EFFECTS OF PYRAZINAMIDE AND/OR ETHAMBUTOL RESISTANCE ON TUBERCULOSIS TREATMENT OUTCOMES BY WHOLE GENOME SEQUENCING, SOUTH AFRICA 2020 - 2022

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DECLARATION

I declare that the content of this research report is original, except where reference is made to the work of others. This report has never been submitted for consideration for any other degree or qualification in this or any other university.

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Agreement by all co-authors

I, Sinothando Samukele Dlamini, student number 1684549, wrote the protocol, acquired ethical approval, cleaned the data, performed data analysis, and wrote the first draft of this research report. Additionally, I received critical comments from all co-authors. By signing this declaration, the co-authors listed below agree to the use of the article by the student as part of his research report**.**

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DEDICATION

I dedicate this report to my late father, whose memory continues to inspire me, and to my family, whose unwavering support and encouragement have been invaluable throughout this journey. Your belief in me has been my greatest strength.

PRESENTATION / PUBLICATION FROM THIS STUDY

- South African Field Epidemiology Training Program – Scientific seminar

ABSTRACT

Introduction:

In South Africa, both pyrazinamide (PZA) and/or ethambutol (EMB) were included in the all-oral short-course regimen despite the high resistance rates to pyrazinamide (PZA) and/or ethambutol (EMB) reported among people with MDR-TB. The effect of resistance to these drugs on treatment outcomes are poorly understood. This study evaluated the effects of PZA and/or EMB resistance on treatment outcomes among MDR-TB cases treated with the all oral short course regimen in South Africa from 2020 to 2022.

Methods: We conducted a retrospective cohort study of people with MDR-TB detected through routine TB diagnostics performed at Braamfontein and Greenpoint laboratories who were treated with the all-oral short-course regimen. Isolates from these laboratories were referred for whole genome sequencing (WGS) as part of a surveillance study. Drug susceptibility patterns for PZA and EMB were classified into four categories: susceptibility to both drugs (EMB-S & PZA-S), resistance to PZA only (EMB-S & PZA-R), resistance to EMB only (EMB-R & PZA-S), and resistance to both drugs (EMB-R & PZA-R). Treatment outcomes were categorized as either successful patient outcomes or unsuccessful patient outcomes. A directed acyclic graph guided the selection of variables for the generalized linear model. Adjusted incident rate ratio (aIRR) were calculated with 95% confidence intervals using modified Poisson regression with robust error variances.

Results:

Among the 598 people with MDR-TB included in the analysis, 60% (356/598) were resistant to one or both TB drugs. There was no evidence that resistance to PZA and/or EMB increased USPO or SPO: resistance to PZA (aIRR = 1.18, 95% CI: 0.86–1.63), resistance to EMB alone (aIRR = 0.94, 95% CI: 0.67–1.32), and resistance to both drugs (aIRR = 0.97, 95% CI: 0.73–1.27).

Conclusion:

This study also highlights PZA or EMB does not affect treatment outcomes in patients with MDR-TB. This observation supports the rationale behind the 2023 adoption of the all-oral MDR-TB regimen. WGS is recommended to guide treatment regimens that include PZA and EMB.

Keywords: Multidrug-resistant tuberculosis, pyrazinamide resistance, ethambutol resistance, whole genome sequencing, treatment outcomes.

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LIST OF ABBREVIATIONS

ART	Antiretroviral treatment
BPaL-L	Bedaquiline, Pretomanid, Linezolid and Levofloxacin
CDC	Centre for Diseases Control
CDW	Corporate Data Warehouse
CI	Confidence Intervals
CTB	Centre for Tuberculosis
DAG	Directed Acyclic Graphs
DR-TB	Drug-resistant Tuberculosis
EDRWeb	Electronic Drug Resistant Software
EMB-R	Ethambutol Resistance
EMB-S	Ethambutol Susceptibility
EPTB	Extra-pulmonary Tuberculosis
HIV	Human Immunodeficiency Virus
INH	Isoniazid
IRR	Incidence Rate Ratio / estimated relative risk
MTB	Mycobacterium Tuberculosis
NGS	Next-generation Sequencing
NHLS	National Health Laboratory Services
NICD	National Institute for Communicable Diseases
Pre-XDR	Pre-Extensively Drug-Resistant Tuberculosis
PTB	Pulmonary Tuberculosis
PZA-R	Pyrazinamide Resistance
PZA-S	Pyrazinamide Susceptibility
RIF	Rifampicin
RR/MDR-TB	Rifampicin Resistant / Multi-drug resistant tuberculosis
SAFETP	South African Field Epidemiology Training Program
SDW	Surveillance Data Warehouse
SPO	Successful Treatment Outcomes
USPO	Unsuccessful Treatment Outcomes
WGS	Whole Genome Sequencing
WHO	World Health Organization
XDR-TB	Extensively Drug-Resistant Tuberculosis

CHAPTER 1: INTRODUCTION

This chapter provides an overview of drug resistant - tuberculosis (DR-TB) epidemiology, DR-TB treatment regimens and information on ethambutol and pyrazinamide. This introduction presents the background of drug-resistant TB, reviewing its epidemiology, relevant literature, and identifying the problem statement and justification for the study. The chapter also outlines the research question, aim, and objectives that guide the investigation.

1.1 Background

Drug-resistant tuberculosis (DR-TB) refers to active tuberculosis (TB) caused by *Mycobacterium tuberculosis* complex (MTB) bacteria that are resistant to one or more anti-TB drugs [1]. The spread of DR-TB is a public health concern in South Africa, threatening TB control in South Africa and globally [2]. According to the WHO Global TB Report 2024, South Africa is included in all three of the WHO high-burden country lists: one for tuberculosis (TB), one for TB/HIV co-infection, and one for multidrug-resistant/rifampicin-resistant TB (MDR/RR-TB) [3].

Globally in 2023, an estimated 10.8 million people (95% uncertainty interval [UI]: 10.1–11.7 million) fell ill with TB (incident cases), a further increase from 10.7 million (95% UI: 10.0–11.5 million) in 2022, the highest since the WHO began monitoring in 1995 [1]. TB caused approximately 1.3 million deaths in 2022 [4]. South Africa's estimated TB incidence rate was 468 per 100,000 population [5]. While antiretroviral treatment (ART) has helped curb TB incidence, the country continues to face challenges in TB control, emerging DR-TB emphasizing the need for strengthened surveillance and TB control [6].

1.1.1 Burden of DR-TB

Globally, the estimated annual number of people who developed MDR/RR-TB was relatively stable between 2020 and 2023, after a slow downward trend between 2015 and 2019. The estimated number in 2023 was 400,000 (95%[UI]: 360,000 – 440,000) [1]. In South Africa the estimated number of incident cases of MDR/RR-TB cases in south Africa was 13000.

In South Africa, the estimated proportion of new people with MDR/RR-TB was 3.4% in 2022, slightly above the global average of 3.3%, while 16% of previously treated individuals had MDR/RR-TB, aligning with the global average of 17% [1]. Additionally, South Africa's high

HIV prevalence exacerbates TB challenges, as immunocompromised individuals face increased susceptibility to TB infection and treatment complications [8].

Despite efforts to improve treatment outcomes, MDR-TB treatment success rates in South Africa remain suboptimal compared to drug-susceptible TB [9]. The South African Drug Resistance Tuberculosis Survey (2012–2014) assessed MDR-TB prevalence across all nine provinces [10]. Led by the Centre for Tuberculosis at the National Institute for Communicable Diseases (NICD), a World Health Organization (WHO) supranational TB Reference Laboratory, the survey was conducted in collaboration with the National Health Laboratory Service (NHLS), national and provincial TB programs, and other partners [10]. A total of 442 healthcare facilities were randomly selected for data collection [10].

Findings indicated an MDR-TB prevalence of 2.1% (95% CI: 1.5%-2.7%) among new patients and 4.6% (95% CI: 3.2%-6.0%) among previously treated patients, with an overall prevalence of 2.8% (95% CI: 2.0%-3.6%) [10]. These figures showed minimal change from the 2001–2002 survey, which reported a prevalence of 2.9% (95% CI: 2.4%-3.5%) [10]. While the prevalence among previously treated cases was lower than the global average of 20% (95% CI: 14%-27%) [10], the persistent burden strengthens the ongoing need for effective TB control measures in South Africa.

1.1.2. Whole Genome Sequencing

Whole genome sequencing (WGS) has emerged as a powerful tool in combating DR-TB by enabling rapid drug resistance detection and transmission analysis, which enhances understanding of DR-TB epidemiology [11]. WGS detects mutations responsible for drug resistance and is currently performed at the Centre for Tuberculosis (CTB) as part of various projects.

Studies show that WGS provides high-resolution, robust drug-resistance data, guiding individual patient management and surpassing other TB diagnostic methods [11]. It can detect resistance to most TB drugs, including pyrazinamide and ethambutol [11]. In 2021, the WHO released its first (Version 1) catalogue of resistance-associated genetic variants, informed by WGS and phenotypic drug susceptibility data from over 38,000 *Mycobacterium tuberculosis complex* isolates [11]. This catalogue serves as a standardized reference for interpreting resistance to first- and second-line TB drugs, including groups A, B, and C, and is included in

the CTB resistance prediction pipeline [11]. The catalogue will be updated as more information becomes available, the version was recently updated in 2023 [11].

