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Rebecca S. Goldstein Date

Utilizing genotypic mutations to determine treatment effectiveness among MDR and XDR TB patients in KwaZulu-Natal, South Africa

By

Rebecca S. Goldstein

Master of Public Health

Hubert Department of Global Health

Neel R. Gandhi, MD

Committee Chair

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By

Rebecca S. Goldstein

B.A., Cornell University

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Thesis Committee Chair: Neel R. Gandhi, MD

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Abstract

Utilizing genotypic mutations to determine treatment effectiveness among MDR and XDR TB patients in KwaZulu-Natal, South Africa

By Rebecca S. Goldstein

Introduction. Tuberculosis (TB) remains the second leading cause of infectious disease death worldwide, and TB drug resistance is associated with dramatically worse treatment outcomes. South Africa's KwaZulu-Natal Province has among the highest caseloads of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB globally. Regimens require at least four likely effective drugs to improve treatment outcomes; yet without sufficient lab capacity, phenotypic drug susceptibility testing is impractical. Genotypic susceptibility testing can fill this need. We utilized sequencing data to estimate susceptibility for drugs used in standardized MDR and XDR regimens, and to examine whether treatment outcomes vary based on different numbers of likely effective drugs.

Methods. We used isolates and data from two studies in KwaZulu-Natal to characterize the frequencies of resistance-conferring mutations among MDR and XDR TB participants. We calculated the number of likely effective drugs that subjects received. In a subset of MDR participants, we examined the association between number of effective drugs and treatment outcomes. We also described the frequency of adverse events, and estimated the odds of unsuccessful treatment using multivariate analysis.

Results. We analyzed sequencing data for 90 MDR and 363 XDR participants (n=453 total). Resistance-conferring mutations were most frequent in the *pncA* (74%) and *inhA* (39%) genes among MDR participants. Over 88% of XDR participants exhibited resistance-conferring mutations for each gene examined. 90% of the MDR, but only 23% of XDR participants received ≥ 4 effective drugs. There was no significant association between the number of effective drugs and treatment outcomes in the subset of MDR participants. The number of adverse events experienced was significantly associated with treatment outcome (aOR=4.1, 95CI 1.3 – 12.9).

Conclusions. Genotypic mutations conferring resistance to antituberculosis drugs were common, and the vast majority of MDR and XDR participants received at least one drug providing no clinical benefit. There was no association between the number of effective drugs and outcomes among MDR patients. However, similar analyses should be performed among XDR populations, where only 23% received at least four effective drugs. The frequency of adverse events was associated with outcomes, and further efforts are needed to minimize their frequency and impact.

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CHAPTER 1.
INTRODUCTION

Introduction and Rationale

Worldwide, tuberculosis (TB) is the second leading cause of death from an infectious disease. The emergence of drug resistance in TB is of great public health concern because increasing resistance results in greater difficulty of treating TB; drug-resistant TB treatment is associated with worse treatment outcomes, and requires the use of second- and potentially third-line TB medications.[1,2] The spread of MDR and XDR TB threatens the goal of TB elimination.[3] South Africa's KwaZulu-Natal Province has among the highest caseloads of MDR and XDR TB globally.[4-7] High-quality, aggressive drug-resistant TB treatment is essential to improve treatment outcomes and control the drug-resistant TB epidemic. This requires appropriate drug regimens, which must include at least four different drugs to which an individual's TB strain is susceptible.[1,8]

Drug susceptibility can be assessed through culture-based phenotypic drug susceptibility testing (DST) methods, or through molecular methods. Phenotypic DST can be impractical where lab capacity is low—including in South Africa[3]—and second-line susceptibility testing is not widely available.[9] Genotypic susceptibility testing can fill a need where phenotypic DST poses serious limitations. Its rapid delivery of results can help patients receive more effective individualized drug regimens earlier, thereby optimizing their treatment, reducing infectious periods, and improving outcomes.[8,10,11] This emphasis on improved treatment is essential because acquired drug resistance is developed and perpetuated by inadequate treatment.[2,12-14] Because drug susceptibility data are not available for all first- and second-line TB drugs, South Africa employs standardized TB treatment regimens for MDR and XDR TB. Standardized regimens are

more feasible on a population level, but can result in lower quality of treatment at the individual patient level.

Problem statement

In South Africa, patients receive a standardized drug regimen, the effectiveness of which is largely dependent on baseline genotypic resistance. However, DST is not widely available for most second-line drugs. Without continued susceptibility testing for second-line antituberculosis drugs or knowledge of the current molecular epidemiology of resistance-conferring genes, the effectiveness the MDR and XDR TB standardized drug regimens is unknown.

Purpose of this Study

This study aims to determine the clinical benefit that MDR and XDR TB patients in KwaZulu-Natal receive from the respective standardized drug regimens. Further, it examines whether treatment outcomes differ among MDR TB patients who receive different numbers of likely effective drugs.

We used isolates and data from two studies in KwaZulu-Natal province, South Africa to describe genotypic, and infer phenotypic, resistance to six specific antituberculosis drugs in MDR and XDR TB patients. After characterizing the frequency of resistance-conferring mutations, we aimed to understand the clinical benefit that patients receive from the drugs in the standardized regimen by evaluating the number of effective drugs received, treatment outcomes, and the frequency of adverse events that patients experienced throughout their course of treatment.

Research Questions

This study aimed to answer the following four research questions:

1. What proportion of MDR and XDR patients has a genotypic mutation with likely phenotypic resistance to six specific antituberculosis drugs (pyrazinamide, ethionamide, kanamycin, capreomycin, moxifloxacin, and high-dose isoniazid)?
2. Based on genotypic resistance data in question 1, how many likely effective drugs did individual MDR and XDR TB patients in this study receive?
3. Do treatment outcomes differ among MDR TB patients who receive different numbers of effective drugs?
4. Is there a difference in the frequency of increased resistance between patients receiving different numbers of effective drugs?

Significance of this Study

By answering the research questions, this study can help to assess how patients benefit from the current standardized drug-resistant TB regimens in South Africa. Accordingly, this information can be used to propose any necessary actions to ensure that the standardized regimens provide sufficient clinical benefit to the large populations of MDR and XDR TB patients in KwaZulu-Natal. Results from this study also can indicate the potential benefit of expanding the use of second-line DST. Finally, this study will explore whether patients receiving more or fewer effective drugs have different treatment or adverse event outcomes, thereby providing evidence that can be used to optimize treatment regimens to maximize successful treatment outcomes and minimize adverse events.

Definition of Terms

Patients in this study either had multidrug-resistant (MDR) TB or extensively drug-resistant (XDR) TB. MDR TB occurs when there is resistance to at least isoniazid and rifampin, the two most powerful first-line antibiotics. Additional resistance to second-line drugs—specifically to a fluoroquinolone and to at least one of the injectable agents—results in XDR TB.

In KwaZulu-Natal province, South Africa, MDR TB patients receive a standardized six-drug regimen that includes kanamycin, moxifloxacin, ethionamide, ethambutol, pyrazinamide, and terizidone for six months, and patients continue on this regimen without kanamycin for an additional 18 months.[5] XDR TB cases receive a similar eight-drug regimen, except that kanamycin is replaced by capreomycin, and XDR patients also receive high-dose isoniazid, para-amino salicylic acid (PAS), and occasionally additional third-line drugs.

We utilized sequencing data to 1) determine if mutations were present, and 2) characterize genetic mutations. Sequences with no mutation at a specific gene were called "wild-type." If a mutation was present, it was further classified as synonymous or non-synonymous. A synonymous mutation is one in which there is a mutation in the isolate's DNA sequence, but it encodes the same amino acid as that of the wild-type sequence. A non-synonymous mutation is one in which a change in the DNA sequence causes the encoded amino acid in a protein to be different. Based on this classification, MDR and XDR TB patients were defined as being likely resistant to a given antituberculosis drug if sequencing results exhibited a non-synonymous mutation in a known resistance-conferring region of the gene. Patients were defined as likely susceptible to a given drug, and expected to be phenotypically susceptible to it, if their isolate's sequence was wild-type or had a synonymous mutation.

A likely effective drug was one to which an MDR TB patient's isolate had no mutation in a corresponding resistance gene, or to which the patient had a synonymous genetic mutation. A likely resistant drug was one to which a patient's isolate exhibited at least one non-synonymous mutation in a corresponding resistance gene.

CHAPTER 2.
COMPREHENSIVE REVIEW OF THE LITERATURE

TUBERCULOSIS EPIDEMIOLOGY

Tuberculosis is an airborne bacterial disease caused by the transmission of *Mycobacterium tuberculosis* from an individual with active tuberculosis (TB) disease. It typically affects the lungs, and is treated with a rigorous and lengthy course of antibiotics.[15]

Global Tuberculosis Epidemiology

Worldwide, in 2013, nine million people were diagnosed with TB, and 1.5 million people died from TB.[3] These World Health Organization (WHO) estimates indicate that TB is second only to HIV/AIDS as the leading cause of death from an infectious disease.[1] While the mortality rate due to TB has decreased 45% worldwide since 1990, TB is still a pressing health problem that disproportionately affects those in the developing world.[3] Similarly, TB is the leading cause of mortality among HIV-infected individuals worldwide.[16] Among the nine million new diagnoses in 2013, 1.1 million (12.2%) were diagnosed among HIV-positive patients, and 360,000 of the tuberculosis deaths (24.0%) were among HIV-positive patients.[3] Africa has the highest worldwide burden of TB-HIV co-infection.[3]

Without rigorous treatment and control, the global burden of TB will remain high. Thus, priority must be placed on achieving proper TB treatment, as well as preventing its transmission and increase in antimicrobial resistance.

Global Drug-Resistant Tuberculosis Epidemiology

A major challenge in TB control and treatment today is drug resistance. Decades of poor TB control have resulted in a large drug-resistant TB epidemic that has recently emerged as a significant threat to advances in TB control and the HIV epidemic.

TB drug resistance can be categorized into two main types. Multidrug-resistant (MDR) TB occurs when there is resistance at least to isoniazid and rifampin, the two most powerful first-line antibiotics. Additional resistance to second-line drugs—specifically, fluoroquinolones and at least one of the injectable agents—is considered extensively drug-resistant (XDR) TB. Increasing resistance to antituberculosis agents results in increased difficulty of treating the TB.

Approximately 5% of TB cases globally are MDR TB.[3] Approximately half a million new MDR TB cases emerge each year. In some nations, serious epidemics threaten progress in eliminating TB and antimicrobial resistance.[3] This indicates a pressing global health challenge.

MDR TB is a concern because it is associated with worse treatment outcomes, and requires greater financial and human resources due to the need to use second-line TB medications. These drugs are less effective, more toxic, and more costly than therapy for drug-susceptible TB. The treatment course for MDR TB is 18-24 months, and costs approximately 60 times more than that of the six months of treatment for drug susceptible TB.[17] In most high-burden MDR TB countries, treatment costs per treatment course per person are more than 100% of the gross national income per capita.[18,19]

XDR TB has been reported among individuals in 100 countries worldwide. Nine percent of MDR TB cases globally are estimated to be XDR TB.[3] Options for XDR TB treatment are severely limited because of resistance to both first- and second-line TB medications. It requires the use of any remaining susceptible first- and second-line antituberculosis drugs, as well as third-line drugs, whose effectiveness against TB is uncertain.[20]

Proper treatment for MDR and XDR TB is essential to improve outcomes and prevent the development of additional antimicrobial resistance. Of patients diagnosed with MDR TB in 2011, only 48% of detected cases were successfully treated—16% died, 12% were not cured despite having received treatment, and 24% either did not have their treatment outcome documented, or their treatment was interrupted.[3] Treatment outcomes among XDR patients are even worse, with only approximately 22% of patients achieving treatment success.[3] Achieving favorable outcomes among drug-resistant TB patients requires improvements in the current treatment practices.

