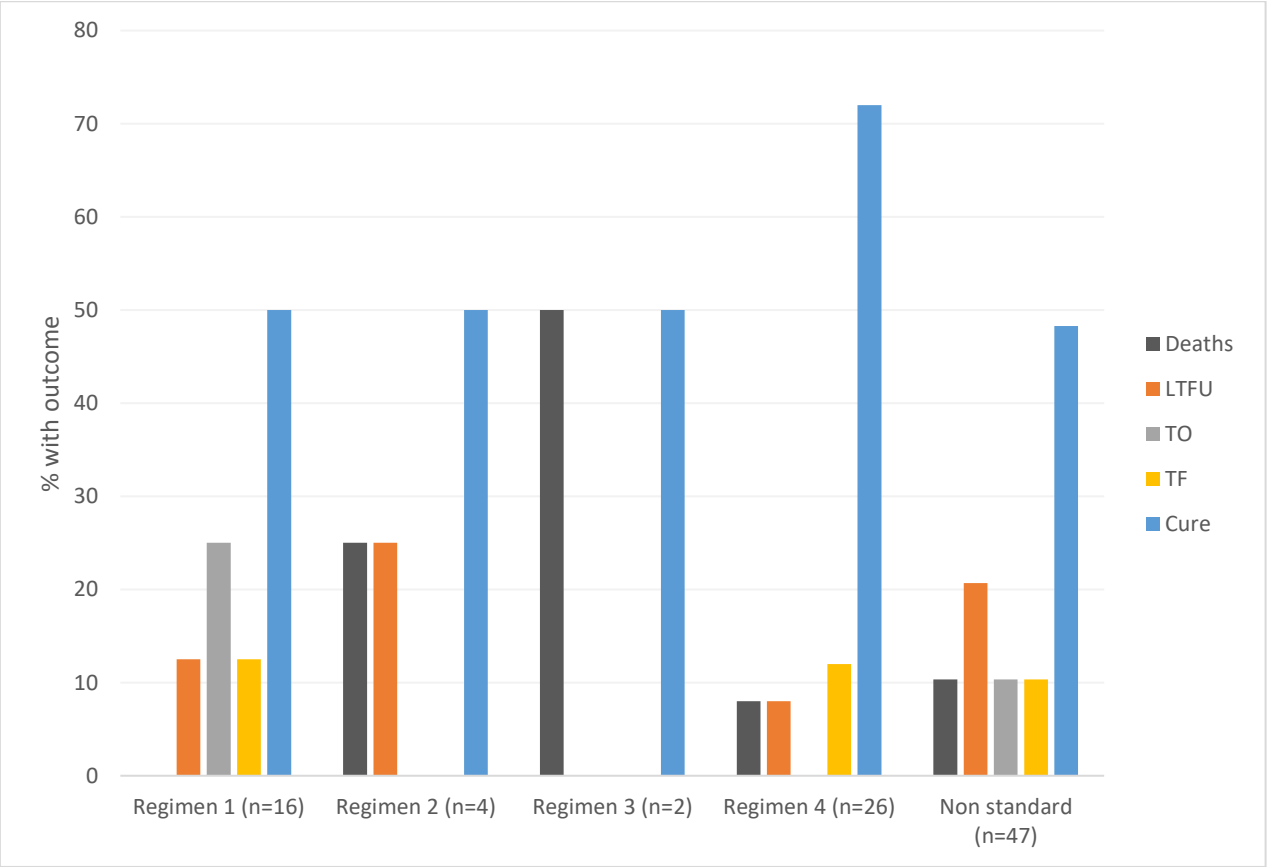

Figure 3: DR TB treatment outcomes according to treatment regimen

Regimen 1= Old long course with injectables (18-20m); Regimen 2= Modified short course with injectables (9-11m); Regimen 3= New long regimen, injectable-free; Regimen 4 = New short regimen, injectable-free, Non-standard regimens, LTFU-Loss to follow-up, TO-Transfer out, TF-Treatment failure.