WGS outperforms traditional diagnostic methods like culture-based phenotypic DST in terms of time to availability of results [11]. It enables precise resistance prediction, supports individualized treatment regimens, and identifies mixed infections and minority populations of resistant bacteria that conventional methods often miss [11]. The implementation of WGS at the CTB in South Africa has provided valuable data and insights into DR-TB resistance trends, and transmission dynamics, allowing the tracking of TB clusters and transmission chains, which are crucial for public health interventions.

Integrating WGS into routine TB diagnostics offers hope for improved TB control by enabling timely and precise resistance detection, ultimately enhancing patient outcomes and reducing transmission [11].

1.1.3. Short Course Regimen

Historically, DR-TB required longer treatment durations, higher pill burdens, and more severe adverse effects compared to DS-TB [12]. South Africa pioneered the all-oral short-course regimen for RR/MDR-TB patients in June 2018, reducing the need for daily injections and pill burden, and thereby enhancing adherence [12]. This regimen, comprising bedaquiline, linezolid, levofloxacin, isoniazid, clofazimine, pyrazinamide, and ethambutol, had a treatment duration of 9–11 months [12]. Updated in 2023, South Africa introduced an even shorter regimen, BPaL-L (Bedaquiline, Pretomanid, Linezolid, and Levofloxacin), which is 6 months [13].

The short regimen is intended for individuals with RR/MDR-TB who have not received second-line anti-TB treatment for more than one month. Eligible individuals included those with RR-TB based on an initial GeneXpert result while awaiting first- and second-line LPA confirmation; rifampicin mono-resistant TB with confirmed susceptibility to isoniazid (validated by reflex phenotypic INH DST); and MDR-TB with resistance to both rifampicin and isoniazid (due to either inhA or katG mutation, but not both) and confirmed susceptibility to fluoroquinolones and injectable agents. Individuals with uncomplicated RR/MDR extrapulmonary TB (e.g., lymphadenopathy or pleural effusion), people living with HIV already on or initiating ART, pregnant women with pulmonary TB (with or without

uncomplicated EPTB) following National Clinical Advisory Committee review, and children under 12 years with confirmed or presumed RR/MDR-TB (treated without injectables and with potential substitution of bedaquiline until age-appropriate dosing data is available) are also eligible.

The management of RR/MDR-TB in children has historically differed from that of adults and adolescents, particularly during the early part of the study period. While efforts were made toward implementing shorter, all-oral regimens in adults and adolescents, children were not immediately included in this shift. Many paediatric patients continued to receive injectable-containing regimens, which are associated with greater toxicity, pain, and long-term adverse effects such as hearing loss, making them less suitable and more burdensome for children. Over time, treatment guidelines evolved to recommend injectable-free regimens for younger children; however, the duration of treatment for paediatric patients typically remained longer, often extending beyond 12 months, compared to the 9–11-month short-course regimen recommended for older age groups. This distinction in treatment duration and composition reflects a cautious approach to regimen adaptation in children, driven by limited safety and pharmacokinetic data for key drugs such as bedaquiline in younger age groups. Recognising this difference is essential for accurately contextualising RR/MDR-TB management in South Africa and for acknowledging the unique challenges faced in paediatric TB care. Moreover, recent literature highlights the need for child-friendly treatment options that are simple, safe, short, and effective, aligning with broader global efforts to ensure equitable TB care across all age groups.

A study by Ndjeka and colleagues found that patients on all-oral regimens had higher treatment success rates, lower mortality, and reduced loss to follow-up compared to those receiving injectable-containing regimens [14]. The removal of injectables from the regimen also eliminated risks such as ototoxicity and nephrotoxicity, making treatment more tolerable [14].

A study conducted by Lempens and colleagues in Bangladesh challenged previous assumptions about the clinical importance of pyrazinamide and ethambutol resistance [15]. This study focused on the shorter MDR-TB treatment regimen and found that resistance to these drugs did not significantly impact treatment outcomes, provided that fluoroquinolone susceptibility was preserved [15]. Their analysis reported an odds ratio of 1.01 (95% CI 0.4 – 2.8), indicating no association between resistance to pyrazinamide or ethambutol and adverse clinical outcomes in people with fluoroquinolone-sensitive MDR-TB [15]. These findings suggest that the

inclusion of these two drugs in some regimens may not be as clinically useful as previously believed, particularly when fluoroquinolones remain effective [15].

1.1.4. Pyrazinamide

Pyrazinamide and ethambutol are companion drugs that assist in preventing resistance in the core drugs. Both ethambutol and pyrazinamide are group C drugs [16]. Pyrazinamide is used for its relapse-preventing properties and assists in the shortening of treatment duration, whilst demonstrating good lung tissue penetration [16]. Common pyrazinamide adverse events include gout, arthralgia, hepatotoxicity, rash, photosensitivity and gastrointestinal upset [16]. Studies show that the prevalence of pyrazinamide resistance increases with resistance to other anti-TB drugs [17]. Pyrazinamide resistance in TB is a complex and driven primarily by diverse mutations in the pncA gene [18]. Phenotypic susceptibility testing for pyrazinamide can be challenging due to the acidic conditions required for the test, which can lead to unreliable results [19].

1.1.5. Ethambutol

Ethambutol is used to prevent TB resistance and inhibits cell wall synthesis by targeting arabinosyl transferase [16]. Its adverse effects include optic neuritis, rash, and headache [16]. Resistance primarily arises from mutations in the embB gene, particularly at codon 306 (embB306), which reduces ethambutol's efficacy [20]. Other mutations in iniA and iniB genes may also contribute to resistance [20]. A study in Russia highlighted the association between embB mutations and specific *Mycobacterium tuberculosis* lineages, suggesting that certain strains are more prone to ethambutol resistance [21].

1.1.Successful Treatment Outcomes and Unsuccessful Treatment Outcomes

The WHO defines successful TB treatment outcomes as patients who are cured or complete the full short-course regimen [22]. A study conducted by Bastos and colleagues evaluated treatment outcomes for patients with MDR-TB and XDR-TB based on drug susceptibility testing for first- and second-line drugs [23]. The findings indicated that resistance to ethambutol and pyrazinamide was associated with poorer treatment outcomes in individuals with MDR-TB. While both drugs are part of standard regimens, their effectiveness in MDR-TB treatment appears reduced when resistance is present [23]. The study highlighted the

importance of drug susceptibility testing-guided therapy to optimise treatment regimens and improve patient outcomes.

1.2. Problem Statement

DR-TB remains a challenge to achieving the WHO End TB Strategy 2035 targets, which aim to reduce TB mortality by 95% and TB incidence by 90% compared to the 2015 baseline [1]. Despite South Africa's efforts to curb the TB burden through interventions such as HIV/TB integration programmes, WGS surveillance, and shorter treatment regimens, the country remains on all three WHO high TB burden lists (TB, RR/MDR-TB, and HIV/TB co-infection) [24].

Pyrazinamide and ethambutol are companion drugs in the MDR-TB short treatment regimen but can cause severe adverse effects [11]. Routine drug susceptibility testing for these drugs is not performed, meaning patients are treated without knowing their resistance status. Previous drug resistance surveys have shown high levels of pyrazinamide and ethambutol resistance among MDR-TB isolates (2012–2014) [10]. However, it remains unclear whether resistance to one or both drugs negatively impacts treatment outcomes, particularly in high TB burden settings like South Africa. This study aims to address this gap by evaluating the effects of pyrazinamide and ethambutol resistance on MDR-TB treatment outcomes.

1.3.Justification

The 2012–2014 South African TB Drug resistant survey found high rates of second-line drug resistance among MDR-TB patients, with 59.1% (95% CI: 49.0–69.1) resistant to pyrazinamide and 44.1% (95% CI: 30.2–58.0) resistant to ethambutol [10]. Despite these resistance rates, the 2019 South African RR-TB Clinical Management Guidelines included both drugs in the all-oral short-course regimen for its full duration [12]. Additionally, neither drug was replaced when withdrawn due to toxicity, and susceptibility testing for these drugs is not routinely performed as part of South Africa's national TB testing algorithm [12]. Given the limited data on the demographic and clinical characteristics of patients with pyrazinamide and/or ethambutol resistance, this study aims to bridge this knowledge gap. Understanding the impact of resistance to these drugs on treatment outcomes in MDR-TB patients receiving the all-oral short-course regimen will contribute to evidence-based treatment strategies in South Africa.

1.4. Research question

Does pyrazinamide and/or ethambutol resistance have an effect on TB treatment outcome amongst people with MDR-TB treated with the all oral short-course regimen recommended for DR-TB treatment in South Africa in the period January 2020 to September 2022?

1.5. Aim

To evaluate the effects of pyrazinamide and/or ethambutol resistance on TB treatment outcome amongst people with MDR-TB treated with the all oral short course regimen in South Africa from January 2020 to September 2022.

1.6.Objectives

- To describe the socio-demographic and clinical characteristics among MDR-TB patients with and without pyrazinamide and/or ethambutol resistance treated with the all oral short-course regimen in South Africa, 2020 - 2022.
- To compare the treatment outcomes of pyrazinamide and/or ethambutol resistant patients to patients with susceptibility to both drugs among MDR-TB patients treated with the all oral short-course regimen in South Africa, 2020 – 2022.