In order to address the global drug-resistant TB crisis, the WHO identified 5 priority actions: 1) Prevent MDR cases through high quality treatment of drug susceptible TB, 2) Scale up and expand rapid testing and detection of resistant TB cases, 3) Provide immediate access to effective treatment and proper care, 4) Prevent transmission of TB through infection control, and 5) Increase political commitment to TB elimination with increased financing.[3]

South Africa Drug-Resistant Tuberculosis Epidemiology

South Africa experiences high rates of TB, and among the highest global rates of TB/HIV co-infection and drug-resistant TB.[3] In 2013, over 300,000 cases of TB were diagnosed in South Africa.[3] Sixty-two percent of these reported TB cases were among HIV-positive patients.[3] South Africa's substantial HIV epidemic has led to great increases in TB incidence. The subsequent high TB burden and a fragmented healthcare system together have fostered a drug-resistant TB epidemic.[21] TB/HIV co-infected populations in South Africa have exhibited excessively high early mortality, particularly among MDR and XDR patients.[22] Specifically, as compared to other

nations, XDR TB patients in South Africa also experience higher mortality likely due to elevated rates of TB/HIV co-infection.[3]

The increased mortality among HIV populations co-infected with drug-resistant TB is particularly striking in South Africa, as South Africa is considered to be one of the 27 high MDR TB burden countries.[3] In 2007, South Africa ranked fourth among countries with the highest estimated number of MDR TB cases.[7,23] Within South Africa, the KwaZulu-Natal province accounted for 38% of MDR TB cases and 50% of XDR TB cases in 2007.[24,25] In KwaZulu-Natal province, the number of MDR TB cases increased tenfold, from 216 cases in 2001 to 2,799 cases in 2007, nearly doubling the MDR TB prevalence.[7] By 2007, the MDR and XDR TB incidence in KwaZulu-Natal were 28 and 2.7 cases per 100,000 population respectively.[7]

Because of this high incidence of MDR TB and a great need to improve treatment outcomes, this thesis will focus on data collected from the KwaZulu-Natal province between 2011 and 2015.[26,27]

DRUG RESISTANCE IN TUBERCULOSIS

First-line TB drugs are those that are used to treat drug-susceptible TB cases. These include several oral agents, such as isoniazid, rifampicin, pyrazinamide, and ethambutol. Streptomycin is an injectable first-line TB drug.[28] Of the first-line drugs, isoniazid and rifampicin are considered the most efficacious, and thus, resistance to these drugs is used for the definition of MDR TB.

MDR TB requires the use of second-line drugs for treatment. These include fluoroquinolones, aminoglycosides (e.g., kanamycin, amikacin), cyclic peptide antibiotics (e.g., capreomycin), and fluoroquinolones (e.g., ofloxacin, moxifloxacin).[28] Fluoroquinolones,

aminoglycosides and capreomycin are considered the most efficacious second-line TB drugs and therefore are used for the definition of XDR TB.

Overview of Drug Resistance

Drug-resistant TB can occur when a genetic mutation impairs the effectiveness of an antituberculosis medication, thereby inhibiting the drug from killing or stopping reproduction of the TB bacteria.[15,29]

Drug-resistant TB cases arise either through one's development of resistance, or through transmission of an already-resistant strain. Resistance in TB cases that have been treated previously is called acquired resistance, and emerges from inadequate treatment.[1,2] New resistant TB cases also can result from infection by an already drug-resistant strain. In other words, a person with acquired resistance can transmit his or her resistant TB strain to others.[1,30] This is called primary resistance. The global drug-resistant TB epidemic is caused by a combination of this acquired and primary resistance, and both must be targeted in prevention strategies.[2]

Understanding Acquired Resistance

Spontaneous, independent, naturally occurring mutations can result in genotypic drug resistance.[1] These mutations can occur from the insertion, deletion, or substitution of a single nucleotide or a codon in any region of *M. tuberculosis* DNA. Such mutations can produce a range of noticeable and unnoticeable effects in amino acid production and consequent resistance to antituberculosis drugs. Most resistant strains are attributable to specific known genetic mutations, yet some drug-resistant strains do not have any known mutations.[1] Selection of strains with these resistance mutations results in acquired drug resistance.

Acquired drug resistance renders the *M. tuberculosis* bacteria resistant to commonly used antituberculosis drugs.[29] Among factors such as malabsorption and poor drug quality, acquired resistance in TB strains can occur from inadequate treatment. Prior to the clinical use of TB drugs, *M. tuberculosis* strains were susceptible to antituberculosis drugs.[1] Such strains of *M. tuberculosis* that have not yet been exposed to antituberculosis drugs are called wild-type strains; these strains typically do not exhibit genetic mutations or phenotypic drug resistance.[1]

The improper clinical use of TB drugs has been cited as one of the major causes of acquired resistance.[1,29] Sub-optimal TB treatment—including the administration of drugs to which a patient is resistant—kills or impairs drug-susceptible *M. tuberculosis* organisms, but leaves drug-resistant mutants. This puts selection pressure on the mutations. As a result, the drug-resistant mutants compose a higher proportion of the disease burden in an individual, leading to the population of *M. tuberculosis* in an infected individual to be increasingly drug-resistant.[1,14,30] Drug resistance that is clinically significant generally emerges after one to two months of inadequate TB treatment.[31]

Improper use of TB drugs can result from the inadequate prescription of antituberculosis medications, or patient non-adherence with the prescribed regimen. This may be due to a high pill burden, adverse side effects from the medications, or an inability for the patient to obtain the appropriate amount of each drug.[32,33]

Understanding Resistance in Practice

It is essential to utilize drug susceptibility results in order to properly diagnose and prescribe adequate treatment for drug-resistant TB.[1] The diagnosis of drug-resistant TB requires

testing for susceptibility to antituberculosis agents either by traditional culture-based DST or rapid molecular diagnostics.[3]

Currently, many TB cases remain untested for resistance. In 2013, only 8.5% of new bacteriologically confirmed TB cases worldwide, and only 17% of those previously treated for TB were tested for drug resistance.[3] While the proportion of new and previously treated cases with DST results has increased since 2012, they remain below the WHO's Global TB Plan's targets.[3]

Detecting Phenotypic Resistance

Phenotypic drug resistance can be assessed using mycobacterial culture and culture-based drug susceptibility testing (DST). Phenotypic DST is commonly used worldwide where lab capacity is sufficient, and can help both to diagnose MDR TB and to design individualized treatment regimens for patients. Through DST, an isolate is deemed resistant to an antituberculosis drug when the cultured *M. tuberculosis* grows in the drug's presence.

Using the proportion method, when more than 1% of a population of organisms is composed of mutants resistant to a given drug, the isolate's strain is considered resistant, and clinical treatment success with this drug is considered unlikely.[1] The proportion of resistant bacilli in the strain is assessed at the critical concentration, which is the level of the drug that inhibits the growth of a wild-type TB strain, but does not suppress the growth of a resistant strain. Thus, if more than 1% of a strain's population continues to grow at the critical concentration, the isolate is considered resistant.[1]

Phenotypic testing is used to design individualized drug-resistant TB regimens, and to provide data to regional surveillance mechanisms to inform standardized treatment regimens and monitor outcomes.[30]

Detecting Genotypic Resistance

Molecular methods can be used to identify mutations associated with drug resistance—termed "genotypic resistance." These methods use nucleic acid amplification tests—most commonly polymerase chain reaction (PCR)—to detect specific mutations that are known to be associated with drug resistance.[1] Rapid molecular tests avoid the need to grow *M. tuberculosis*, which grows very slowly, and instead utilizes PCR to detect DNA mutations that are associated with resistance.[34,35]

Applications and Limitations of Phenotypic and Genotypic DST Methods

Traditionally, culture-based methods have been the gold standard for diagnosis and DST.[36-38] Phenotypic resistance testing is valuable because it allows clinicians to gauge whether or not a drug will be effective for a particular patient based on his or her specific DST results. Using this method, however, drug resistance is generally considered to be binary—an isolate is deemed either to be resistant or susceptible to a specific drug. This paradigm fails to recognize that there is a gradient of resistance, and that for some drugs, such as ethambutol, much resistance occurs at the critical concentration.[12] In cases like this, it is often difficult to distinguish a strain's true level of resistance. This limits the sensitivity and accuracy of phenotypic testing, and poses a challenge in diagnosing and treating drug-resistant TB.

Furthermore, phenotypic testing is a time-consuming and laboratory-intensive procedure that is often difficult to accomplish in low-resource settings. Culture-based DST can take at least eight weeks to produce susceptibility results due to the slow-growing nature of the TB bacteria. It also requires sophisticated laboratories with biocontainment infrastructure. These facilities are often unavailable in low resource settings, where TB incidence can be highest.[9,21,30,38] Additionally, for many antituberculosis drugs, phenotypic tests are technically difficult to carry

out, especially in low-resource settings.[39] Thus, DST is not available for some first- and second-line drugs due to difficulties related, but not limited, to *in vitro* drug instability, varied critical concentrations, drug loss caused by protein binding, heat inactivation, and varying drug potency.[40]

Some molecular methods for detecting resistance require very simple sample preparation, have improved biosafety, and can produce results of diagnostics and some susceptibility testing within hours or days instead of weeks or months.[3,41] This faster time to diagnosis is important because early identification of MDR TB and consequent early linkage to care have been shown to improve survival outcomes.[41] Because of the rapid turnaround of results, some molecular assays have emerged recently as cost-effective resistance detection tools in certain settings.[34,35,42-45] This push to expand rapid molecular DST technologies may allow for better resistance testing in remote settings, and can lead to the ability to begin proper treatment promptly and to maximize patient retention.[35]

There are, however, several limitations posed by molecular DST methods. These include the sparse and insensitive technologies to detect resistance especially to second-line drugs, the need for consistent power, temperature control, as well as the lack of capacity and trained personnel to carry out these tests.[38,40,46,47] Additionally, the cost can be prohibitive in some settings, and some technologies have low sensitivity.[41,47,48] When incorporating these new molecular technologies, health systems must be strengthened to meet the operational demands associated with this rapid technology.[36,49] Despite these limitations, there has been a recent growing trend from relying only on phenotypic resistance testing, towards utilizing genotypic or molecular-based resistance testing.

In South Africa, of the confirmed MDR TB cases in 2013, 88% (n=8,763) had drug susceptibility test (DST) results.[3] There, however, the lab capacity to perform DST tests is low—for every five million people, the nation has approximately 1.4 labs that can perform DST testing.[3] Even among these few labs that have DST capability, DST tests are not available for many antituberculosis drugs, especially for second-line drugs used to treat drug-resistant TB.[9] Thus, the limited ability to gain widespread and thorough DST results for patients reduces the nation's ability to provide individualized treatment regimens, and indicates a need to scale up lab capacity.

TB Drugs and Resistance

Once drug resistance is suspected or identified in a patient, susceptibility tests for first- and second-line TB drugs should be requested as soon as possible in order to determine and rapidly begin the best course of treatment for a patient.[1]

Research about the molecular mechanisms of each antituberculosis agent has led to the identification of specific genetic polymorphisms that confer resistance. Below is a discussion of specific antituberculosis drugs and their known resistance patterns.