Table 5: TB treatment outcomes comparing regimens with an injectable to bedaquiline based regimens (N=105)

Outcome	Injectable (n=57)	Bedaquiline only (n=35)	Both injectables and Bedaquiline (n=13)	X^2 p-value (Injectable vs BDQ excluding those with both)
Death	4 (7.0)	4 (11.4)	1 (7.7)	0.466
LTFU	9 (15.8)	6 (17.1)	2 (15.4)	0.865
Transfer out	10 (17.5)	1 (2.9)	0	0.035
Treatment Failure	6 (10.5)	3 (8.6)	2 (15.4)	0.759
Cure	28 (49.1)	21 (60.0)	8 (61.5)	0.310

LTFU= loss to follow up; Both= Patients that initially received an injectable regimen but were subsequently changed to a bedaquiline regimen due to contra-indications to injectables

7. Frequency of adverse events

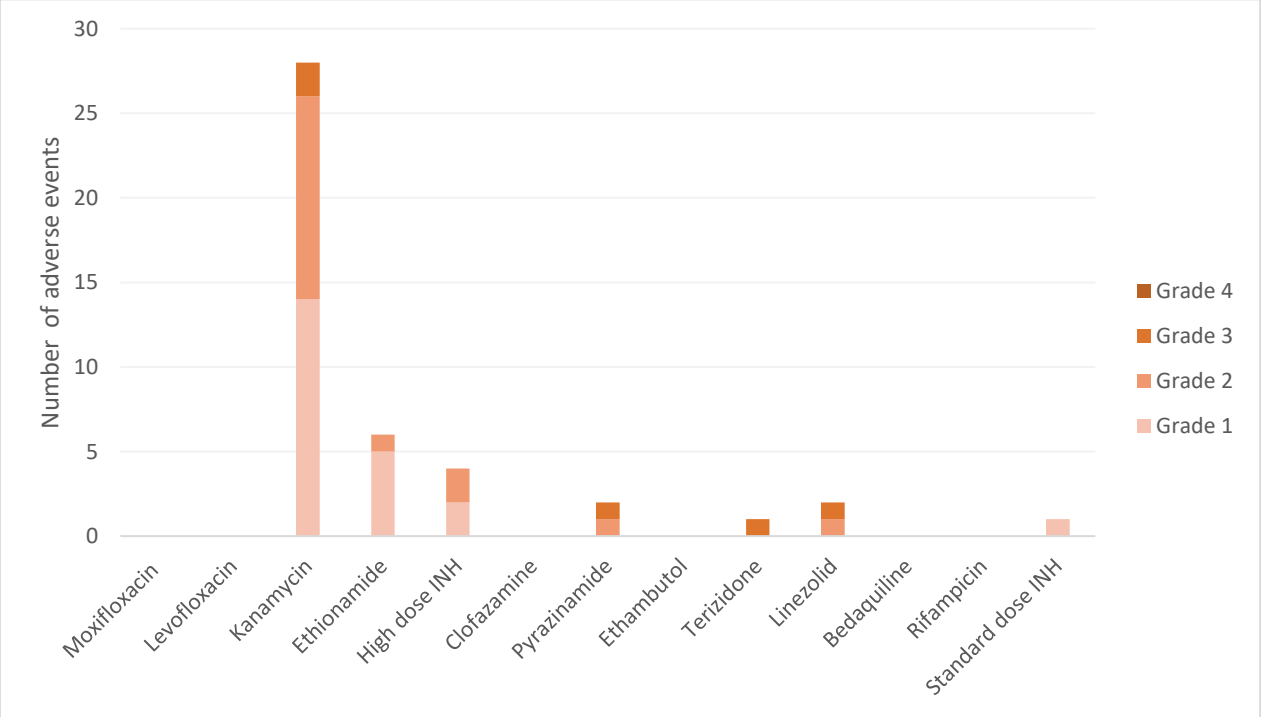

Figure 4: Frequency of adverse events by severity of adverse events

2.7 Discussion:

South Africa has a disproportionately high number of cases of drug resistant TB.[7] The drug resistant TB clinic at CMJAH caters for patients residing in central Johannesburg, South Africa's largest urban population. Our study documents the outcomes of patients with MDR-TB in a high HIV prevalence setting. The study was intended to describe the various drug regimens that were used at this facility over the period and report on the outcomes recorded for this data set.