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CHAPTER 2 – MANUSCRIPT

This chapter includes a brief introduction, highlighting the problem statement and justification, followed by a detailed explanation of the methods, results, and discussion of the effects of pyrazinamide and/or ethambutol resistance on treatment outcomes. The journal guidelines followed for this study were those of PLOS ONE, a global, open-access, peer-reviewed journal that publishes scientifically rigorous research across diverse fields. The guidelines are attached as an appendix.

Abstract

Introduction:

In South Africa, both pyrazinamide (PZA) and/or ethambutol (EMB) were included in the all-oral short-course regimen despite the high resistance rates to pyrazinamide (PZA) and/or ethambutol (EMB) reported among people with MDR-TB. The effect of resistance to these drugs on treatment outcomes are poorly understood. This study evaluated the effects of PZA and/or EMB resistance on treatment outcomes among MDR-TB cases treated with the all oral short course regimen in South Africa from 2020 to 2022.

Methods: We conducted a retrospective cohort study of people with MDR-TB detected through routine TB diagnostics performed at Braamfontein and Greenpoint laboratories who were treated with the all-oral short-course regimen. Isolates from these laboratories were referred for whole genome sequencing as part of a surveillance study. Drug susceptibility patterns for PZA and EMB were classified into four categories: susceptibility to both drugs (EMB-S & PZA-S), resistance to PZA only (EMB-S & PZA-R), resistance to EMB only (EMB-R & PZA-S), and resistance to both drugs (EMB-R & PZA-R). Treatment outcomes were categorized as either successful patient outcomes (SPO) or unsuccessful patient outcomes (USPO). A directed acyclic graph (DAG) guided the selection of variables for the generalized linear model. Adjusted incident rate ratio (aIRR) were calculated with 95% confidence intervals using modified Poisson regression with robust error variances.

Results:

Among the 598 people with MDR-TB included in the analysis, 60% (356/598) were resistant to one or both TB drugs. There was little evidence that resistance to PZA and/or EMB increased USPO: resistance to PZA (aIRR = 1.18, 95% CI: 0.86–1.63), resistance to EMB alone (aIRR = 0.94, 95% CI: 0.67–1.32), and resistance to both drugs (aIRR = 0.97, 95% CI: 0.73–1.27).

Conclusion:

This study also highlights PZA or EMB does not affect treatment outcomes in patients with MDR TB. This observation supports the rationale behind the 2023 adoption of the all-oral MDR TB regimen. WGS is recommended to guide treatment regimens that include PZA and EMB.

Keywords: Multidrug-resistant tuberculosis, pyrazinamide resistance, ethambutol resistance, whole genome sequencing, treatment outcomes.

Background

Drug-resistant tuberculosis (DR-TB) refers to tuberculosis (TB) caused by *Mycobacterium tuberculosis* resistant to one or more anti-TB medications [1]. Rifampicin-resistant TB (RR-TB) is TB resistant to rifampicin, the most effective first-line anti-TB drug [2]. The spread of RR-TB poses a significant public health challenge, threatening TB control efforts in South Africa [3]. Despite advances in TB drugs and diagnostic tests in recent decades, South Africa is classified amongst the World Health Organization's (WHO) list of high-burden countries for TB, HIV-associated TB, and multidrug-resistant TB (MDR-TB) [4]. High-burden countries account for 87% of all estimated TB cases worldwide [5]. South Africa has also identified high resistance to pyrazinamide and ethambutol [6], but the effects of these resistant strains remain poorly understood.

Globally in 2023, an estimated 10.8 million people (95% uncertainty interval [UI]: 10.1–11.7 million) fell ill with TB (incident cases), a further increase from 10.7 million (95% UI: 10.0–11.5 million) in 2022, the highest since the WHO began monitoring in 1995 [1]. In South Africa, the TB incidence rate was 468 per 100,000 population. While the scale-up of antiretroviral treatment (ART) has helped curb the mortality rate, the country's TB epidemic remains high [8].

From 2012 to 2014, the National Institute for Communicable Diseases (NICD), a WHO Supranational TB Reference Laboratory, conducted a nationwide DR-TB survey in collaboration with the national Department of Health [6]. A total of 442 healthcare facilities across South Africa's nine provinces were randomly selected [6]. The survey revealed an MDR-TB prevalence of 2.1% (95% CI: 1.5%–2.7%) among new cases and 4.6% (95% CI: 3.2%–6.0%) among re-treatment cases, with an overall prevalence of 2.8% (95% CI: 2.0%–3.6%) [6]. The proportion of resistant strains on MDR Isolates for pyrazinamide was 59.1% (95% CI: 49.0-69.1) while it was 44.1% (95% CI: 30.2-58.0) for ethambutol [6].

Next-generation sequencing (NGS), enables rapid characterisation of drug-resistance profiles and analysis of transmission strains, providing valuable insights into DR-TB epidemiology [9 – 11]. Whole genome sequencing (WGS), performed at the Centre for Tuberculosis, is a powerful tool for detecting drug-resistant mutations. Studies demonstrate that WGS provides robust, high-resolution drug-resistance data, surpassing traditional methods like culture-based drug susceptibility testing (DST) in speed and accuracy [9]. It predicts resistance to most TB

drugs, including pyrazinamide and ethambutol, facilitating individualised treatment regimens [9].

The introduction of all-oral short-course regimens in 2018 was revolutionary for TB treatment by eliminating injections, reducing pill burden, and potentially improving adherence [12]. This regimen included a combination of seven drugs; bedaquiline, linezolid, levofloxacin, high-dose isoniazid, clofazimine, pyrazinamide, and ethambutol, with a total treatment duration of 9–11 months [12]. In September 2023, South Africa introduced a shorter 6-month regimen (BPaL-L: Bedaquiline, Pretomanid, Linezolid, with or without Levofloxacin) to replace the 9-month and longer (>18 months) regimens [3]. However, patients already on the short-course regimen continued their treatment through 2023–2024 [3].

A study conducted by Lempens and colleagues in Bangladesh area investigated the impact of pyrazinamide and ethambutol resistance on the effectiveness of the shorter multidrug-resistant tuberculosis (MDR-TB) regimen in Bangladesh [13]. Their findings indicated that resistance to these companion drugs did not significantly affect treatment outcomes (OR 1.01, 95% CI 0.4–2.8), provided fluoroquinolone susceptibility was maintained [13]. Consequently, the authors argue that initial resistance to these drugs should not be a criterion for excluding patients from the shorter regimen [13].

Despite advancements in reducing mortality over the past decade, the emerging DR-TB remains a significant barrier to achieving the WHO End TB Strategy 2035, which aims to reduce TB deaths by 95% and incidence by 90% compared to 2015 [1,14]. South Africa faces challenges, including high resistance to pyrazinamide and ethambutol among MDR-TB isolates [12]. Despite this, both drugs were included in the short-course all-oral regimen and furthermore, were not routinely tested for susceptibility in the national TB drug resistance testing algorithm [12]. Limited data exist on the demographic and clinical patterns of how resistance to these drugs affects treatment outcomes. This study evaluated the effect of pyrazinamide and/or ethambutol resistance on TB treatment outcomes among MDR-TB patients treated with the all-oral short-course regimen in South Africa.

Methods

Study Setting

This study is a secondary analysis of data collected from a primary study that evaluated the application of WGS to enhance microbiological and epidemiological surveillance of DR-TB in South Africa. The primary study focused on two districts from high DR-TB burden provinces: City of Johannesburg district in Gauteng province and the City of Cape Town Metro in the Western Cape province.

The primary study focused on two referral laboratories: The Greenpoint TB Laboratory in the Western Cape and the Braamfontein TB Laboratory in Johannesburg. The Greenpoint TB Laboratory, located in the old City Hospital complex in Greenpoint, supports the Western Cape and parts of the Northern Cape. It serves 32 district hospitals, five regional hospitals, six TB hospitals, four psychiatric institutions, and over 400 primary health care facilities.

The Braamfontein TB Laboratory provides services to Ekurhuleni, West Rand, Sedibeng, and the City of Johannesburg districts, as well as referrals from Limpopo, Mpumalanga, North West provinces, and private mining hospitals.

Study Design

A retrospective cohort study design using isolates from the laboratories; Braamfontein TB Laboratory in Gauteng and the Greenpoint TB Laboratory in the Western Cape that had MDR-TB from 2020 – 2022.

Data Sources

The WGS data, as part of a surveillance project funded by the Centers for Disease Control and Prevention (CDC), included only the exposure of interest (pyrazinamide and/or ethambutol resistance) and was linked to the Electronic Drug-Resistant TB Register (EDRWeb), which contained socio-demographic information and final treatment outcomes. Data sources were linked using a combination of deterministic and probabilistic methods, with manual review.

Study Population

All MDR-TB cases on the all oral short course regimen recorded in the EDRWeb matched to isolates referred from the Braamfontein and Greenpoint laboratory and processed for WGS from January 2020 – September 2022**.**

Data Management

All statistical analyses were performed using Stata Statistical Software, Release 18 (StataCorp LLC, College Station, TX, 2023). The dataset was examined for duplicates, outliers, and errors, and deduplication was performed. New variables were created, defined and labelled as appropriate such as the exposure and the outcome variable. The variable 'unsuccessful treatment outcome on TB Rx' (USPO on Rx) was generated by tracing the treatment outcomes of MDR-TB cases from 2 years ago, using EDRWeb data from 2018. Probabilistic record linkage was performed using Stata Statistical Software (Release 18, StataCorp LLC, College Station, TX, 2023). Records were matched based on a combination of key variables, including name, date of birth, Person ID, facility, and the drug-resistant TB (DR-TB) province. These variables were used to estimate the likelihood that records referred to the same individual, allowing for minor discrepancies across datasets.