Isoniazid

Isoniazid is one of the four drugs that form the basis of first-line TB treatment.[29] It is a bactericidal pro-drug that only operates against metabolically active replicating bacilli, and it requires activation by the catalase/peroxidase enzyme *katG*, which is encoded by the *katG* gene.[29,30,50,51] INH kills *M.tb* by inhibiting essential mycolic acid synthesis most effectively in dividing cells.[30]

The two main molecular mechanisms of isoniazid resistance are associated with mutations in the *katG* and *inhA* genes, specifically in the *inhA* promoter region.[29,52] The most prevalent mutation conferring isoniazid resistance is S315T in the *katG* gene. This mutation, common in MDR TB strains, results in the isoniazid product lacking the ability to form the isoniazid-NAD adduct that is needed for isoniazid to exert its antimicrobial activity. This leads to high levels of resistance.[29,53,54] With higher doses of isoniazid, however, this resistance can potentially be overcome.[20]

The next most prevalent mutation conferring isoniazid resistance is in the *inhA* promoter region. This mutation, most commonly -15C/T, can cause overexpression of *inhA* or a mutation in its active site. Consequently, INH decreases its affinity for the isoniazid-NAD adduct, and is associated with a low level of resistance.[29,53]

Side effects of isoniazid use include hepatotoxicity/hepatitis, nausea/vomiting, and dose-related peripheral neuropathy.[28,30]

Ethambutol

Ethambutol is bacteriostatic against multiplying bacilli interfering with the biosynthesis of arabinogalactin in the cell wall. This inhibits lipid and cell wall metabolism.[29,30,55]

Mutations in the operon coding for arabinosyl transferase in the *embCAB* genes result in resistance.[29] Specifically, mutations most commonly at codon 306 of the *embB* gene cause the amino acid methionine to be substituted for either valine or isoleucine. These substitutions can increase the hydrophobicity of the surrounding region, resulting in the inaccessibility of ethambutol to reach its binding site, thus conferring resistance.[29,50,56,57] Other molecular mechanisms can result in varying levels of ethambutol resistance.[50]

Ethambutol can cause visual deficits—including optic neuritis—as well as nausea and vomiting in patients.[28]

Pyrazinamide

Pyrazinamide is a bactericidal pro-drug that is inhibits semi-dormant bacilli that reside in acidic environments, such as in TB lesions.[29,30,58] Pyrazinamide needs to be converted to its active form—pyrazinoic acid—by the enzyme pyrazinamidase/ nicotinamidase, which is encoded by the *pncA* gene.[29,59,60] Once converted to its active form, pyrazinoic acid disrupts the bacterial membrane energetics, thus inhibiting membrane transport. Instead of entering a cell by passive diffusion, this active form is excreted by a weak efflux pump, resulting in cell damage.[29,61]

Resistance to pyrazinamide is often attributed to mutations in the *pncA* gene. Mutations in *pncA* are diverse and scattered along the gene, although there is often some clustering of the mutations.[50] Most of these mutations occur in a 561-base pair region in the *pncA* gene's open reading frame, or in an 820-base pair region of its putative promoter.[29,62,63] A mutation on *pncA* at codon 159 is common.[50] Pyrazinamide resistance is uncommon in the absence of resistance to other first-line antituberculosis drugs.[1]

Some challenges in determining pyrazinamide resistance are that pyrazinamide resistance can occur without mutations in the *pncA* gene.[1,50] Additionally, it can be difficult to determine whether an isolate is resistant to pyrazinamide in a laboratory setting because pyrazinamide functions in acidic environments, which are difficult to replicate in a culture.[29]

Adverse effects of pyrazinamide use include hepatotoxicity or hepatitis, nausea/vomiting, arthropathy, and hyperuricemia.[28,30]

Ethionamide

Ethionamide is a bacteriostatic derivative of isonicotinic acid that is structurally similar to isoniazid.[29,30] It is a pro-drug that requires activation by a monooxygenase encoded by the *ethA* gene.[29] Like isoniazid, ethionamide functions by interfering with mycolic acid synthesis by forming an adduct with NAD. This adduct inhibits the enoyl-ACP reductase enzyme.[29,64,65]

Due to their structural similarity, ethionamide and isoniazid share the same target. Thus, similar DNA mutations confer resistance to both drugs. Mutations in the *inhA* promoter region can cause resistance to ethionamide as well as INH.[29,66-68] In addition, mutations in *ethA* and *ethR* can cause resistance to ethionamide.[29,67,69]

Common adverse events from ethionamide use are hypothyroidism and peripheral neuropathy. Additional side effects include nausea/vomiting, gastritis, and psychotic symptoms.[28]

Aminoglycosides (Streptomycin, Kanamycin, and Amikacin)

Aminoglycosides are a class of injectable second-line antituberculosis drugs that are bactericidal, and inhibit protein synthesis through the disruption of ribosomal function.[29,30]

<u>Streptomycin</u>

Streptomycin is an aminocyclitol glycoside that works against actively growing bacilli. It inhibits the initiation of the translation in protein synthesis.[29,70]

Mutations in *rpsL* and *rrs* are the major markers for resistance, accounting for 60 to 70% of known streptomycin resistance.[16,71] The most commonly reported mutation in the *rpsL* gene usually entails a substitution from lysine to arginine in codon 43. This produces a high level of resistance. The most common mutation in the *rrs* gene occurs around nucleotides 530 and 915.[29]

The remaining 30 to 40% of known streptomycin resistance occurs from diverse mechanisms beyond mutations in either of these two genes.[29]

Kanamycin

Kanamycin inhibits protein synthesis by alteration at the level of the 16S rRNA.[29] The most common mutations conferring this high-level kanamycin resistance occur at position 1400 and 1401 of the *rrs* gene, but mutations have also been reported at position 1483.[29,64,65] Low levels of resistance to kanamycin are also associated with mutations in the promoter region of the *eis* gene, which encodes aminoglycoside acetyltransferase. Mutations at position -10 and -35 of the *eis* promoter can result in an overexpression of the protein, and consequent low levels of resistance to kanamycin.[29,72]

Amikacin

Like kanamycin, amikacin inhibits protein synthesis by alteration at the level of the 16S rRNA.[29] Compared to other aminoglycosides in vitro, amikacin is highly mycobactericidal.[30] Mutations in the *rrs* gene confer resistance to amikacin as well as kanamycin, however complete cross-resistance between these two aminoglycosides is not ubiquitous.[29,73,74]

Side Effects of Aminoglycosides

A common side effect of aminoglycoside administration is pain at the injection site; less common effects include cochlear toxicity and consequent hearing loss, as well as increased risk of renal insufficiency.[30]

Cyclic Peptide Antibiotics (Capreomycin)

Capreomycin is an injectable cyclic peptide antibiotic that affects the methylation of ribose in rRNA. Mutations in the *tlyA* gene—which is an rRNA methyltransferase specifically

for 2'-O-methylation of ribose in rRNA—can lead to the absence of methylation activity.[29,75] Cross-resistance is often reported in varying degrees between capreomycin and kanamycin.[30] Like with aminoglycosides, side effects include pain at the injection site, ototoxicity, and nephrotoxicity.[30]

Fluoroquinolones

Fluoroquinolones are likely bactericidal agents that inhibit the DNA gyrase topoisomerase II. This DNA gyrase is a critical enzyme for bacterial viability.[30] Fluoroquinolones include ciprofloxacin and ofloxacin, which are both synthetic derivatives of the parent compound nalidixic acid.[29,76] Moxifloxacin and gatifloxacin are newer fluoroquinolones.[29]

Mutations in *gyrA* and *gyrB* inhibit topoisomerase II. The genes *gyrA* and *gyrB* code two alpha and beta subunits respectively, forming the type II topoisomerase that catalyzes the supercoiling of DNA.[29,77] Mutations in these genes, most commonly in position 90 and 94 of *gyrA*, can lead to quinolone resistance.[29,78,79]

Cross-resistance generally occurs between fluoroquinolones, and is near complete between first-generation fluoroquinolones.[29,30,74]

TREATMENT AND OUTCOMES IN MULTIDRUG-RESISTANT TUBERCULOSIS

MDR TB Treatment Outcomes

MDR TB treatment outcomes can be classified into successful treatment, and unsuccessful treatment. Several indicators can be used to classify patients' treatment outcomes.

Successful MDR TB Treatment

Successful treatment of MDR TB results in cure, which indicates that the patient no longer has *M.tb* infection. A cure occurs when a patient that has competed treatment according to protocol, has at least five consecutive negative cultures from samples collected at least 30 days apart from each other during the final 12 months of treatment.[28,80]

For treatment success to occur, MDR TB patients must be prescribed a regimen of drugs that will effectively kill or halt *M.tb* while preventing the development of further resistance.[2] Beyond consecutive negative sputum cultures, survival is a favorable outcome of treatment. Additionally, as severe weight loss is a common side effect of TB disease, weight gain can indicate successful treatment.[81]

Unsuccessful MDR TB Treatment

Patient death, treatment failure, and treatment default are typically considered unsuccessful treatment outcomes.[28,80] Treatment failure means that after a full course of treatment, the patient is still infected with *M.tb*. This occurs when two or more of the five cultures during the final 12 months of MDR TB treatment are positive, or if any one of the final three cultures is positive.[28,80] Treatment default occurs when an MDR TB patient's MDR TB treatment is interrupted for any reason for at least two consecutive months.[28,80]

In addition to positive sputum culture results, laboratory and clinical indicators can signify negative outcomes associated with toxicity from antituberculosis drugs. Adverse events due to drug regimens, are common among MDR TB patients, and can include pain, peripheral neuropathy, gastritis, psychosis, hepatitis, hypothyroidism, dermatologic abnormalities, renal effects, hearing loss, visual deficits, and death.[8,82-84] These can be reported by patients in clinical

histories, witnessed at clinical visits, or assessed through blood-based laboratory results. Almost all patients experience at least one mild adverse event throughout treatment, and fewer patients (approximately 10-20%) experience more severe adverse events.[82,85]

Additional Markers of Treatment Progress and Outcomes

Sputum culture conversion is defined as two sets of consecutive negative smears and cultures taken 30 days apart.[28] Culture conversion occurs at an average of 60 days into MDR TB treatment, and is associated with favorable MDR TB treatment outcomes.[30,86] Additionally, any changes or lack of changes in drug resistance is important to note when assessing treatment outcomes. Increased resistance is a negative outcome, but the absence of increased resistance can be viewed as a favorable treatment outcome.

Approaches to Treatment and Management of MDR TB

In order to improve treatment outcomes and control the MDR TB epidemic, high-quality, aggressive MDR TB treatment is necessary. This is especially imperative in areas with high HIV prevalence due to the increased TB incidence.[30,87] Proper treatment and management of MDR TB cases requires appropriate drug regimens.

For adequate treatment, MDR TB patients must receive at least four different drugs to which their isolates are known or likely to be susceptible. This should include any first-line drugs that remain susceptible based on DST, a fluoroquinolone, an injectable agent (an aminoglycoside or capreomycin), and additional oral second-line drugs to construct a regimen with four to five likely effective medications.[1,8] Worldwide, there are several approaches to constructing MDR TB drug regimens, ranging from individualized treatment to standardized treatment.