The clinic achieved a cure rate for the period under review of 54%. The success rates in available reviews varies between 60-70%.[10] The relatively low cure rate in our clinic is in variance with clinical trials in which higher cure rates (between 78-80%) are documented but better than the global cure rate, which according to a recent WHO report is 48%.[1,11] The higher rates in trials can be attributed to clinical trial selection bias and the comprehensive patient support provided to trial participants.[11]

The median age of patients included in our cohort was 35 years, similar to that reported by Farley et al (36.5 years).[8] They reviewed patients with MDR-TB in a South African cohort.

In our study the majority (75%) of patients were HIV positive. In South Africa almost 63% of people with newly diagnosed TB are HIV positive, but in Gauteng HIV/TB co-infection is much higher (75%).[7] Furthermore, the higher proportion of HIV co-infection in patients with MDR-TB echoes the findings of a systematic review which examined the relationship between the two infections. The authors concluded that HIV infection had a significant association with primary MDR-TB and a definite association with secondary MDR-TB.[9]

The proportions of patients who had rifampicin monoresistance and multi-drug resistance in our study (68% and 31% respectively) were strikingly similar to that reported in a 2014 South African study which looked at the prevalence of drug-resistant TB. In the study by Ismail et al rifampicin resistance was confirmed in 70% and MDR-TB in 30% of patients surveyed.[7] Our numbers do not wholly match global surveillance data, which reported multi-drug resistance in 82% and mono-drug resistance in the remaining 18%.[1] One of the reasons for this inconsistency would be the unknown isoniazid susceptibility in many patients included in the global data.[11]

Unfortunately, only 29% of subjects in this study had resistance mutations tested by line probe assay. The *katG* mutation was the most frequent mutation detected and this finding is consistent with a study from the Free State which found this to be the predominant isoniazid resistance-conferring mechanism among MDR-TB isolates.[12] The 71.4% of the study group that did not have a mutation recorded can be attributable to *rpoB* gene mutations as the Cepheid Gene Xpert detects for this mutation prior to a full line probe assay being performed.

In our subject group that received a bedaquiline based regimen 60% achieved cure, 49% of the injectable group and 62% that received a combination of injectable and bedaquiline therapy during their modified regimen achieved cure, respectively. In phase 2 of the Diacon et al TMC207 multi-centre trial, similar results were noted when comparing a bedaquiline based regimen against the standard injectable therapy with cure rates of 79% in the bedaquiline arm and 58% in the non-bedaquiline arm.[4]

There were 58 patients who received an individualized drug regimen (55%). This was attributed to two factors; contra-indications to various drugs including ototoxicity related to aminoglycoside injectable therapy, ethionamide associated thyroid dysfunction as well as isoniazid related neuropathy and hepatotoxicity. The second factor was the period under which the drug resistance clinic was reviewed. The study covered a period of transition between published treatment guidelines which changed due to the availability of new drugs.[2] Initially patients that showed intolerance to injectable regimens were the first to be converted from the injectable regimens to the bedaquiline based regimens, with new patients being initiated on a bedaquiline based regimen from the first quarter of 2018. Some international studies on individualized regimens have been more favourable than standardized drug regimens with some trials reporting cure rates of 80% using this approach.[13] The drawback of this approach is that it requires access to specialized health care where individual treatment regimens can be designed by trained health care workers analysing drug sensitivity profiles.

The most significant drug intolerance was related to the injectable aminoglycoside (kanamycin), with 28 patients having documented adverse reactions to this treatment, largely hearing loss and local tissue damage at the intra-muscular injection site. Side-effects related to amikacin are well described, including a South African study which looked at the

incidence of ototoxicity and found it to be as high as 57%.[14] We found 42% of our patients experienced ototoxicity resulting in change of their regimen.

A mortality rate of 9% in our study with loss to follow up of 11% was an improvement when compared to trials done in South Africa on similar population groups where HIV prevalence is high. A similar cohort by Farley et al. found a 23% mortality rate and a 21% default rate in 2011.[8]

The new bedaquiline short regimen achieved the most favourable cure and mortality rates. This is in congruence with local and international studies including the STREAM trial which is still ongoing.[5] There could be several factors contributing to this outcome. The short bedaquiline regimen, initially reserved for patients with relatively uncomplicated drug resistant tuberculosis, was constituted of drugs with a more favourable side-effect profile and had the shortest duration of all the regimens. A more equal comparison would be to compare the bedaquiline short regimen against the injectable short regimen and the bedaquiline long regimen against the injectable long regimen. Unfortunately, only two patients received the bedaquiline long regimen so comparison of these outcomes was not possible.