Exposure

WGS identified resistance to PZA and EMB by detecting mutations in key genes associated with drug resistance. WGS provides a high-resolution, comprehensive method for detecting resistance patterns, allowing for a more precise classification of *Mycobacterium tuberculosis* isolates. One exposure variable was generated with four level categories. The primary exposure to pyrazinamide and/or ethambutol resistance results in four exposure levels of interest:

- Resistant to both ethambutol and pyrazinamide (EMB-R, PZA-R)
- Susceptible to ethambutol and resistant to pyrazinamide (EMB-S, PZA-R)
- Resistant to ethambutol and susceptible to pyrazinamide (EMB-R, PZA-S)
- Susceptible to both ethambutol and pyrazinamide (EMB-S, PZA-S)

Understanding these resistance patterns is clinically important, as they may influence treatment outcomes.

a) **EMB-R & PZA-R**: Dual resistance may indicate more complex treatment challenges, as both drugs are part of standard TB regimens.

b) **EMB-S & PZA-R**: PZA resistance is concerning for MDR-TB and treatment failure risk, especially in retreatment cases.

c) **EMB-R & PZA-S**: EMB resistance alone may suggest early resistance development or issues in first-line therapy.

d) **EMB-S & PZA-S**: This is the fully susceptible group, serving as the reference for comparison.

Outcome - EDRWeb

The TB treatment outcomes analysed in this study included cured, treatment completed, treatment failure, died, and loss to follow-up [15]. The outcomes were categorized as a binary measure: successful treatment outcomes (SPO) and unsuccessful treatment outcomes (USPO). SPO was defined as the sum of cured and treatment completed, while USPO was defined as the sum of died, treatment failure, and loss to follow-up. The binary treatment outcome was categorized according to the WHO definitions. A successful treatment outcome included patients who were either cured or completed treatment. An unsuccessful treatment outcome included those who died, were lost to follow-up, or experienced treatment failure. These categorizations align with WHO's standard reporting framework for tuberculosis treatment outcomes.

Inclusion and Exclusion

After deduplication and linking the CDC WGS and EDRWeb databases, TB cases with an outcome date earlier than the specimen collection date were excluded. To address multiple records across the databases, only cases where treatment commenced within 90 days were retained. Furthermore, the analysis was limited to TB cases on the all-oral short-course regimen and those diagnosed with MDR-TB (Figure 1).

Statistical Analysis

To describe socio-demographic and clinical characteristics, categorical data were summarized as frequencies and proportions, stratified by pyrazinamide and/or ethambutol susceptibility.

To compare the treatment outcomes of people with pyrazinamide and/or ethambutol resistance to those with susceptibility to both drugs, a causal modelling approach was conducted using a directed acyclic graph (DAG) developed with DAGitty V3.1. This was informed by epidemiological knowledge, literature review and expert input. Prior to the analysis, the DAG was used to identify the minimally sufficient set of variables to be included in the model. Our modelling strategy was intentionally focused on estimating the association between drug resistance and outcome. In line with guidance from Westreich and Greenland (2013) in "The Table 2 Fallacy," we were cautious not to fall into the common misinterpretation of coefficients from variables that are not central to the primary causal question. A Generalized Linear Model

(GLM) with a Poisson distribution and robust error variance was used to estimate relative risks. GLM with a Poisson distribution and robust error variance is used to estimate relative risks (or risk ratios) for binary outcomes, especially when the outcome is common and logistic regression may overestimate odds ratios. The Poisson distribution models count data, but when used with binary outcomes, the robust error variance corrects for over dispersion and provides valid standard errors. The GLM with a Poisson distribution and robust error variance was the best choice for this study because it allowed for direct estimation of relative risks, a more interpretable measure than odds ratios, especially when the outcome (treatment success or failure) is common. Unlike logistic regression, which can overestimate effect sizes in such cases, this method provides accurate and stable estimates. Used widely in epidemiological studies and supported by The Stata Journal, it also handles binary outcomes effectively when paired with robust standard errors, correcting for model misspecification and ensuring valid inference.

This method, as recommended in The Stata Journal, is particularly suitable for estimating relative risks in studies with binary outcomes, especially when the outcome is common. It offers a stable and interpretable alternative to logistic regression, which tends to overestimate odds ratios in such contexts. The robust error variance corrects for potential overdispersion and model misspecification, ensuring valid inference. This approach was chosen to assess the association between drug resistance and treatment outcomes.

The GLM assessed the absolute effect of the exposure (PZA and/or EMB resistance) on the outcome. This model included the exposure, the outcome, and variables identified from the DAG as the minimal sufficient adjustment set, namely province/community and previous USPO on TB treatment (Figure 2). The IRR with their corresponding 95% confidence intervals (CIs) were reported.

We chose not to include p-values in the final model table, in line with epidemiological best practices that emphasize the interpretation of effect estimates (adjusted IRRs) and their 95% CIs over p-values. Confidence intervals provide more information than p-values alone, they indicate both the magnitude and precision of the association, and whether the interval includes the null value. Moreover, relying on p-values can lead to misinterpretation of statistical significance, especially in observational studies where the focus is on estimating associations rather than hypothesis testing.

Ethical Approval

Ethical clearance was obtained from the Human Research Ethics Committee (Medical) at the University of the Witwatersrand (REF: M240219).

Results

The study utilized data from two primary sources: the CDC WGS dataset (N = 2,326) and the EDRWeb dataset (N = 2,939). After removing duplicates (n=26), missing final sample outcomes (n=181), and missing person IDs (n=359), 1,760 individuals remained in the CDC WGS dataset. Following the merging of both datasets, the combined dataset contained 3,298 records. Records were excluded due to unsuccessful merging (n=359), outcome date errors (n=119), treatment initiation after 90 days (n=711), invalid outcomes (n=5), duplicates (n=477), records outside the study period (n=163), and individuals not diagnosed with MDR-TB (n=866). The final dataset retained 598 individuals for analysis (Figure 1).

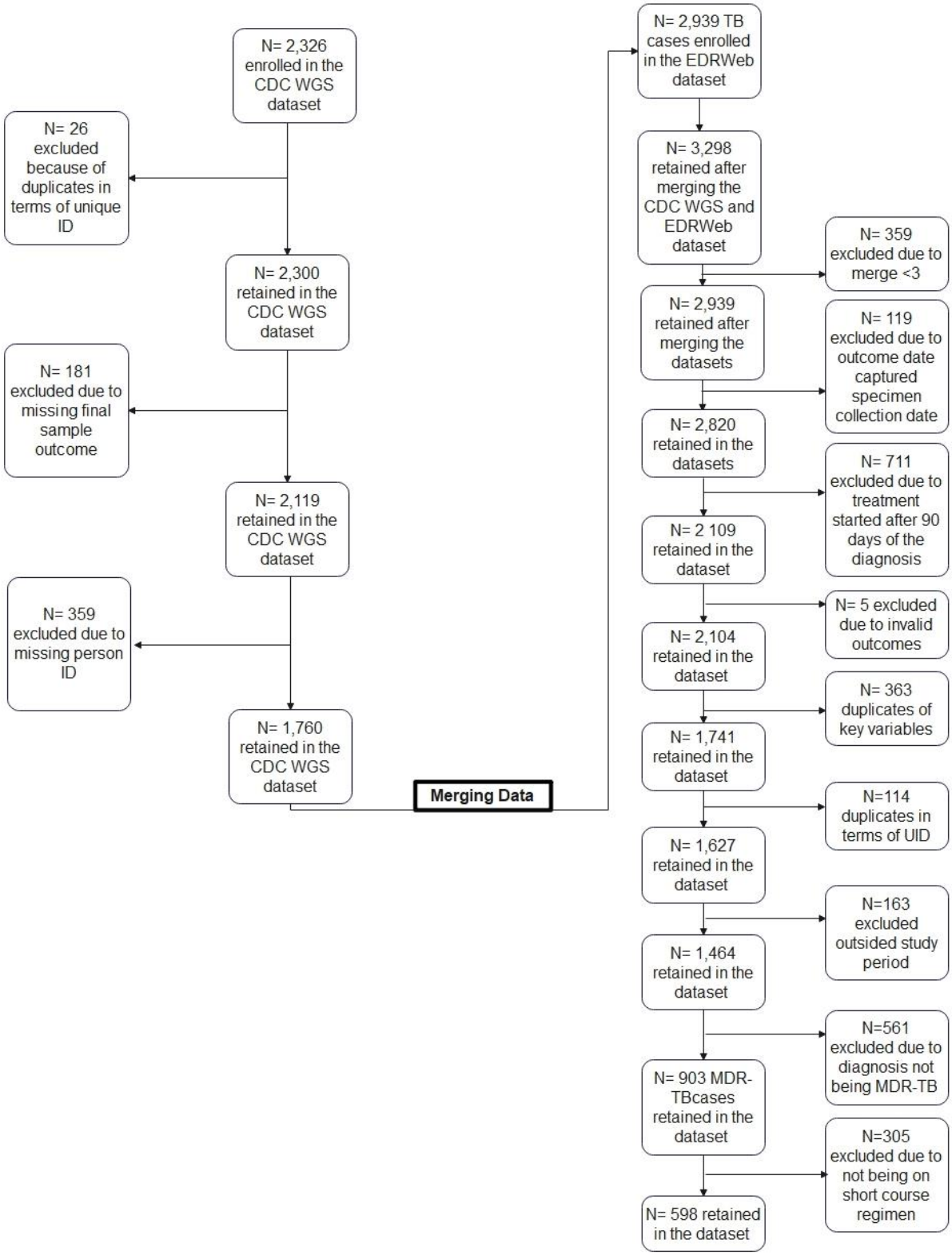


Figure 1: Algorithm of the inclusion and exclusion criteria for MDR-TB cases in the CDC WGS and EDRWeb database, January 2020–September 2022.