Individualized drug regimens are designed based on a patient's particular DST results and his or her history of previous antituberculosis treatment.[28] An individualized treatment approach allows patients to receive regimens that are tailored specifically to their strain's resistance patterns. While an individualized approach may provide a higher quality of treatment for an individual patient,[30] it can be more costly, require more time from physicians and pharmacists, be more resource-intensive in laboratories and clinics, and thus can burden public health capacity.[88] A considerable proportion of MDR TB patients have substantial delays in seeking healthcare, so these patients are likely to be excluded from health facility-based reporting of MDR TB burden, thus precluding the design of effective standardized regimens.[30,89,90]

Standardized drug regimens are used where DST capacity is limited, including in most high-incidence TB settings. Standardized regimens are designed using drug resistance surveillance data from periodic surveys, and do not utilize individual patients' DST results. Based on surveillance data, patients in a defined treatment group or category—such as MDR TB patients in a given country—all receive the same treatment regimen.[28,30,89] The same standardized regimens are used to treat the vast majority of patients. Thus, in order for each patient in a given treatment category to receive a minimum of four effective drugs, standardized regimens often include five or six drugs to provide effective treatment for patients with most resistance patterns.[28] While this strategy may enable more patients to access adequate care, it also increases the pill burden, cost, and potential side effects of treatment for patients requiring fewer antituberculosis agents. Furthermore, without an individualized treatment regimen, a patient may receive a drug to which he or she has resistance. Due to this, the patient may experience severe and potentially irreversible adverse effects from an antituberculosis medication, and

simultaneously receive no clinical benefits from the drug due to resistance. Despite this limitation, standardized treatment is used in most of the world, including in South Africa.[28]

Additionally, prescribing too many medications to patients can result in decreased adherence. Several studies have shown that higher pill burden is associated with worse adherence to medication regimens among HIV and TB patients.[32,33] Thus, prescribing patients antituberculosis medications to which they are resistant not only can cause potentially unnecessary adverse side effects, but also can result in decreased adherence to the medication regimen. Ultimately, this can cause poor treatment outcomes and potentially increase resistance.

Hybrid regimens utilize standardized regimens as a basis for treatment, but make individualized adjustments to regimens based on DST if results are available.[28]

RATIONALE FOR THIS STUDY AND RESERCH QUESTIONS

Rationale for Completing this Study

Research on MDR TB to date indicates that acquired resistance is developed and perpetuated by inadequate treatment; therefore, there is a great need to strengthen MDR TB treatment by increasing and better utilizing drug susceptibility and resistance data at an individual patient level.[2,12,14,30] Phenotypic DST is difficult for many drugs, and can be impractical where lab capacity is low. Genotypic susceptibility testing can fill a need where phenotypic DST is too difficult to be done. Utilizing genotypic susceptibility testing may allow patients to receive more effective drugs earlier, and optimize their treatment, thereby improving patient outcomes and decreasing the spread of MDR TB.[8,10,11]

Having fewer than four likely effective TB drugs available at baseline is a known risk factor for developing increasing resistance during treatment.[1,14,28] Thus, there is a need to better

understand genotypic and phenotypic resistance patterns among MDR TB patients, and how both treatment outcomes and adverse events differ among those receiving more or fewer effective drugs under a standardized MDR TB regimen.

Utilizing data from two studies that have been conducted in KwaZulu-Natal province, South Africa, the present study will describe genotypic and infer phenotypic resistance to six specific antituberculosis drugs in MDR and XDR TB patients. Additionally, we sought to understand how the patients' MDR TB treatment regimens are associated with treatment and clinical outcomes, based on their resistance patterns. Four research questions were designed to accomplish this.

Research Questions for this Study

1. What proportion of MDR and XDR patients have a genotypic mutation with likely phenotypic resistance to six specific antituberculosis drugs (pyrazinamide, ethionamide, kanamycin, capreomycin, moxifloxacin, and high-dose isoniazid)?
2. Based on genotypic resistance data in question 1, how many likely effective drugs did individual MDR and XDR TB patients in this study receive?
3. Do treatment outcomes and adverse events differ among MDR TB patients who receive different numbers of effective drugs?
4. Is there a difference in the frequency of increased resistance between patients receiving different numbers of effective drugs?

CHAPTER 3.

Utilizing genotypic mutations to determine treatment effectiveness among MDR and XDR TB patients in KwaZulu-Natal, South Africa

By

Rebecca S. Goldstein

B.A., Cornell University

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Thesis Committee Chair: Neel R. Gandhi, MD

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Contribution of Student to Manuscript

RSG and NRG conceptualized this study. RSG assisted with retrieval and quality control for the MDR TB data. RSG cleaned the data, performed analyses of the MDR and XDR TB data, wrote the manuscript, and developed the tables. NRG critically reviewed the manuscript, and both RSG and NRG approved the final version.

Utilizing genotypic mutations to determine treatment effectiveness among MDR and XDR TB patients in KwaZulu-Natal, South Africa

AUTHORS
Rebecca S. Goldstein, Salim Allana, Barun Mathema, James C.M. Brust, Thuli Mthiyane, Koleka Mlisana, Pravi Moodley, N. Sarita Shah, Neel R. Gandhi

ABSTRACT

Introduction. Tuberculosis (TB) remains the second leading cause of infectious disease death worldwide, and TB drug resistance is associated with dramatically worse treatment outcomes. South Africa's KwaZulu-Natal Province has among the highest caseloads of multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB globally. Regimens require at least four likely effective drugs to improve treatment outcomes; yet without sufficient lab capacity, phenotypic drug susceptibility testing is impractical. Genotypic susceptibility testing can fill this need. We utilized sequencing data to estimate susceptibility for drugs used in standardized MDR and XDR regimens, and to examine whether treatment outcomes vary based on different numbers of likely effective drugs.

Methods. We used isolates and data from two studies in KwaZulu-Natal to characterize the frequencies of resistance-conferring mutations among MDR and XDR TB participants. We calculated the number of likely effective drugs that subjects received. In a subset of MDR participants, we examined the association between number of effective drugs and treatment outcomes. We also described the frequency of adverse events, and estimated the odds of unsuccessful treatment using multivariate analysis.

Results. We analyzed sequencing data for 90 MDR and 363 XDR participants (n=453 total). Resistance-conferring mutations were most frequent in the *pncA* (74%) and *inhA* (39%) genes among MDR participants. Over 88% of XDR participants exhibited resistance-conferring mutations for each gene examined. 90% of the MDR, but only 23% of XDR participants received ≥ 4 effective drugs. There was no significant association between the number of effective drugs and treatment outcomes in the subset of MDR participants. The number of adverse events experienced was significantly associated with treatment outcome (aOR=4.1, 95CI 1.3 – 12.9).

Conclusions. Genotypic mutations conferring resistance to antituberculosis drugs were common, and the vast majority of MDR and XDR participants received at least one drug providing no clinical benefit. There was no association between the number of effective drugs and outcomes among MDR patients. However, similar analyses should be performed among XDR populations, where only 23% received at least four effective drugs. The frequency of adverse events was associated with outcomes, and further efforts are needed to minimize their frequency and impact.

INTRODUCTION

Tuberculosis (TB) remains the second leading cause of infectious disease death worldwide. A major challenge in TB control is drug resistance.[1,2] Multidrug-resistant (MDR) TB occurs when there is resistance to at least isoniazid and rifampin, the two most powerful first-line antibiotics. Additional resistance to a fluoroquinolone and at least one injectable agent results in extensively drug-resistant (XDR) TB. Approximately 5% of TB cases globally are MDR, and 9% of MDR cases are XDR.[3] Drug-resistant TB is especially concerning because its treatment is associated with worse outcomes, and requires the use of second-line and potentially third-line TB medications. The emergence of MDR and XDR TB threatens the goal of TB elimination.[3]

High-quality, aggressive drug-resistant TB treatment is essential to improve outcomes and control the drug-resistant TB epidemic. This requires treatment regimens with at least four different drugs to which an individual's TB strain is susceptible.[1,8] For MDR TB, this includes a minimum of any susceptible first-line drugs, one fluoroquinolone, one injectable agent (an aminoglycoside or capreomycin), and additional oral second-line drugs to construct a regimen with four to five likely effective medications.[1] In XDR TB, additional second- and third-line TB medications should replace fluoroquinolones and injectable agents to which the strain is resistant.[20]

Phenotypic drug susceptibility testing (DST) helps identify drugs that will likely be effective for an individual patient. These culture-based methods, however, are time-consuming, taking eight to ten weeks to return results, and are difficult to perform for many of the TB drugs, due to poorly reproducibility.[39] Consequently, phenotypic DST is impractical where lab capacity is low; even in the most sophisticated laboratories, susceptibility information is not available for all drugs in a drug-resistant TB regimen. This lack of DST data causes individualized drug

regimens—tailored to individuals using their own susceptibility information—to be impractical in most settings. Instead, many countries employ national standardized regimens, which are based on population-level drug susceptibility surveillance data.

Standardized regimens often include five or six drugs to ensure that any drug-resistant TB patient receives a minimum of four effective drugs.[28] Inadvertently, this strategy increases the pill burden, cost, and potential side effects of treatment. Despite this, standardized treatment is used in most of the world, including South Africa.[28]

In contrast to phenotypic DST, molecular methods test for genotypic resistance using nucleic acid amplification tests.[1] These technologies generally require simple sample preparation, have improved biosafety, and can produce diagnostic and some susceptibility results within hours or days rather than weeks or months.[3,41] Genotypic susceptibility testing can potentially fill a need where phenotypic DST is limited, by allowing patients to receive more effective drugs earlier, thereby improving patient survival, outcomes, and decreasing the spread of MDR TB.[8,10,11]

In this study, we examined sequencing data from MDR and XDR TB patients in KwaZulu-Natal province, South Africa to describe genotypic mutations and infer susceptibility for six specific antituberculosis drugs in which phenotypic DST is not routinely available. We determined the number of likely effective drugs that each patient received from the standardized regimen, and characterized the clinical benefit of MDR and XDR TB standardized drug regimens, by examining whether treatment outcomes differ among participants who received different numbers of likely effective drugs.

METHODS

Setting and Standardized TB Treatment

KwaZulu-Natal province, South Africa has among the highest caseloads of MDR and XDR TB globally.[4-7] The number of MDR TB cases in KwaZulu-Natal increased tenfold between 2001 and 2007.[4,5,7] By 2007, the MDR and XDR TB incidence in KwaZulu-Natal were 28 and 2.7 cases per 100,000 population, respectively.[7]

Lab capacity to perform DST in South Africa is limited.[3] DST tests are not available for many TB drugs, especially second-line drugs;[9] thus, national guidelines recommend standardized treatment regimens for MDR and XDR TB. The standardized MDR regimen includes six drugs: kanamycin, moxifloxacin, ethionamide, ethambutol, pyrazinamide, and terizidone.[5] XDR cases receive a similar eight-drug regimen, except capreomycin replaces kanamycin, and they receive high-dose isoniazid and para-amino salicylic acid (PAS).

Study Population and Data Sources

Data from a longitudinal MDR TB study and cross-sectional XDR TB study conducted in KwaZulu-Natal[27,91] were used for this study's primary objectives: to characterize the frequency of genetic mutations associated with resistance to TB medications; and to determine the number of likely effective drugs that patients received. The secondary objectives examining treatment outcomes, adverse events, and increased resistance were performed in a subset of MDR TB patients.

Participants in the MDR study were males and females, ≥18 years old, with laboratory-confirmed MDR TB, a documented HIV status, and initiated MDR TB treatment at one of three public MDR clinics within 14 days of their screening visit. Patients in the XDR study were male or female residents of KwaZulu-Natal, age 0-99, diagnosed with culture-confirmed XDR TB.

In both studies, baseline sputum samples were collected, and patients' *Mycobacteria tuberculosis* isolates were shipped to the TB Center at Public Health Research Institute in New Jersey for targeted sequencing of five resistance-conferring regions: *katG*, *inhA*, *pncA*, *gyrA*, and *rrs*. A total of 206 MDR TB and 404 XDR TB patients were enrolled in the parent studies; isolates were available for analysis in the current study in a subset of 90 MDR TB and 363 XDR TB participants.

Monthly clinical and laboratory data from the MDR TB study were used for the secondary study objectives. Clinical follow-up included structured patient interviews and physician reports, identifying clinical adverse events. Laboratory follow-up included sputum tests, as well as thyroid, liver, and renal toxicity tests.