There were eleven patients in the study group who failed to convert to culture negativity after more than six months of therapy (10%). Six of the patients received the injectable regimen, three received the bedaquiline based regimen while two patients initially started on the injectable regimen and were subsequently converted to the Bedaquiline regimen. All the patients who failed to convert were documented to have had one or more episodes of non-compliance to treatment during their treatment period. This is in keeping with local treatment failure rates documented elsewhere. Farley et al. studying a similar population set also noted treatment failure in 9.8% of their study group.[8]

2.8 Limitations and Sentiments:

A minimum of three visits to the clinic was required before a patient would be included into the study group. This was done as a number of the data point variables for completion of the data sheet took at least 3 visits before they were attainable.

There were a number of patients who attended the clinic less than 3 times. This was because some of these patients were referred on to Sizwe Tropical Medicine Hospital from the outset

if they were diagnosed with XDR-TB. Others were lost to follow up within the initial treatment phase and some chose to relocate to facilities closer to their homes for the period for which they were required to take treatment.

Missing data from patients who attended more than 3 clinic visits was omitted when secondary variables were reported and when data for the primary endpoints were not available, they were displayed in our results as *"missing".* The final outcomes described in the data set identified an incomplete capture of 5 subjects (4.8%).

Prior to the roll-out of bedaquiline in 2018, patients failing the injectable regimen at CMJAH were referred to a specialist TB hospital like Sizwe Tropical Hospital for unique drug regimens that could be monitored daily on an in-patient basis. Once bedaquiline and linezolid were made accessible, it allowed the unit to cater for more complicated drug resistant TB cases as there was an increased number of drug options available with sufficient bactericidal activity.

To limit the level of confounders and bias it was ensured that all patient files that met the inclusion criteria were recorded while the exclusion criteria were limited to those where an outcome was not obtainable. The patient names were not overtly discernable when the data was captured as their study numbers were allocated prior to the date of capture. The data capturing was performed by an experienced medical professional who understood the material however it would have been advantageous if the capturing of the data set could have been out-sourced in order to avoid further bias.

The thought behind improved outcomes from the new regimen is postulated to be related to the improved side-effect profile of the regimen as well as the improved compliance to treatment as a result of a regimen that no longer requires the patient to attend a health facility daily in order to receive their injectable antibiotic. The new regimen allows for completely oral therapy for a shorter treatment period than ever before which will encourage patients diagnosed with MDR-TB to complete the course of therapy.

2.9 Conclusion:

The review of CMJAH DR-TB clinic over the period of evolving drug treatment regimens elucidates key developments in the progression of drug resistant therapy. While the older injectable regimens showed comparable efficacy when cure rate as an outcome was studied

in isolation, the improvement in treatment completion rate and adverse event rate identified the areas of significant improvement in instituting a drug regimen that has a shorter duration of therapy, fewer side effects and easier administration. With a drug regimen that is better tolerated by the patient, a more sustainable method of eradicating drug resistant tuberculosis could be on the horizon.

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CHAPTER 3 APPENDICES:

3.1 Definitions

Culture conversion:

Two negative cultures of a liquid sputum medium that were taken at least 25 days apart while on a drug resistant anti-tuberculous regimen.[2]

Cure:

A patient who has undergone culture conversion and maintained a negative culture for three consecutive specimens, each 30 days apart, during the continuation phase of therapy. Completion of at least nine months of therapy.[2]

Loss to follow up:

Treatment interruption of equal to or more than two months.[2]

Treatment failure:

1. Failure to culture convert after six months of treatment.
2. Two or more positive cultures during the initial six months of therapy with deterioration in clinical condition.
3. Culture positivity recorded after culture conversion has occurred.[2]

Transferred out:

Referral of the patient to another treatment facility whereby further recordings of treatment progress is carried out at that facility.[2]

Death:

Patients with confirmed death certification during the period of treatment studied.[2]

Adverse events:

These were defined as per the Division of Microbiology and Infectious Disease adult toxicity tables[6]:

Grade 1

Mild; mild discomfort, no medical intervention needed.