Characteristics of pyrazinamide and/or ethambutol susceptibility

A total of 598 individuals with MDR-TB were included in the analysis. Among the people with MDR-TB, 40% (241/598) were susceptible to both drugs (EMB-S and PZA-S), while 60% (357/598) were resistant to one or both drugs (EMB-S and PZA-R, EMB-R and PZA-S, EMB-R and PZA-R). Despite the resistance, overall 64% (352/547) of people with MDR-TB achieved successful treatment outcomes. The majority of people with MDR-TB were in the age groups 25–34 years and 35–44 years, each accounting for 28%. The youngest age group, 0–14 years, accounted for just 1% of the total people with MDR-TB, with very few people in this age range across all resistance groups.

Males made up 60% (361/598) of the people with MDR-TB. Among the males, a large proportion (68%) were resistant to both drugs (EMB-R & PZA-R). Most people with MDR-TB (510/598, 85%) were from the Western Cape province, while only 15% were from Gauteng province.

A small proportion of people had previous DR-TB episodes (10/598, 2%), with a slightly higher frequency of previous DR-TB episodes in the EMB-R, PZA-R group (2%) compared to the other groups. In addition, the majority of individuals in the EMB-S & PZA-S group had no prior DR-TB episodes, suggesting that they were less likely to have had previous resistant infections.

The majority of people with MDR-TB across all groups were classified as 'new' TB cases (396/598, 66%), indicating that individuals with no prior TB diagnosis constituted the largest proportion of people with MDR-TB. This was followed by MDR-TB individuals that relapsed (144/598, 24%).

Pulmonary tuberculosis (PTB) was the predominant form of TB in all groups, accounting for 98% (589/598) of the individuals with MDR-TB. The HIV status of MDR-TB cases showed that more than half of the individuals were HIV-negative (332/598, 56%) **(Table 1.1).**

Among the total population assessed (N=547), 48.3% (264/547) were individuals living with HIV (HIV positive), while 60.7% (332/547) were HIV-negative. These figures suggest that the burden of disease, or the condition being studied, affected both HIV-positive and HIV-negative individuals, with a slightly higher proportion among those who were HIV-negative.

This distribution should be considered when evaluating potential risk factors and stratifying treatment outcomes by HIV status.

Table 1: Socio-demographic and clinical characteristics of the people with MDR-TB in Western Cape and Gauteng Provinces from 2020 – 2022.

	PZA and/or EMB susceptibility				
	PZA-S & EMB-S 241 (40.3%)	**PZA-S & EMB-R 73 (12.2%)**	**PZA-R & EMB-S 104 (17.4%)**	**PZA-R & EMB-R 180 (30.1%)**	**Total 598 (100.0%)**
Treatment Outcome					
Successful Patient Outcome	140 (64.8%)	38 (55.9%)	67 (67.0%)	107 (65.6%)	352 (64.4%)
Unsuccessful Patient Outcome	76 (35.2%)	30 (44.1%)	33 (33.0%)	56 (34.4%)	195 (35.6%)
Age Category					
0 - 14 years	5 (2.1%)	0 (0.0%)	2 (1.9%)	1 (0.6%)	8 (1.3%)
15 - 24 years	45 (18.8%)	7 (9.6%)	24 (23.3%)	35 (19.4%)	111 (18.6%)
25 - 34 years	66 (27.5%)	22 (30.1%)	26 (25.2%)	51 (28.3%)	165 (27.7%)
35 - 44 years	70 (29.2%)	24 (32.9%)	28 (27.2%)	47 (26.1%)	169 (28.4%)
45 - 64 years	48 (20.0%)	20 (27.4%)	19 (18.4%)	42 (23.3%)	129 (21.6%)
65 years & older	6 (2.5%)	0 (0.0%)	4 (3.9%)	4 (2.2%)	14 (2.3%)
Sex					
Female	103 (42.7%)	31 (42.5%)	45 (43.3%)	58 (32.2%)	237 (39.6%)
Male	138 (57.3%)	42 (57.5%)	59 (56.7%)	122 (67.8%)	361 (60.4%)
DR-TB Province					
Gauteng	38 (15.8%)	3 (4.1%)	22 (21.2%)	25 (13.9%)	88 (14.7%)
Western Cape	203 (84.2%)	70 (95.9%)	82 (78.8%)	155 (86.1%)	510 (85.3%)
Previous DR-TB Episode					

No	236 (97.9%)	73 (100.0%)	103 (99.0%)	176 (97.8%)	588 (98.3%)
Yes	5 (2.1%)	0 (0.0%)	1 (1.0%)	4 (2.2%)	10 (1.7%)
Patient Category					
New	146 (60.6%)	49 (67.1%)	76 (73.1%)	125 (69.4%)	396 (66.2%)
Relapse	71 (29.5%)	18 (24.7%)	16 (15.4%)	39 (21.7%)	144 (24.1%)
TF1	15 (6.2%)	4 (5.5%)	5 (4.8%)	13 (7.2%)	37 (6.2%)
TF2	0 (0.0%)	0 (0.0%)	1 (1.0%)	0 (0.0%)	1 (0.2%)
Treatment after loss to follow-up	7 (2.9%)	1 (1.4%)	6 (5.8%)	2 (1.1%)	16 (2.7%)
Other	2 (0.8%)	1 (1.4%)	0 (0.0%)	1 (0.6%)	4 (0.7%)
HIV Status					
Negative	129 (53.8%)	40 (54.8%)	62 (60.2%)	101 (56.1%)	332 (55.7%)
Positive	111 (46.2%)	33 (45.2%)	41 (39.8%)	79 (43.9%)	264 (44.3%)
Bedaquiline Susceptibility					
Sensitive	239 (99.2%)	72 (98.6%)	102 (98.1%)	170 (94.4%)	583 (97.5%)
Resistant	2 (0.8%)	1 (1.4%)	2 (1.9%)	10 (5.6%)	15 (2.5%)
Fluoroquinolone Susceptibility					
Sensitive	240 (99.6%)	72 (98.6%)	103 (99.0%)	169 (93.9%)	584 (97.7%)
Resistant	1 (0.4%)	1 (1.4%)	1 (1.0%)	11 (6.1%)	14 (2.3%)

*Ethambutol (EMB), Pyrazinamide (PZA), Susceptible (S), Resistant, (R), Drug-resistant tuberculosis (DR-TB), Treatment failed in regimen 1 (Tf1), Treatment failed in regimen 2 (TF2), Pulmonary tuberculosis (PTB), Extra-pulmonary tuberculosis (EPTB).

Table 2: Incidence of MDR-TB Among Individuals with and without HIV in the Western Cape and Gauteng Provinces, 2020–2022.

HIV Status	**Incidence rate**	**Percentage (%)**
HIV Status Positive (+)	264 / 547 X 100	48.3%
HIV Status Negative (-)	332 / 547 X 100	60.7%