Statistical Analysis and Definition of Terms

Frequency of Resistance-Conferring Mutations among MDR and XDR Participants

We described the frequency of mutations in the following resistance genes: *katG* for resistance to high-dose isoniazid, *inhA* for ethionamide, *pncA* for pyrazinamide, *gyrA* for moxifloxacin, and *rrs* for kanamycin and capreomycin resistance. Sequencing data was not available for genes that confer resistance to ethambutol, terizidone, and PAS.

We utilized sequencing data to determine whether patients had a non-synonymous or synonymous mutation, or a wild-type sequence in each gene. Missing sequencing data were interpolated. MDR and XDR TB patients were defined as likely resistant if they exhibited a non-synonymous mutation. Participants were defined as likely susceptible to a given drug if their isolate's sequence was wild-type or had a synonymous mutation.

Maximum Number of Likely Effective Drugs for MDR and XDR Participants

We then determined the maximum number of likely effective drugs that each MDR and XDR patient received. A likely effective drug was one to which a participant's isolate had no resistance-conferring mutation. At least one resistance-conferring mutation indicated a likely resistant drug. Drugs for which mutation data were not available (ethambutol, terizidone, and PAS) were assumed to be effective. We assumed that participants received the standard drug regimen for their TB type in KwaZulu-Natal.

Treatment Outcomes in MDR TB Participants

Among MDR TB participants, we examined the association between number of effective drugs and patients' treatment outcomes. We also examined risk factors for unsuccessful TB treatment outcomes in multivariate analysis, and described the number of patients with increased resistance. Successful primary treatment outcomes included cure, treatment completion, and patients still on treatment and having converted their cultures to negative. Unsuccessful primary treatment outcomes included death, treatment failure, treatment default, and recurrence of TB. Standard international definitions were used.[80]

Adverse Events among MDR TB Participants

We described the frequency of adverse events among MDR participants. Clinical adverse events occurred when participants reported peripheral neuropathy, vomiting, or visual change during monthly follow-up. We examined monthly thyroid stimulating hormone, albumin, alkaline phosphatase, alanine aminotransferase, and creatinine levels. Laboratory-confirmed toxicity events from these tests were considered adverse events if they were grade 1 or 2 on the

DAIDS grading scale, or severe adverse events if they were grade 3 or 4.[92] Adverse events from the study period were pooled for each participant, and divided by the number of study visits to create a ratio describing each participant's proportional frequency of adverse events.

Bivariate analyses were performed utilizing two-sided Wilcoxon rank-sum and Fisher's exact tests. Multivariate analyses were performed using logistic regression. We used SAS statistical software, version 9.4, and a significance level of p=0.05.

Both the MDR and XDR studies were approved by the ethics committees of Emory University, Albert Einstein College of Medicine, and the University of KwaZulu-Natal.

RESULTS

Among the participants enrolled in the parent studies from 2011 to 2014, sequencing data were available for 90 MDR and 363 XDR TB subjects. We analyzed one sample from each of these 453 participants.

Participants were mainly black South Africans (99%); 64% of MDR and 59% of XDR subjects were female, and the median age was 33 years (IQR 26-41 and 29-45, respectively). The HIV co-infection rate was 73% and 77%, respectively, of whom 81% of MDR and 91% of XDR participants were receiving antiretroviral therapy at enrollment. The median baseline CD4 count was 309 cells/mm (IQR 185-476) and 283 cells/mm (IQR 162-450), respectively, and 69% of MDR and 66% of XDR participants had virologic suppression (<400 copies/mL).

Frequency of Mutations

Table 2 describes the frequency of resistance-conferring mutations in the *katG*, *inhA*, *pncA*, *gyrA*, and *rrs* genes. The vast majority (95%) of participants—74 (82%) of MDR and 354

(98%) of XDR TB participants—exhibited non-synonymous *katG* mutations, potentially conferring resistance to high-dose isoniazid. Similarly, 367 participants (81%) had non-synonymous mutations in the *inhA* promoter, likely conferring ethionamide resistance. The proportion of those with an *inhA* promoter mutation was notably different between MDR and XDR subjects; the vast majority of the XDR participants (92%), whereas only 39% of the MDR subjects had an *inhA* promoter mutation. The majority of participants (92%) were likely resistant to pyrazinamide, as 74% of MDR and 96% of XDR participants had a non-synonymous *pncA* mutation. An overwhelming proportion of XDR subjects (91%) exhibited mutations in *gyrA*, and thus are likely resistant to moxifloxacin. Conversely, only 6 MDR participants (10%) had a resistance-conferring *gyrA* mutation. Further, 329 (73%) participants exhibited a non-synonymous mutation in *rrs*. Accordingly, only 12% of MDR subjects were likely resistant to kanamycin, but 88% of XDR subjects were likely resistant to capreomycin.

Maximum Number of Likely Effective Drugs

Table 3 describes the maximum number of likely effective drugs received by participants in this study. The majority (90%) of MDR TB participants received at least four effective drugs, while only nine (10%) received a maximum of three effective drugs. Of the 363 XDR subjects, 280 (77%) received only three likely effective drugs among the eight drugs in the standardized XDR regimen—these participants exhibited resistance-conferring mutations in all five genes that we examined.

Treatment Outcomes and Adverse Events

Primary TB treatment outcomes and adverse events were examined for 76 of the 90 MDR

TB patients included in the previous analyses. In this subset, 56 participants (74%) had successful treatment, and 20 (26%) had unsuccessful outcomes. Age, sex, HIV status, diabetes, and smoking status did not have statistically significant associations with type of treatment outcome ($p>0.05$). Similarly, the number of effective drugs was not significantly associated with type of treatment outcome ($p=0.20$).

The number of adverse events experienced throughout the course of treatment had a statistically significant association with treatment outcome ($p=0.03$). The ratio describing the number of adverse events per number of clinic visits throughout treatment also was significantly associated with treatment outcome ($p=0.04$). While the frequency of adverse events was found to be significantly associated with treatment outcome type, the severity of adverse events was not ($p=0.56$).

Multivariate logistic regression was used to estimate the association between demographic and clinical factors with treatment outcome type among MDR patients (Table 5). Controlling for age and gender, patients that experienced more adverse events per visit than the median (0.35 AEs per visit) had 4.1 times the odds of unsuccessful treatment, as compared to those with fewer adverse events ($p=0.02$). No other covariates had statistically significant associations with treatment outcome. Thus, taking into account age and sex, a higher proportion of adverse events per visit was found to be an important predictor of unsuccessful TB treatment.

Increased Resistance

Only four (5.3%) patients experienced treatment failure with increased resistance. Two (50%) of them were among the only six (10%) of MDR participants that received a maximum of three effective drugs.

DISCUSSION

In this study, we sought to understand how treatment outcomes compare among patients receiving different numbers of likely effective drugs. We utilized data from sequencing five resistance-conferring genes to determine the number of likely effective drugs participants were receiving in their standardized MDR and XDR TB treatment regimens. We found that most MDR TB patients were likely receiving at least four effective medications; however, among XDR TB patients, under a quarter were receiving at least four effective medications. Our secondary analysis showed that there was no association between the number of effective drugs received and primary TB treatment outcome in a subset of only MDR TB patients. A similar study should be conducted among XDR TB patients, where the number of effective drugs was lower. Finally, we incidentally found that the frequency of adverse events may play an important role in predicting outcomes.

Our analysis of sequencing data to determine genotypic resistance may provide insight into the current effectiveness of the standardized MDR and XDR TB drug regimens in South Africa. The majority (90%) of MDR TB patients in this study received at least four likely effective drugs; this number has been associated with TB treatment success.[8] Accordingly, the standardized MDR TB regimen in South Africa seemed to be sufficient for participants in this study—they were largely susceptible to the drugs in the regimen. In contrast, the genetic analysis of XDR TB participants had striking results. More than 77% of the XDR participants had genotypic resistance to all drugs examined. Thus, at best, they were receiving three likely effective drugs, and this may be an overestimate, since susceptibility data to these three remaining drugs were not available, neither genotypically nor phenotypically. These patients were therefore receiving treatment regimens with too few drugs to have a likely clinical benefit.

This finding is consistent with data from an earlier study from the same province,[9] in which 68% of XDR TB isolates exhibited genotypic resistance to all eight drugs tested. Our findings may help explain the low treatment success rates (22%) of XDR TB patients exhibited in this setting.[93]

We also found that nearly all of the MDR and XDR TB participants (95% and 96%, respectively) received at least one drug to which they were resistant and thus likely receive no clinical benefit. Genotypic resistance was high for pyrazinamide (74% in MDR and 96% in XDR) and ethionamide (39% in MDR and 92% in XDR). Participants may have experienced unnecessary nausea/vomiting, hepatotoxicity, and hypothyroidism without receiving clinical benefit from pyrazinamide and ethionamide. We also found that 98% of XDR participants had a mutation in the *katG* gene, which likely confers resistance to high-dose isoniazid. This substantial proportion of participants with resistance indicates that high-dose isoniazid provides very little benefit in the standardized XDR TB drug regimen. Patients may experience adverse events such as hepatotoxicity and peripheral neuropathy due to their treatment course with high-dose isoniazid, but simultaneously receive little benefit. This finding can be broadened to other drugs in the standardized XDR regimen as well; XDR patients experience a multitude of adverse events due to their medications,[94] yet for the majority of patients, at least five of the drugs in their regimen likely provide no clinical benefit.

The issue of unnecessary or potentially preventable adverse events is important particularly in light of our finding that a higher frequency of adverse events per clinical visit was associated with unsuccessful MDR TB treatment. The types, frequency, and risk factors for adverse events have been described among MDR TB patients,[82-84,95] but there is little literature linking MDR TB adverse events to treatment outcomes. Our finding highlights that the

frequency of adverse events may play an important role in TB treatment outcomes. More research on this linkage is needed, and can help to indicate whether the prevention of adverse events can improve treatment outcomes.

We assumed that genotypic susceptibility to a drug indicates that the drug will provide clinical benefit. However, in this study, those receiving more effective drugs did not have significantly better treatment outcomes. This may be because we assessed the maximum number of effective drugs, instead of the actual number of effective drugs—we may have overestimated the number of effective drugs that patients receive. Additional explanations can be our small sample size, and the limited distribution of participants receiving low numbers of effective drugs. Including phenotypic susceptibility data to these drugs at baseline would help create more sensitive estimates of the number of effective drugs. Previous studies have found differences in treatment success based on the number of effective drugs received in the initial phase;[8] despite our inconclusive findings, future studies should examine the relationship between the number of effective drugs and treatment outcomes throughout the entire treatment course. Future analyses also should take into account patients' adherence to their drug regimen to better understand how treatment impacts outcomes.

This study also aimed to understand whether differences in number of effective drugs were associated with increased resistance. Only four of the 76 MDR participants experienced treatment failure with increased resistance. This small number of participants limits our ability to draw conclusions. However, among six participants with three or fewer effective drugs, 33% (two of six) experienced increased resistance, compared to only 3% (2 of 70) of those with four or more drugs. This indicates a need to further explore these differences with a larger sample.

There are several potential limitations of this study. The small sample size of MDR TB participants decreased the statistical power in the analysis, thereby reducing internal validity of the treatment outcome and adverse event analyses. As the MDR TB study is still ongoing, this analysis will be performed again upon completion of the study, when outcome data is available for more participants. With more complete data and a larger sample size, we expect that the significant association between proportional frequency of adverse events and treatment outcome will remain significant, but that the confidence interval will tighten. We also are interested in continuing to explore whether the number of effective drugs is predictive of treatment outcome in a larger sample. Second, the number of genes for which sequencing data was available limited our analyses. We attributed drug resistance to only one gene for all drugs included in this study. In reality, however, resistance to several of the drugs included can occur from mutations in more than just one gene. For example, we utilized mutations in the *inhA* promoter to indicate ethionamide resistance, but resistance to ethionamide can also occur through mutations in *ethA* and *ethR*. Similarly, resistance to capreomycin can occur from mutations in *tlyA*, but as this data was not available, we used only *rrs* mutations to indicate capreomycin resistance. Because of this, we may have underestimated resistance to the drugs included in this analysis. Furthermore, we assumed susceptibility to drugs for which no sequencing data in resistance-conferring genes was available. As a result, we proposed only minimum estimates of the degree of drug resistance in MDR and XDR patients. This indicates that the degree of resistance may have been greater than we estimated, and the current standardized MDR and XDR TB drug regimens in South Africa may be less adequate than we concluded. We will use whole-genome sequencing and phenotypic DST in the future to more completely estimate drug resistance in our cohorts.