Grade 2

Moderate; minimal supportive medical care required.

Grade 3

Severe; marked limitation to activity, medical therapy required with possible hospital admission.

Grade 4

Life threatening; marked limitation to activity with hospital admission, significant medical intervention required.

<u>Drug regimens:</u>

Old long course with injectables (18-20 months):

Moxifloxacin(fluoroquinolone)+Pyrazinamide+Ethionamide+Terizodone+Kanamycin (Second line injectable group)

Modified short course with injectables (9-11 months):

Intensive phase (4 -6 months):

Moxifloxacin+Kanamycin+High dose INH+Ethionamide+Clofazamine+Pyrazinamide+Ethambutol

Continuation phase (5 months):

Moxifloxacin+Clofazamine+Pyrazinamide+Ethambutol

New long regimen, injectable-free:

Intensive phase (6-8 months):

Linezolid+Bedaquiline+Levofloxacin+Clofazamine+Terizidone

Continuation phase (12 months):

Bedaquiline+Levofloxacin+Clofazamine+Terizidone

New short regimen, injectable-free:

Intensive phase (4-6 months):

Bedaquiline(24weeks)+Linezolid(8weeks)+Levofloxacin+Clofazamine+Pyrazinamide+ High dose Isoniazid+Ethambutol

Continuation phase (5months):

Levofloxacin+Clofazamine+Pyrazinamide+High dose INH+Ethambutol

3.2 Data collection sheet

Confidential

MDR TB capture form

Record ID	_____
Study Number	_____
Age	_____
Sex	☐ Male ☐ Female
HIV status	☐ Positive ☐ Negative ☐ Unknown
CD4 Count	☐ < 100 ☐ 100-200 ☐ >200 ☐ N/A (For HIV positive only)
HIV viral load	☐ < 1000 ☐ >1000 ☐ N/A (For HIV positive only)
Drug resistance pattern	☐ INH mono ☐ Rif mono ☐ MDR
Specific mutation	☐ KatG ☐ INHA ☐ rpoB
Method of diagnosis	☐ Sputum ☐ Histology ☐ Aspirate

Drug List

	0-4 months	>4 months	Drug related adverse event
Moxifloxacin	☐	☐	☐
Levofloxacin	☐	☐	☐
Kanamycin/injectable	☐	☐	☐
Ethionimide	☐	☐	☐
High dose INH	☐	☐	☐
Clofazamine	☐	☐	☐

Pyrazinamide	☐	☐	☐
Ethambutol	☐	☐	☐
Terizidone	☐	☐	☐
Linezolid	☐	☐	☐
Bedaquiline	☐	☐	☐
Rifampicin	☐	☐	☐
Standard dose INH	☐	☐	☐

Time to Second culture negative	☐ < 4 months ☐ 4-6 months ☐ 6-12 months ☐ 12-16 months ☐ >16 months
Mortality	○ Yes ○ No
Loss to Follow-up	○ Yes ○ No
Treatment failure	○ Yes ○ No
Cure	○ Yes ○ No
Drug related adverse event	○ Yes ○ No
Grade of adverse event	☐ Grade 1 ☐ Grade 2 ☐ Grade 3 ☐ Grade 4 (Only if adverse event "yes")

3.3 Ethical clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Jared Tulloch

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M190866 MED19-06-127

NAME: (Principal Investigator)	Dr Jared Tulloch
DEPARTMENT:	Internal Medicine Charlotte Maxeke Academic Hospital
PROJECT TITLE:	A review of the efficacy of multi-drug resistant tuberculosis regimens used at Charlotte Maxeke Academic Hospital
DATE CONSIDERED:	30/08/2019
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	
APPROVED BY:	Dr C Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	20/09/2019

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed August and will therefore be due in the month of August each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

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SENATE PLAGIARISM POLICY: APPENDIX ONE

I Jared Tulloch (Student number: 363590) am a student registered for the degree of Master of medicine (internal medicine) in the academic year 2020.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
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