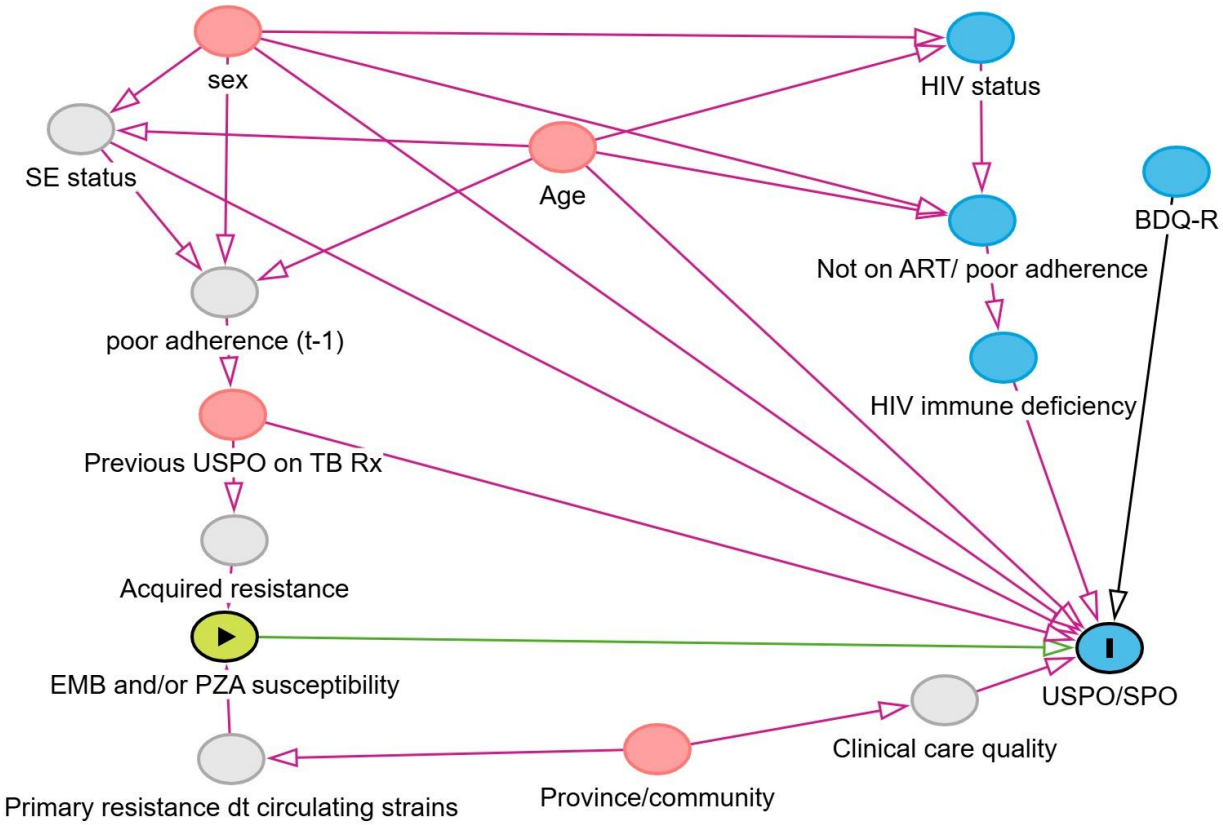
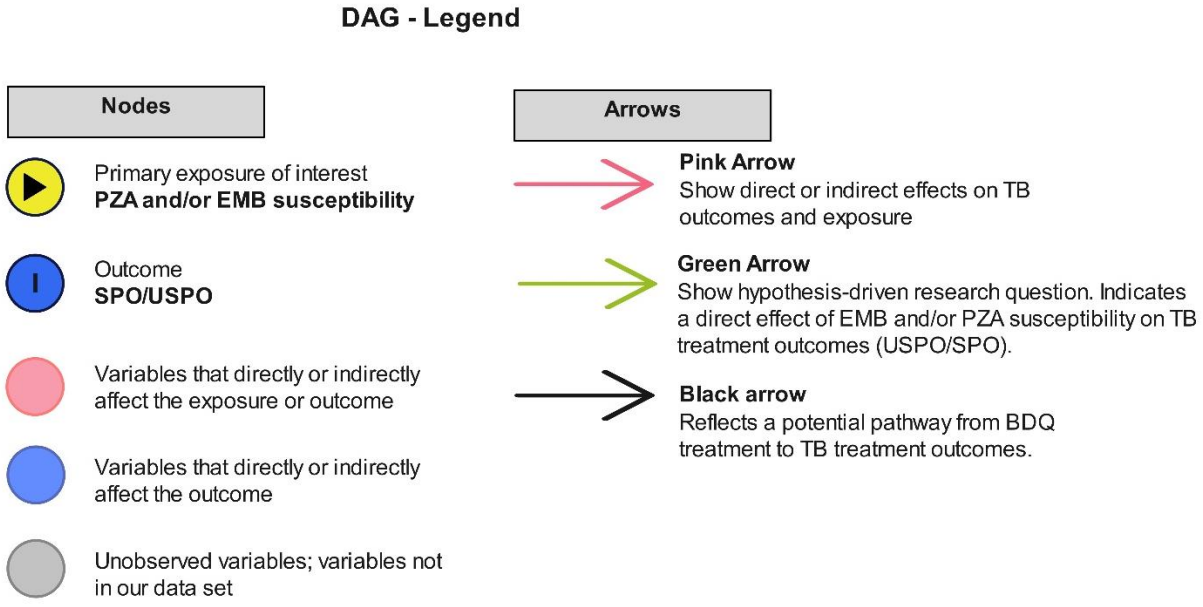


Figure 2: Factors influencing TB treatment outcomes in relation to Ethambutol (EMB) and/or Pyrazinamide (PZA) susceptibility.



Multivariable analysis

None of the categories, either PZA and/or resistance, affected the IRR of TB treatment compared to those susceptible to both drugs (PZA-S & EMB-S). People with MDR-TB and only PZA resistance had a slightly higher adjusted IRR for USPO (adjusted IRR = 1.18, 95% CI = 0.86–1.63) while those resistant to only EMB had slightly lower IRR (adjusted IRR = 0.94, 95% CI = 0.67–1.32) though the confidence intervals included the null in both cases. Resistance to both drugs also did not influence treatment outcomes (adjusted IRR = 0.97, 95% CI = 0.73–1.27) **(Table 3).**

Table 3: Factors associated with unsuccessful treatment outcomes among MDR-TB cases in Gauteng and Western Cape provinces from 2020 - 2022.

Variable Name	Adjusted IRR (95% CI)
Pyrazinamide and/or ethambutol resistance	
EMB-S & PZA-S	Ref
EMB-S & PZA-R	1.18 (0.86 - 1.63)
EMB-R & PZA-S	0.94 (0.67 - 1.32)
EMB-R & PZA-R	0.97 (0.73 - 1.27)
Province	
Gauteng	Ref
Western Cape	1.78 (1.16 - 2.74)
Previous USPO on TB Rx	
No	Ref
Yes	-

***IRR – incidence risk ratio, *EMB – ethambutol, PZA – Pyrazinamide, S – susceptible, R – resistant, USPO – unsuccessful treatment outcome, TB – tuberculosis, Rx – Treatment**

Discussion

This study evaluated the effects of pyrazinamide and/or ethambutol resistance on treatment outcomes among MDR-TB individuals treated with the all-oral short-course regimen in South Africa from January 2020 to September 2022. A considerable proportion (40%) of individuals with MDR-TB were susceptible to both ethambutol and pyrazinamide, while the majority showed resistance to either one or both of these drugs. Despite the high resistance, the majority achieved successful treatment outcomes. The largest proportions of individuals with MDR-TB were within the 25–34 years and 35–44 years age categories, with men having more than twice the proportion of resistance to both drugs compared to women. Most individuals with MDR-TB were HIV-negative. None of the resistance categories affected the estimated relative risk of TB treatment outcomes when compared to individuals susceptible to both drugs.

Descriptive Findings

This study found a high proportion of individuals with MDR-TB were resistant to either pyrazinamide and/or ethambutol. These findings align with the South African Drug Resistance Survey (2012 – 2014) which found that there was a high proportion of resistance to pyrazinamide and ethambutol among MDR-TB isolates [6].

Despite this high level of resistance, this study found that the majority achieved successful treatment outcomes. These findings align with the study by Lempens and colleagues which found that despite resistance to drugs like pyrazinamide and ethambutol which are companion drugs most individuals still achieve successful treatment outcomes [13]. Their findings suggest that the shorter MDR-TB regimen remains effective even in individuals with resistance to companion drugs, showing that such resistance does not necessarily result in unsuccessful treatment outcomes [13].

The highest proportion of MDR-TB was observed in the 25–34 years and 35–44 years age groups, with men showing more than double the proportion of resistance to both drugs compared to women. These findings are consistent with the South African TB prevalence study, which also found that TB prevalence peaked in the 35–44 years age group for overall pulmonary TB [15]. Furthermore, the finding that men have a higher proportion of drug resistance aligns with both the South African study and global studies [16], which report that men have a significantly higher overall prevalence of TB compared to women [15]. Other studies have suggested that higher exposure risk and differences in healthcare-seeking behaviour may contribute to men's increased susceptibility to both TB and MDR-TB [5]. Age-

targeted interventions for 25–44-year-olds, including the working class, students, and the unemployed, are essential. Expanding workplace screenings, mobile clinics, and strengthening community health worker capacity may improve TB access in underserved areas.

HIV Negative

This study also found a higher proportion of individuals with MDR-TB among HIV-negative individuals. This aligns with previous research in South Africa indicating a burden of TB among those who are HIV-negative [15]. In this study, the majority of individuals with MDR-TB were HIV-negative. While people living with HIV are known to be at higher risk of TB-related mortality due to immunosuppression, it is important to note that HIV-negative individuals also represent a significant proportion of the MDR-TB burden and can experience poor outcomes due to delayed diagnosis, treatment challenges, or comorbidities. Literature shows that those who are at higher risk of mortality were LTFU from ART, whereas those who were on ART and accessing care regularly had better outcomes. This aligns with existing literature. People living with HIV (PLHIV) who are not on ART or are lost to follow-up (LTFU) from HIV care face significantly higher mortality risks compared to those who are retained in HIV care and on ART, who tend to have better TB treatment outcomes due to immune reconstitution and access to integrated services.