The ongoing epidemic of MDR and XDR TB in South Africa requires emphasis on increasing detection of resistance, and optimizing treatment regimens in order to maximize clinical benefit and decrease adverse events. Although our findings suggest that the standardized MDR TB drug regimen may be adequate, there is a need to increase second-line drug susceptibility efforts among MDR patients in KwaZulu-Natal to ensure that the standardized regimen maintains clinical value. Increased second- and third-line drug susceptibility testing is also important in the XDR population, as our results indicate that the standardized XDR TB drug regimen in inadequate. Finally, this study provides evidence that more research should be done on adverse events, as they may be significant predictors of treatment outcome.

Tables and Figures

Table 1. Demographic and clinical characteristics of participants with multidrug-resistant (MDR) and extensively drug-resistant (XDR) TB at baseline (n=603), n (%)

	MDR	XDR
Total patients enrolled	206	397
Age, median yrs (IQR)	33 (26-41)	33 (29-45)
Sex: female	131 (64)	234 (59)
Diabetes	6 (3)	15 (4)
Current smoking *	47 (23)	40 (10)
HIV positive	150 (73)	292 (77)
Receiving ARV	121 (81)	266 (91)
CD4 count at baseline: median (IQR)	309 (185-476)	283 (162–450)
Virologic suppression (<400 copies/mL)	77 (69) **	176 (66)
# with sequencing results	90 (43)	363 (91)

* Smoking history among MDR participants; current smokers among XDR participants
** 77 of the 112 with viral load results among MDR participants

Table 2. Frequency of genotypic mutations conferring resistance to antituberculosis drugs among MDR and XDR TB participants

		Frequency of mutations by gene, n (%)[1]		
Gene	**Medication(s) Affected**	**MDR (n=90)**	**XDR (n=363)**	**Total (n=453)**
katG	High-Dose Isoniazid[3]	74 (82)	354 (98)	428 (95)
inhA	Ethionamide[2,3]	35 (39)	332 (92)	367 (81)
pncA	Pyrazinamide[2,3]	67 (74)	348 (96)	415 (92)
gyrA	Moxifloxacin[2,3]	6 (10)	331 (92)	337 (74)
rrs	Kanamycin[2] Capreomycin[3]	11 (12)	318 (88)	329 (73)

[1] % calculated (# with mutation / # for which sequencing data is available for this gene)
[2] Denotes that this drug is part of the standardized MDR drug regimen
[3] Denotes that this drug is part of the standardized XDR drug regimen

Table 3. Maximum number of likely effective drugs received by MDR and XDR TB participants, based on genotypic resistance

Maximum number of likely effective drugs received, n (%)	MDR	XDR	Total
≤3	9 (10)	280 (77)	289 (64)
4	27 (30)	43 (12)	70 (15)
5	35 (39)	22 (6)	57 (13)
6	19 (21)	8 (2)	27 (6)
7	—	7 (2)	7 (2)
8	—	3 (1)	3 (1)
Total	90 (20)	363 (80)	453

Table 4. Baseline demographic and clinical characteristics associated with treatment outcomes among participants with MDR TB

	Successful Treatment	Unsuccessful Treatment	p-value
# Participants	56 (74)	20 (26)	—
Age, median (IQR)	33.8 (25.9 - 37.8)	30.8 (27.2 - 38.6)	0.9718
Sex, n (%)			0.2895
Female	34 (69)	15 (31)	
Male	22 (818)	5 (19)	
HIV Status, n (%)			0.3311
HIV+	44 (71)	18 (29)	
CD4, median (IQR)	224 (136 - 391)	175 (54 - 257)	0.1654
Virologic suppression (<400 copies/mL), n (%)	49 (78)	14 (22)	0.0913
HIV-	12 (86)	2 (14)	
Diabetes, n (%)			0.2632
Diabetic	0 (0)	1 (100)	
Non-diabetic	56 (75)	19 (25)	
Smoking, n (%)			0.7778
Smoker	15 (71)	6 (29)	
Non-smoker	41 (75)	14 (25)	
Max. # Effective Drugs, n (%)			0.2015
≤3	3 (50)	3 (50)	
4	20 (87)	3 (13)	
5	21 (72)	8 (28)	
6	12 (67)	6 (33)	
# AEs, n (%)			**0.0301**
0-3	5 (45)	6 (55)	
4-9	29 (85)	5 (15)	
10-15	11 (85)	2 (15)	
>15	11 (61)	7 (39)	
# AEs per Visit, n (%)			
≤0.196	17 (94)	1 (6)	**0.0381**
0.196 – 0.350	16 (80)	4 (20)	
0.351 – 0.67	12 (63)	7 (37)	
> 0.67	11 (58)	8 (42)	
Severe AE, n (%)			0.5588
≥1 grade 3 AE	13 (68)	6 (32)	
No grade 3 AE	43 (75)	14 (25)	

Bold values indicate statistically significant results at a significance level of p=0.05
AE = Adverse Event

Table 5. Odds of unsuccessful treatment among MDR TB participants

	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Sex	1.9 (0.62-6.10)	0.2564	2.0 (0.57-7.00)	0.2758
Age	1.0 (0.96-1.07)	0.5667	1.02 (0.96-1.08)	0.5796
# AEs per Visit				
≤ 0.35	Ref	Ref	Ref	Ref
> 0.35	4.3 (1.37-13.51)	**0.0124**	4.1 (1.27-12.88)	**0.0178**

Bold values indicate statistically significant results at a significance level of p=0.05
AE = Adverse Event

CHAPTER 4.
CONCLUSIONS AND RECOMMENDATIONS

This study utilized sequencing data to estimate participants' susceptibility to drugs used in standardized MDR and XDR TB regimens, and to examine whether treatment outcomes vary based on different numbers of likely effective antituberculosis drugs received. Our findings have several broad implications.

Adequacy of Standardized TB Treatment Regimens

MDR TB Standardized Regimen

We inferred phenotypic resistance from the analysis of genotypic mutations in MDR TB subjects. We found that there were high frequencies of resistance to pyrazinamide (74%), high-dose isoniazid (82%), and ethionamide (39%). There were lower frequencies of resistance to moxifloxacin (10%) and kanamycin (12%). Based on this, we estimated that 90% of MDR TB participants received at least four likely effective drugs, which is the standard for adequate treatment.[1,8] The KwaZulu-Natal TB control program should consider exploring alternate drugs to replace pyrazinamide in MDR TB treatment, and should not consider introducing high-dose isoniazid into the current standardized regimen. As moxifloxacin, kanamycin, and, to some extent, ethionamide still provide clinical benefit to the majority of MDR TB patients, they should be kept in the standardized regimen. Although our data indicate that the standardized MDR TB regimen may be adequate, there is a need for ongoing and expanded DST to continue monitoring the clinical value that this regimen provides for patients.

XDR TB Standardized Regimen

In this study, XDR participants exhibited extremely high rates of resistance to each drug examined, and an overwhelming proportion (77%) received at best three effective drugs. These results emphasize the stark inadequacy of treatment options available for XDR TB patients. Poor treatment outcomes among XDR TB patients are now well described,[3,12,93] and our results further illustrate the urgency of developing new drugs that can treat XDR TB, as the standardized XDR regimen did not provide participants in this study with enough effective drugs.

Optimizing Standardized Regimens

TB control programs must ensure that their recommended regimens will provide patients with adequate drug-resistant TB treatment. To do so, evaluation of current standardized TB drug regimens must occur regularly, and must be informed by DST data, including that of second- and third-line drugs. Optimizing standardized treatment regimens will help improve treatment outcomes and prevent the acquisition of greater resistance,[11,14] which in turn, can prevent primary transmission of drug-resistant TB.[91]

Furthermore, several new antituberculosis drugs are in the development pipeline or have been rolled out recently.[29] Their effectiveness is still being evaluated. This is important progress, as continued efforts to expand the standardized TB regimens—especially that for XDR TB—are necessary next steps. Members of the KwaZulu-Natal TB control program should keep abreast of the effectiveness studies for new TB drugs, and depending on their results, they should consider replacing highly resistant drugs with these new pharmaceuticals, with potentially greater susceptibility, in the standardized TB treatment regimens. This is of the utmost importance, as there is a great need for better, fast-acting, affordable drugs to control the TB epidemic and make progress towards its elimination.

Number of Effective Drugs and Treatment Outcomes among MDR TB Participants

Bivariate analysis indicated that there was no statistically significant association between the number of effective drugs received and treatment outcomes among MDR subjects. There are several possible explanations for this finding. First, we assessed the maximum number of effective drugs, instead of the actual number of effective drugs received. Consequently, we may have overestimated the number of effective drugs that patients receive, potentially masking a true association. Additionally, our small sample size (n=76) and the limited distribution of participants receiving low numbers of effective drugs may have affected this analysis.

While our data do not show a significant association, we still believe that the number of effective drugs received may be an important predictor of treatment outcomes.[8,12] Accordingly, we will perform this analysis on a larger sample of patients once the MDR TB study ends, and treatment outcome data becomes available for more participants. Beyond our analysis, future studies also should examine the number of effective drugs received among MDR TB participants. This will help indicate the adequacy of the standardized TB regimens, and it can add to the existing body of literature regarding the optimal number of drugs to be included in individual and standardized drug-resistant TB regimens. Furthermore, similar studies examining the relationship between the number of effective drugs received and treatment outcomes should be performed in XDR TB populations, where only 33% of participants received at least four effective drugs.

Accessibility of Susceptibility Testing Technologies

Low lab capacity in many low-resource, high-burden TB settings results in the underdiagnosis of TB, and reduced detection of drug-resistance. Failing to capture and treat TB cases is a public health threat, as TB can be so widely spread through populations, especially those of low-income and high HIV infection rates. Failure to diagnose and treat any TB case puts that untreated patient's community at risk. Expanding second-line DST capacity will allow for earlier diagnosis and more timely initiation of drug-resistant TB treatment.[30,96] Further, inadequate treatment of TB can foster the development of increased resistance. By failing to detect resistance and continuing to treat patients with suboptimal regimens containing too few effective drugs, drug-resistant *M. tb* mutants will be selected, and greater resistance acquired.[11] Priority must be placed on expanding lab capacity, and developing low-cost second-line molecular methods to test for resistance in resource-limited areas.

Developing and expanding second-line drug susceptibility efforts will also help provide tools to better monitor the clinical utility of standardized treatment regimens. With more available second-line DST data, policymakers in TB control programs can be better informed about the current clinical value of standardized treatment regimens, and they can make updates to standardized regimens more frequently, and with a greater evidence base.

The improvement and expansion of second-line DST methods may ultimately allow for a shift in treatment management in South Africa. A greater capacity for individual-level second-line DST testing is an important development that could, in part, lead to the ability to design individualized drug regimens.

Adverse Events

Incidentally, our results suggest that the frequency of adverse events may play an important role in predicting treatment outcomes. The types, frequency, and risk factors for adverse events have been described among MDR TB patients,[82-84,95] but there is little literature linking MDR TB adverse events to treatment outcomes. More research on this linkage is needed, and can help to indicate whether the prevention of adverse events can improve treatment outcomes.