Another study by Bustard and colleagues found that while people living with HIV who are co-infected with MDR-TB had a higher fatality rate, they also had a higher treatment success rate compared to people who are HIV-negative but have MDR-TB (64.0% vs 53.2%) [17]. Additionally, the loss to follow-up rate was significantly higher among HIV-negative patients (22.0% vs. 8.4%) [17]. This suggests that while HIV-positive individuals face greater challenges in terms of mortality, they also might receive more comprehensive care and support, leading to better treatment adherence and outcomes [18]. On the other hand, people who were HIV-negative and had MDR-TB might not receive the same level of integrated care, which could contribute to higher rates of unsuccessful treatment outcomes.

Model

This study found no evidence that resistance to pyrazinamide and/or ethambutol affects TB treatment outcomes. Resistance to these drugs did not influence treatment success or failure. This may suggest that in the context of the overall short-course treatment regimen, resistance to these specific drugs does not independently drive treatment success or failure. Resistance to

both drugs also did not affect treatment outcomes among people with MDR-TB who are treated with the all oral short course regimen.

Literature indicates that resistance to pyrazinamide and/or ethambutol does not significantly compromise the overall treatment success of MDR-TB [13]. In contrast, another study found that patients with pncA mutations (genotypic resistance to PZA) had a significantly longer time to sputum culture conversion compared to those without such mutations [19, 22]. Furthermore, the inclusion of pyrazinamide in treatment regimens, even when resistance is present, has not been shown to improve outcomes.

Although resistance to pyrazinamide and/or ethambutol was not found to compromise MDR-TB treatment outcomes, these drugs despite their known severe adverse effects [20] were still prescribed as part of the all-oral short-course regimens. It is important to assess whether these drugs are necessary for the full duration of the regimen in people with MDR-TB, despite resistance to them.

Ethambutol is associated with several side effects that can impact patient adherence to treatment regimens [21]. Common side effects include decreased visual acuity, optic neuritis, and colour blindness [20]. These side effects can be particularly challenging for patients, leading to difficulties in maintaining consistent treatment. In severe cases, ethambutol can cause irreversible vision loss, which necessitates careful monitoring and management [20]. The WHO has introduced new treatment regimens for DR-TB, including the BPaL-L regimen [12]. This regimen has shown improved treatment success rates and fewer adverse events without including pyrazinamide and ethambutol [12].

The study period overlapped with the global COVID-19 pandemic, which significantly disrupted TB services in South Africa and globally. National lockdowns, reallocation of healthcare resources, reduced patient mobility, and fear of attending health facilities all contributed to delays in TB diagnosis, interruptions in treatment, and poorer overall health outcomes. Although all participants in the study were affected by the same pandemic context thereby limiting differential bias between exposure groups. It is important to note that the pandemic may have contributed to a general increase in unsuccessful treatment outcomes. This broader impact does not undermine the internal comparisons made within the study but may limit the generalisability of the findings to non-pandemic periods.

In support of this, the WHO Global Tuberculosis Report (2022) noted a significant decline in TB case detection and treatment completion during the pandemic, with South Africa

experiencing decreased notifications and increased mortality. A modelling study by McQuaid and colleagues (2020) also projected increased TB-related deaths due to COVID-related health system strain [23]. These findings align with observations that TB outcomes were adversely affected during the pandemic and justify our acknowledgment of this as a potential background influence on the overall treatment outcomes in our study population.

Conclusion

This study provided insights into the effect of pyrazinamide and ethambutol resistance on treatment outcomes among people with MDR-TB receiving the all-oral short-course regimen in South Africa from 2020 to 2022. Resistance to pyrazinamide and/or ethambutol did not show any evidence that it affected the overall treatment outcome. The study also highlighted the disproportionate burden of MDR-TB among HIV-negative individuals.

Strengths

This study addresses an important gap in knowledge regarding the effects of pyrazinamide and/or ethambutol resistance on treatment outcomes among individuals with MDR-TB in South Africa, one of the 30 high-burden countries. Despite their high resistance rates and adverse effects, these drugs were included for the full duration of the all-oral short-course regimen. This study provides insights, finding no evidence that their inclusion affects the overall TB treatment outcomes. Additionally, it highlights the burden of MDR-TB among HIV-negative individuals and the ongoing challenges with pyrazinamide and ethambutol resistance. It is important to clarify that this study was designed not only to estimate the association between pyrazinamide and/or ethambutol resistance and treatment outcomes, but also to contribute to the growing body of research in drug-resistant TB. The analytic approach and model outputs are intended to inform future epidemiological investigations and guide hypothesis generation. In this context, the study serves as a foundational analysis upon which subsequent work, potentially with prospective designs or larger datasets, can build. Furthermore, it highlights the higher resistance observed in men, taking into account that a greater proportion of males are infected with TB than females.

Limitations

A limitation of this study is the presence of unobserved variables in the DAG. The absence of certain variables may lead to residual confounding, which could affect the accuracy of the associations depicted. This limitation highlights the potential for omitted variable bias, as we

may not have fully accounted for all underlying factors influencing the relationship between the exposure and outcome. While our findings provide important preliminary evidence, the observational nature of the study and the specific model assumptions warrant cautious interpretation. The intention is to promote methodological dialogue and provide a platform for other researchers to refine and expand upon this work using alternative modelling strategies or additional data sources.

This study was conducted during the COVID-19 pandemic (2020–2022), a period marked by widespread disruptions to TB services in South Africa. Although all study groups were equally exposed to pandemic-related challenges, the overall increase in unsuccessful TB treatment outcomes during this time may have influenced the results. As such, the findings may not be fully generalisable to non-pandemic periods.

Linkage of the CDC WGS and EDRWeb databases resulted in additional missing outcome data. Due to the duration of the study period, some individuals with MDR-TB in the database who were enrolled in 2022 do not have the final TB outcomes in the EDRWeb.

Recommendations

This study did not find that resistance to either PZA and/or EMB significantly affected treatment outcomes. The study alignment with the findings of Lampens in Bangladesh, which similarly demonstrated that resistance to PZA or EMB does not significantly impact treatment outcomes. This is further supported by the 2023 adoption of the all-oral MDR TB regimen, which excludes both PZA and EMB. Patients on this regimen should undergo routine screening for severe side effects at every visit, and the two drugs should be discontinued immediately if adverse reactions occur. We recommend strengthening surveillance systems, particularly through electronic linkages to enable rapid programmatic responses and ongoing evaluation of MDR-TB treatment regimens. Additionally, routine use of the TB screening form during clinical consultations, regardless of HIV status, may support early detection and timely treatment, and could complement broader surveillance efforts.

We acknowledge that most patients are currently being treated with the Bedaquiline, Linezolid, Levofloxacin (BPaL-L) regimen, and agree that prioritising whole genome sequencing (WGS) surveillance for drugs currently in use is crucial for optimizing treatment effectiveness. However, we also note that resistance to pyrazinamide and ethambutol remains relevant, especially for individuals who may not be eligible for BPaL-L or who are initiated on alternative regimens due to contraindications, drug stock-outs, or clinical considerations. As

such, expanding WGS surveillance to include pyrazinamide and ethambutol can still provide valuable insights into resistance patterns and help guide regimen selection in real-world programmatic settings. This recommendation has been revised to better reflect these priorities.

We recommend conducting a follow-up study with a larger sample size, potentially including all provinces, to improve the generalizability of the findings. Additionally, future research should aim to incorporate all relevant variables with a more complete dataset to build upon this study and account for all factors included in the DAG.

This study serves as a foundation for broader research and aims to initiate dialogue for future investigations. It focused on evaluating the effects of pyrazinamide and/or ethambutol resistance on treatment outcomes, which remain poorly understood. Future studies should explore resistance patterns to other drugs and their impact on treatment outcomes.

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CHAPTER 3: EXTENDED METHODS

This section provides a detailed description of the primary study, the completeness of the variables used, and the method used to measure the susceptibility of pyrazinamide and ethambutol.

Primary Study

Description of Primary Study

This study evaluated the application of whole genome sequencing (WGS) to enhance microbiological and epidemiological surveillance of drug-resistant tuberculosis (DR-TB) in South Africa.

Study Setting:

The National Health Laboratory Service (NHLS) has a nationwide footprint with 203 laboratories servicing approximately 4,700 healthcare facilities, many equipped with GeneXpert systems for TB detection. For this study, two referral laboratories were included: the Greenpoint TB Laboratory in the Western Cape and the Braamfontein TB Laboratory in Johannesburg. The Greenpoint TB Laboratory, located in the old City Hospital complex on Portwood Road, supports the entire Western Cape Province and parts of the Northern Cape, including 32 district hospitals, five regional hospitals, six TB hospitals, four psychiatric institutions, and over 400 primary healthcare facilities. The Braamfontein Laboratory supports regions in Greater Gauteng, including Ekurhuleni, West Rand, Sedibeng, and the City of Johannesburg, as well as referring sites from Limpopo, Mpumalanga, Northwest Provinces, and private mining hospitals.

Study Population:

The study included DR-TB patient isolates (MDR-TB, pre-XDR, and XDR) from two high-burden districts: The City of Johannesburg in Gauteng and the City of Cape Town Metro in the Western Cape. These isolates were collected between October 1, 2019, and September 30, 2022.

Inclusion Criteria:

All rifampicin-resistant/multidrug-resistant (RR/MDR-TB), pre-XDR, and XDR isolates, classified per WHO definitions, were included if collected in Johannesburg or Cape Town between October 1, 2019, and September 30, 2022.

Exclusion Criteria:

Patients with drug-susceptible TB and those with mono-resistance to rifampicin (RIF) or isoniazid (INH) were excluded from this study

Data Collection and Management:

Data were collected continuously from 2019 to 2022. Routine drug resistance test results were recorded at study sample registration and used to classify patients according to updated WHO definitions for TB drug resistance. Patient outcomes were extracted from the Electronic Drug Resistant Tuberculosis Register (EDRWeb), which includes all patients started on DR-TB regimens. Data from the Cape Town and Johannesburg referral laboratories were entered into a web-based laboratory information system, then stored in the Corporate Data Warehouse (CDW) and further processed for DR-TB reporting in the Surveillance Data Warehouse (SDW) The CDW, managed by the NHLS, serves as a central database for public sector laboratory data nationally, while the SDW was specifically created for surveillance activities. All data are stored on secure servers with restricted access and are protected by firewalls and corporate IT security policies.

Limitation:

Due to limited funding from the Centers for Disease Control and Prevention (CDC), the study was limited to two districts from high-burden provinces.

Other Objectives

3.2. Before the analysis, variables were assessed for completeness and type using the codebook and by examining the proportion of completeness for each variable.

3.3. Additional analysis focused on the proportion of pyrazinamide and ethambutol resistance. Counts and proportions were reported**.**

CHAPTER 4: EXTENDED RESULTS

This section presents the results on variable completeness and the proportion of pyrazinamide and ethambutol resistance among MDR-TB patients treated with the all-oral short-course regimen in South Africa (2020–2022).

The dataset showed high levels of completeness for most variables, with over 90% completeness across key demographic and clinical characteristics. Variables such as *Sex*, *DR-TB Province*, *Previous DR-TB Episode*, and *PTB or EPTB* were 100% complete. Slightly lower completeness was observed for *Age Category* (99%) and *HIV Status* (99%) and *Treatment Outcome* (92%) and *ART Status* (42%). However, *CD4 Count* (3%) and *Viral Load* (2%), had the lowest levels completeness **(Table 1).**

Table 1: Variable name, classifications, and completeness for each variable among MDR-TB cases in Gauteng and Western Cape province, from 2020 – 2022.

Variable Name	Variable Type	Categories	Completeness
Ethambutol and/or Pyrazinamide Susceptibility	Categorical (Nominal)	EMB-S PZA-S, EMB-S PZA-R, EMB-R PZA-S, EMB-R PZA-R	598 (100%)
Sex	Categorical (Binary)	Female, Male	598 (100%)
DR-TB Province	Categorical (Nominal)	Gauteng, Western Cape	598 (100%)
Previous DR-TB Episode	Categorical (Binary)	No, Yes	598 (100%)
Patient Category	Categorical (Nominal)	New, Relapse, Tf1, Tf2, TAL, Other	598 (100%)
PTB or EPTB	Categorical (Binary)	PTB, EPTB	598 (100%)
Previous USPO on TB Rx	Categorical (Binary)	No, Yes	598 (100%)
Bedaquiline	Categorical (Binary)	Sensitive, Resistant	598 (100%)
Pyrazinamide	Categorical (Binary)	Sensitive, Resistant	598 (100%)
Ethambutol	Categorical (Binary)	Sensitive, Resistant	598 (100%)

Age Category	Categorical (Ordinal)	0–14, 15–24, 25–34, 35–44, 45–54, 55–64, 65+	596 (99%)
HIV Status	Categorical (Binary)	Negative, Positive	596 (99%)
Treatment Outcome	Categorical (Binary)	Successful Treatment Outcome, Unsuccessful Treatment Outcome	547 (92%)
ART Status	Categorical (Binary)	No, Yes	254 (42%)
CD4 Count	Continuous (Ratio)	24 - 811	19 (3%)
Viral Load	Continuous (Ratio)	<20 – 296,658	13 (2%)

Among the 598 MDR-TB individuals analysed, 42% (253/598) were resistant to pyrazinamide, while 58% (345/598) were sensitive. Resistance to ethambutol was observed in 48% (284/ 598), of individuals who had MDR-TB whereas 53% (314/598) remained sensitive. These findings indicate a substantial proportion of individuals who had MDR-TB were resistant to pyrazinamide and ethambutol who were treated with the all oral short course regimen from 2020 – 2022.

Table 2: Susceptibility of pyrazinamide and ethambutol among individuals with MDR-TB from 2020 - 2022

Name of drug	**Summary** **598**
Pyrazinamide	
Sensitive	345 (58%)
Resistant	253 (42%)
Ethambutol	
Sensitive	314 (53%)
Resistant	284 (48%)

Table 3: HIV and ART status among individuals with MDR-TB from 2020 - 2022

Characteristics	Incidence X 100, 000	Total N (%)
People living with HIV with MDR-TB	264 / 596	0.44 X 100,000 = 44.3%
HIV Negative individuals with MDR-TB	332 / 596	0.56 X 100,000 = 55.7%
People living with HIV on ART	246 / 254	0.96 X 100,000 = 96.9%
People who are HIV not ART	8 / 254	0.03 X 100,000 = 3.1%

CHAPTER 5: OVERALL DISCUSSION, CONCLUSION AND RECOMMENDATION

This section presents the overall discussion, conclusion, and recommendations, highlighting key findings of the study the hypothesis driven research. It also explains how the primary study informed the secondary analysis, discusses major strengths and limitations, and examines the impact of data completeness on the analysis.

Discussion

This study investigated the effects of pyrazinamide and/or ethambutol resistance on the treatment outcomes of MDR-TB patients treated with an all-oral short-course regimen in Gauteng and Western Cape between January 2020 and September 2022. The findings indicate a substantial proportion of MDR-TB individuals were resistant to pyrazinamide and ethambutol. However, this resistance did not affect the overall TB final treatment outcomes in those treated with the all-oral short-course regimen.

The primary study formed the basis of this research, as we analysed MDR-TB isolates from two laboratories in high DR-TB provinces of South Africa. These isolates were identified using WGS to detect resistance to pyrazinamide and ethambutol, a method that provides more accurate and comprehensive results than traditional phenotypic drug susceptibility testing, especially for these drugs [1- 3]. This study reinforces the adoption of WGS for surveillance, given the growing resistance among MDR-TB isolates.

The treatment outcome variable was 92% complete meaning some of the individuals in the data did not have treatment outcomes. This missing treatment outcome was a limitation, potentially introducing bias that could either overestimate or underestimate the true effect. Additionally, variables such as ART initiation, CD4 count, and viral load were less than 80% complete, which was also a limitation given that this study was based on a DAG. As Westreich and colleagues highlight, inappropriate adjustment or misinterpretation of confounder coefficients especially when missing data is present can lead to misleading conclusions about causal relationships [4]. Missing data in key confounders can introduce selection bias, distort the assumed causal structure, or result in incomplete adjustments, affecting the estimated effect of drug resistance on treatment outcomes.

RECOMMENDATIONS

This study did not find evidence supporting the inclusion of pyrazinamide and ethambutol in the all-oral short-course regimen. Therefore, the adoption of BPAL-L should be strengthened, as it has demonstrated better efficacy and reduced mortality [5], while the use of the all-oral short-course regimen should be phased out immediately. BPAL-L has shown better efficacy and reduced mortality. Expanding WGS surveillance for pyrazinamide and ethambutol resistance in MDR-TB individuals is also key, with a goal of increasing coverage by at least 30% in high DR-TB provinces over the next two years in individuals on the all oral short course regimen. Strengthening TB data integration by creating a unified system to track treatment outcomes will help reduce missing data by 50% in two years through better collaboration and electronic records.

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FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Sinothando Samukele Dlamini (Student number: 1684549) am a student registered for the degree of MSc Field Epidemiology in the academic year 2024.

I hereby declare the following:

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- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
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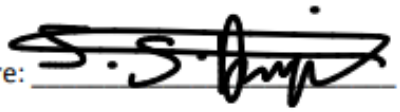
Signature:  Date: 01 November 2024

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Miss Sinothando Dlamini et al M240219 MED24-01-379

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

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NAME: (Principal Investigator)	Miss Sinothando Dlamini et al
STUDENT/STAFF NO:	1684549
DEGREE:	Msc Epidemiology and Biostatistics
DEPARTMENT:	School of Public Health Epidemiology and Biostatistics Centre for Tuberculosis National Institute for Communicable Disease
PROJECT TITLE:	Effects of pyrazinamide and/or ethambutol resistance on tuberculosis treatment outcomes by whole genome sequencing, South Africa, 2020 - 2022
DATE CONSIDERED:	01/03/2024
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Harry Moultrie
APPROVED BY:	Paul Ruff Paul Ruff (Jun 12, 2024 12:39 GMT+2) Prof P Ruff, Chair, HREC (Medical)
DATE OF APPROVAL:	03/05/2024 **EXPIRY DATE:** 03/05/2029

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

DECLARATION OF INVESTIGATORS

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to Committee. **I agree to submit a yearly progress report** in July each year until study is closed. Failure to submit annual report will result in ethics clearance certificate suspension. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in February and will therefore be due in the month of February each year. Unreported changes to the application invalidate the clearance given by the HREC (Medical). Email signed copy of this ethics clearance certificate prior to commencing with the study hrec-medical.researchoffice@wits.ac.za

Principal Investigator Signature — Date: 12 Jun 2024

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