Side effects from antituberculosis medications are common and can be severe; accordingly, adverse events are known factors that contribute to decreased regimen adherence.[32] Poor adherence has been associated with unsuccessful treatment outcomes, and greater likelihood of increased TB drug resistance.[32,97] While there is a need for more research on adherence to TB drugs generally, there is a particular need for future research to explore the links between adherence, adverse events, and treatment outcomes.

Additionally, future studies should aim to understand the burden of adverse events attributable to drugs from which patients receive no clinical benefit. Under a standardized regimen, a patient may receive a drug to which he or she exhibits phenotypic resistance. As a result, the patient may experience severe and potentially irreversible adverse effects from an antituberculosis medication, and simultaneously receive no clinical benefits from the drug due to resistance. In our study, 95% of participants received at least one drug to which they had genotypic resistance. Understanding the burden of adverse events attributable to excess drugs can help to hone standardized regimens, reduce unnecessary adverse events, and increase treatment benefit. Additionally, these adverse events attributable to unnecessary drugs may result in decreased adherence to TB drug regimens, thereby jeopardizing treatment outcomes. Examining patients' adverse events attributable to drugs that provide them no clinical benefit

also can highlight the additional burden that the healthcare system bears due to the use of standardized drug regimens that may not be effective for all patients.

Moreover, despite the significant association between the frequency of adverse events and treatment outcomes, there was no significant association between the severity of adverse events and outcomes in our study. This could be due to the limited number of participants (19 of 76) having experienced a severe (≥ grade 3) adverse event. Thus, future studies should perform a more robust analysis of the relationship between severe adverse events and treatment outcomes.

Conclusion

The ongoing epidemic of MDR and XDR TB in South Africa requires that emphasis be placed on increasing detection of resistance, and optimizing treatment regimens in order to maximize clinical benefit and decrease adverse events. Our findings indicate a pressing need to improve the drug-resistant TB treatment options, especially for XDR TB patients. Thus, we must continue to support the development of new antituberculosis drugs. Additionally, more DST technologies must be developed to optimize timely and accurate TB diagnosis and detection of resistance. To do this, more effort must be placed on developing and honing second-line DST technologies that can be integrated into low-resource health systems and remote locations. Finally, this study provides evidence that more research must be done on adverse events and their relationship with adherence, as they may be significant predictors of treatment outcome.

REFERENCES

1. Braun JTD, Charles L,; Desmond, Edward P.; Flood, Jennifer M.; Kawamura, L. Masae; Loeffler, Ann M.; Mase, Sundari R.; Pai, Madhukar; Peloquin, Charles; Raftery, Ann; Salfinger, Max; Schecter, Gisela; Seaworth, Barbara J.; Smith, Karen Lee; Wallis, Kay. Drug-Resistant Tuberculosis: A Survival Guide for Clinicians. Second ed. Francis J. Curry National Tuberculosis Center and California Department of Public Health; 2008.

2. Gandhi NR, Nunn P, Dheda K, et al. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. *Lancet* 2010; **375**(9728): 1830-43.

3. WHO. Global Tuberculosis Report 2014: World Health Organization, 2014.

4. Brust JC, Lygizos M, Chaiyachati K, et al. Culture conversion among HIV co-infected multidrug-resistant tuberculosis patients in Tugela Ferry, South Africa. *PloS one* 2011; **6**(1): e15841.

5. Gandhi NR, Andrews JR, Brust JC, et al. Risk factors for mortality among MDR- and XDR-TB patients in a high HIV prevalence setting. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2012; **16**(1): 90-7.

6. Gandhi NR, Moll A, Sturm AW, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet* 2006; **368**(9547): 1575-80.

7. Wallengren KS, F; Nunn, P; Margot, B; Buthelezi, SSS; Williams, B; et al. Drug-Resistant Tuberculosis, KwaZulu-Natal, South Aftrica, 2001-2007. *Emerg Infect Dis* 2011; **17**(10).

8. Ahuja SD, Ashkin D, Avendano M, et al. Multidrug resistant pulmonary tuberculosis treatment regimens and patient outcomes: an individual patient data meta-analysis of 9,153 patients. *PLoS medicine* 2012; **9**(8): e1001300.

9. Shah NS, Richardson J, Moodley P, et al. Increasing drug resistance in extensively drug-resistant tuberculosis, South Africa. *Emerg Infect Dis* 2011; **17**(3): 510-3.

10. Millen SJ, Uys PW, Hargrove J, van Helden PD, Williams BG. The effect of diagnostic delays on the drop-out rate and the total delay to diagnosis of tuberculosis. *PloS one* 2008; **3**(4): e1933.

11. Van Rie A, Enarson D. XDR tuberculosis: an indicator of public-health negligence. *Lancet* 2006; **368**(9547): 1554-6.

12. Bastos ML, Hussain H, Weyer K, et al. Treatment outcomes of patients with multidrug- and extensive drug-resistant tuberculosis according to drug susceptibility testing to first- and second-line drugs: an individual patient data meta-analysis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2014.

13. Mitnick CD, Shin SS, Seung KJ, et al. Comprehensive treatment of extensively drug-resistant tuberculosis. *The New England journal of medicine* 2008; **359**(6): 563-74.

14. Cegielski JP, Dalton T, Yagui M, et al. Extensive drug resistance acquired during treatment of multidrug-resistant tuberculosis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2014; **59**(8): 1049-63.

15. National Institute of Allergy and Inefectious Diseases (NIAID). Tuberculosis (TB). 2012. http://www.niaid.nih.gov/topics/tuberculosis/understanding/whatistb/pages/tbdefinitions.aspx2014).

16. Pawlowski A, Jansson M, Skold M, Rottenberg ME, Kallenius G. Tuberculosis and HIV co-infection. *PLoS pathogens* 2012; **8**(2): e1002464.

17. WHO. MDT-TB Scale-Up Initiative. 2014. http://www.unitaid.eu/en/mdr-tb-scale-up (accessed 5 December 2014 2014).

18. Nathanson E, Nunn P, Uplekar M, et al. MDR Tuberculosis — Critical Steps for Prevention and Control. *New England Journal of Medicine* 2010; **363**(11): 1050-8.

19. WHO. Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response. Geneva, Switzerland: World Health Organization, 2010.

20. Caminero JA, Sotgiu G, Zumla A, Migliori GB. Best drug treatment for multidrug-resistant and extensively drug-resistant tuberculosis. *The Lancet Infectious Diseases* 2010; **10**(9): 621-9.

21. Wells CD, Cegielski JP, Nelson LJ, et al. HIV infection and multidrug-resistant tuberculosis: the perfect storm. *The Journal of infectious diseases* 2007; **196 Suppl 1**: S86-107.

22. Gandhi NR, Shah NS, Andrews JR, et al. HIV coinfection in multidrug- and extensively drug-resistant tuberculosis results in high early mortality. *American journal of respiratory and critical care medicine* 2010; **181**(1): 80-6.

23. WHO. Global tuberculosis control: epidemiology, strategy, financing: report 2009: World Health Organization, 2009.

24. Tuberculosis strategic plan for South Africa, 2007–2011: Department of Health, Republic of South Africa, 2007.

25. Statistical notes: Extremely drug resistant tuberculosis (XDR-TB) in South Africa.: Department of Health, Republic of South Africa, 2008.

26. Gandhi N, Moodley P, Mthiyane T, et al. Transmission of HIV-Associated XDR TB in South Africa (TRAX). Emory University; 2011.

27. Gandhi NR. Impact of HIV, Antiretroviral Therapy, and TB Genotype on Survival in MDR TB. Emory University; 2010.

28. WHO. Guidelines for the programmatic management of drug-resistant tuberculosis: World Health Organization; 2006.

29. Palomino JCM, Anandi. Drug Resistance Mechanisms in *Mycobacterium tuberculosis*. *Antibiotics* 2014; **3**(3): 317-40.

30. Mitnick CD, Appleton SC, Shin SS. Epidemiology and treatment of multidrug resistant tuberculosis. *Seminars in respiratory and critical care medicine* 2008; **29**(5): 499-524.

31. Medical Research Council (MRC). Streptomycin Treatment of Pulmonary Tuberculosis: A Medical Research Council Investigation. *British Medical Journal* 1948; **2**(4582): 769-82.

32. Gebremariam MK, Bjune GA, Frich JC. Barriers and facilitators of adherence to TB treatment in patients on concomitant TB and HIV treatment: a qualitative study. *BMC public health* 2010; **10**: 651.

33. Nachega JB, Parienti JJ, Uthman OA, et al. Lower pill burden and once-daily antiretroviral treatment regimens for HIV infection: A meta-analysis of randomized controlled trials. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* 2014; **58**(9): 1297-307.

34. Palomino JC. Newer diagnostics for tuberculosis and multi-drug resistant tuberculosis. *Current opinion in pulmonary medicine* 2006; **12**(3): 172-8.

35. Wells WA, Boehme CC, Cobelens FG, et al. Alignment of new tuberculosis drug regimens and drug susceptibility testing: a framework for action. *The Lancet Infectious diseases* 2013; **13**(5): 449-58.

36. Dlamini-Mvelase NR, Werner L, Phili R, Cele LP, Mlisana KP. Effects of introducing Xpert MTB/RIF test on multi-drug resistant tuberculosis diagnosis in KwaZulu-Natal South Africa. *BMC infectious diseases* 2014; **14**(1): 442.

37. Gholoobi A, Masoudi-Kazemabad A, Meshkat M, Meshkat Z. Comparison of Culture and PCR Methods for Diagnosis of Mycobacterium tuberculosis in Different Clinical Specimens. *Jundishapur journal of microbiology* 2014; **7**(2): e8939.

38. Narasimooloo R, Ross A. Delay in commencing treatment for MDR TB at a specialised TB treatment centre in KwaZulu-Natal. *South African medical journal = Suid-Afrikaanse tydskrif vir geneeskunde* 2012; **102**(6 Pt 2): 360-2.

39. Kim SJ. Drug-susceptibility testing in tuberculosis: methods and reliability of results. *The European respiratory journal* 2005; **25**(3): 564-9.

40. Barrera L, Cooreman E, Iragena JdD, et al. Policy guidance on drug-susceptibility testing (DST) of second-line antituberculosis drugs: World Health Organization, 2008.

41. Drobniewski F, Nikolayevskyy V, Maxeiner H, et al. Rapid diagnostics of tuberculosis and drug resistance in the industrialized world: clinical and public health benefits and barriers to implementation. *BMC medicine* 2013; **11**: 190.

42. Lawn SD, Mwaba P, Bates M, et al. Advances in tuberculosis diagnostics: the Xpert MTB/RIF assay and future prospects for a point-of-care test. *The Lancet Infectious diseases* 2013; **13**(4): 349-61.

43. Lin SY, Desmond EP. Molecular diagnosis of tuberculosis and drug resistance. *Clinics in laboratory medicine* 2014; **34**(2): 297-314.

44. Pantoja A, Fitzpatrick C, Vassall A, Weyer K, Floyd K. Xpert MTB/RIF for diagnosis of tuberculosis and drug-resistant tuberculosis: a cost and affordability analysis. *The European respiratory journal* 2013; **42**(3): 708-20.

45. Vassall A, van Kampen S, Sohn H, et al. Rapid diagnosis of tuberculosis with the Xpert MTB/RIF assay in high burden countries: a cost-effectiveness analysis. *PLoS medicine* 2011; **8**(11): e1001120.

46. Boehme CC, Nicol MP, Nabeta P, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. *The Lancet*; **377**(9776): 1495-505.

47. Kranzer K. Improving tuberculosis diagnostics and treatment. *The Lancet*; **377**(9776): 1467-8.

48. Dheda K, Theron G, Welte A. Cost-effectiveness of Xpert MTB/RIF and investing in health care in Africa. *The Lancet Global Health*; **2**(10): e554-e6.

49. Cohen GM, Drain PK, Noubary F, Cloete C, Bassett IV. Diagnostic delays and clinical decision making with centralized Xpert MTB/RIF testing in Durban, South Africa. *J Acquir Immune Defic Syndr* 2014; **67**(3): e88-93.

50. Jnawali HN, Hwang SC, Park YK, et al. Characterization of mutations in multi- and extensive drug resistance among strains of Mycobacterium tuberculosis clinical isolates in Republic of Korea. *Diagnostic microbiology and infectious disease* 2013; **76**(2): 187-96.

51. Zhang Y, Heym B, Allen B, Young D, Cole S. The catalase-peroxidase gene and isoniazid resistance of Mycobacterium tuberculosis. *Nature* 1992; **358**(6387): 591-3.

52. Ramaswamy SV, Reich R, Dou SJ, et al. Single nucleotide polymorphisms in genes associated with isoniazid resistance in Mycobacterium tuberculosis. *Antimicrobial Agents and Chemotherapy* 2003; **47**(4): 1241-50.

53. Rozwarski DA, Grant GA, Barton DH, Jacobs WR, Jr., Sacchettini JC. Modification of the NADH of the isoniazid target (InhA) from Mycobacterium tuberculosis. *Science (New York, NY)* 1998; **279**(5347): 98-102.

54. Vilcheze C, Jacobs WR, Jr. The mechanism of isoniazid killing: clarity through the scope of genetics. *Annual review of microbiology* 2007; **61**: 35-50.

55. Takayama K, Kilburn JO. Inhibition of synthesis of arabinogalactan by ethambutol in Mycobacterium smegmatis. *Antimicrobial Agents and Chemotherapy* 1989; **33**(9): 1493-9.

56. Sreevatsan S, Stockbauer KE, Pan X, et al. Ethambutol resistance in Mycobacterium tuberculosis: critical role of embB mutations. *Antimicrobial Agents and Chemotherapy* 1997; **41**(8): 1677-81.

57. Telenti A, Philipp WJ, Sreevatsan S, et al. The emb operon, a gene cluster of Mycobacterium tuberculosis involved in resistance to ethambutol. *Nature medicine* 1997; **3**(5): 567-70.

58. Mitchison DA. The action of antituberculosis drugs in short-course chemotherapy. *Tubercle* 1985; **66**(3): 219-25.

59. Konno K, Feldmann FM, McDermott W. Pyrazinamide susceptibility and amidase activity of tubercle bacilli. *The American review of respiratory disease* 1967; **95**(3): 461-9.

60. Scorpio A, Zhang Y. Mutations in pncA, a gene encoding pyrazinamidase/nicotinamidase, cause resistance to the antituberculous drug pyrazinamide in tubercle bacillus. *Nature medicine* 1996; **2**(6): 662-7.

61. Zhang Y, Mitchison D. The curious characteristics of pyrazinamide: a review. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2003; **7**(1): 6-21.

62. Jureen P, Werngren J, Toro JC, Hoffner S. Pyrazinamide resistance and pncA gene mutations in Mycobacterium tuberculosis. *Antimicrobial Agents and Chemotherapy* 2008; **52**(5): 1852-4.

63. Scorpio A, Lindholm-Levy P, Heifets L, et al. Characterization of pncA mutations in pyrazinamide-resistant Mycobacterium tuberculosis. *Antimicrobial Agents and Chemotherapy* 1997; **41**(3): 540-3.

64. Alangaden GJ, Kreiswirth BN, Aouad A, et al. Mechanism of resistance to amikacin and kanamycin in Mycobacterium tuberculosis. *Antimicrobial Agents and Chemotherapy* 1998; **42**(5): 1295-7.

65. Suzuki Y, Katsukawa C, Tamaru A, et al. Detection of kanamycin-resistant Mycobacterium tuberculosis by identifying mutations in the 16S rRNA gene. *Journal of clinical microbiology* 1998; **36**(5): 1220-5.

66. Banerjee A, Dubnau E, Quemard A, et al. inhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. *Science (New York, NY)* 1994; **263**(5144): 227-30.

67. Brossier F, Veziris N, Truffot-Pernot C, Jarlier V, Sougakoff W. Molecular Investigation of Resistance to the Antituberculous Drug Ethionamide in Multidrug-Resistant Clinical Isolates of Mycobacterium tuberculosis. *Antimicrobial Agents and Chemotherapy* 2011; **55**(1): 355-60.

68. Larsen MH, Vilcheze C, Kremer L, et al. Overexpression of inhA, but not kasA, confers resistance to isoniazid and ethionamide in Mycobacterium smegmatis, M. bovis BCG and M. tuberculosis. *Molecular microbiology* 2002; **46**(2): 453-66.

69. DeBarber AE, Mdluli K, Bosman M, Bekker LG, Barry CE, 3rd. Ethionamide activation and sensitivity in multidrug-resistant Mycobacterium tuberculosis. *Proceedings of the National Academy of Sciences of the United States of America* 2000; **97**(17): 9677-82.

70. Moazed D, Noller HF. Interaction of antibiotics with functional sites in 16S ribosomal RNA. *Nature* 1987; **327**(6121): 389-94.

71. Gillespie SH. Evolution of drug resistance in Mycobacterium tuberculosis: clinical and molecular perspective. *Antimicrobial Agents and Chemotherapy* 2002; **46**(2): 267-74.

72. Zaunbrecher MA, Sikes RD, Jr., Metchock B, Shinnick TM, Posey JE. Overexpression of the chromosomally encoded aminoglycoside acetyltransferase eis confers kanamycin resistance in Mycobacterium tuberculosis. *Proceedings of the National Academy of Sciences of the United States of America* 2009; **106**(47): 20004-9.

73. Kruuner A, Jureen P, Levina K, Ghebremichael S, Hoffner S. Discordant resistance to kanamycin and amikacin in drug-resistant Mycobacterium tuberculosis. *Antimicrobial Agents and Chemotherapy* 2003; **47**(9): 2971-3.

74. Von Groll A, Martin A, Jureen P, et al. Fluoroquinolone resistance in Mycobacterium tuberculosis and mutations in gyrA and gyrB. *Antimicrobial Agents and Chemotherapy* 2009; **53**(10): 4498-500.

75. Johansen SK, Maus CE, Plikaytis BB, Douthwaite S. Capreomycin binds across the ribosomal subunit interface using tlyA-encoded 2'-O-methylations in 16S and 23S rRNAs. *Molecular cell* 2006; **23**(2): 173-82.

76. Goss WA, Deitz WH, Cook TM. Mechanism of action of nalidixic acid on escherichia coli.ii. Inhibition of deoxyribonucleic acid synthesis. *Journal of bacteriology* 1965; **89**: 1068-74.

77. Takiff HE, Salazar L, Guerrero C, et al. Cloning and nucleotide sequence of Mycobacterium tuberculosis gyrA and gyrB genes and detection of quinolone resistance mutations. *Antimicrobial Agents and Chemotherapy* 1994; **38**(4): 773-80.

78. Cheng AF, Yew WW, Chan EW, Chin ML, Hui MM, Chan RC. Multiplex PCR amplimer conformation analysis for rapid detection of gyrA mutations in fluoroquinolone-resistant Mycobacterium tuberculosis clinical isolates. *Antimicrobial Agents and Chemotherapy* 2004; **48**(2): 596-601.

79. Sun Z, Zhang J, Zhang X, Wang S, Zhang Y, Li C. Comparison of gyrA gene mutations between laboratory-selected ofloxacin-resistant Mycobacterium tuberculosis strains and clinical isolates. *International journal of antimicrobial agents* 2008; **31**(2): 115-21.

80. Laserson KF, Thorpe LE, Leimane V, et al. Speaking the same language: treatment outcome definitions for multidrug-resistant tuberculosis. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2005; **9**(6): 640-5.

81. van Crevel R, Karyadi E, Netea MG, et al. Decreased plasma leptin concentrations in tuberculosis patients are associated with wasting and inflammation. *The Journal of clinical endocrinology and metabolism* 2002; **87**(2): 758-63.

82. Brust JC, Shah, N. S., van der Merwe, T. L., Bamber, S., Ning, Y., Heo, M., Moll, A. P., Loveday, M., Lalloo, U. G., Friedland, G. H. & Gandhi, N. R. Adverse events in an integrated home-based treatment program for MDR-TB and HIV in KwaZulu-Natal, South Africa. *J Acquir Immune Defic Syndr* 2013; **62**(4): 436-40.

83. Jacobs TQR, A. Adverse effects profile of multidrug-resistant tuberculosis treatment in a South African outpatient clinic. *South African Family Practice* 2012; **54**(6): 531-9.

84. Sagwa E, Ruswa N, Musasa JP, Mantel-Teeuwisse AK. Adverse events during treatment of drug-resistant tuberculosis: a comparison between patients with or without human immunodeficiency virus co-infection. *Drug safety : an international journal of medical toxicology and drug experience* 2013; **36**(11): 1087-96.

85. Furin JJ, Mitnick CD, Shin SS, et al. Occurrence of serious adverse effects in patients receiving community-based therapy for multidrug-resistant tuberculosis. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2001; **5**(7): 648-55.

86. Holtz TH, Sternberg M, Kammerer S, et al. Time to sputum culture conversion in multidrug-resistant tuberculosis: predictors and relationship to treatment outcome. *Annals of internal medicine* 2006; **144**(9): 650-9.

87. Nelson LJ, Talbot EA, Mwasekaga MJ, et al. Antituberculosis drug resistance and anonymous HIV surveillance in tuberculosis patients in Botswana, 2002. *Lancet* 2005; **366**(9484): 488-90.

88. WHO. 3, Standard treatment regimens. Treatment of Tuberculosis: Guidelines. 4th ed. Geneva: World Health Organization; 2010.

89. Rich ML, Socci AR, Mitnick CD, et al. Representative drug susceptibility patterns for guiding design of retreatment regimens for MDR-TB. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2006; **10**(3): 290-6.

90. Storla DG, Yimer S, Bjune GA. A systematic review of delay in the diagnosis and treatment of tuberculosis. *BMC public health* 2008; **8**: 15.

91. Shah NS, Brust JC, Mathema B, et al. Majority of XDR TB Cases are due to Transmission in a High HIV Prevalence Setting. Conference on Retroviruses and Opportunistic Infections. Seattle, Washington; 2015.

92. AIDS Do. Table for Grading the Severity of Adult and Pediatric Adverse Events. 1 ed; 2004.

93. O'Donnell MR, Padayatchi N, Kvasnovsky C, Werner L, Master I, Horsburgh CR, Jr. Treatment outcomes for extensively drug-resistant tuberculosis and HIV co-infection. *Emerg Infect Dis* 2013; **19**(3): 416-24.

94. Shean K, Streicher E, Pieterson E, et al. Drug-associated adverse events and their relationship with outcomes in patients receiving treatment for extensively drug-resistant tuberculosis in South Africa. *PloS one* 2013; **8**(5): e63057.

95. Bloss E, Kuksa L, Holtz TH, et al. Adverse events related to multidrug-resistant tuberculosis treatment, Latvia, 2000-2004. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2010; **14**(3): 275-81.

96. Hoffner S. The role of drug susceptibility testing in controlling drug resistant tuberculosis: Challenges and possibilities. *International Journal of Mycobacteriology* 2015; **4, Supplement 1**(0): 4-5.

97. Shargie EB, Lindtjorn B. Determinants of treatment adherence among smear-positive pulmonary tuberculosis patients in Southern Ethiopia. *PLoS medicine* 2007; **4**(2): e37.