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PHARMACOLOGIC AND CLINICAL RISK FACTORS OF POOR TUBERCULOSIS TREATMENT OUTCOMES IN PATIENTS WITH RIFAMPICIN-SUSCEPTIBLE TUBERCULOSIS IN SELECTED HOSPITALS IN GHANA

BY

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DECLARATION

I declare that this thesis is the product of my own research except where references have been made to work done by other people. This research was conducted in three districts in the Ashanti Region and one district in the Bono East Region of Ghana under the supervision of my academic supervisors. This thesis has not been submitted to any institution for the award of any other degree.

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ABSTRACT

Introduction: The global reduction in Tuberculosis (TB) incidence and deaths since 2015 is not progressing as rapidly as required to meet the established milestones of a 50% reduction in the 2015 incidence rate and a 75% reduction in the 2015 mortality rate. The rate of decline in the African region is even slower. However, the milestones are achievable with early diagnosis and appropriate treatment. Adequate plasma concentration of the recommended multi-drug regimen for each patient is important for treatment success. Current evidence linking plasma concentration to treatment outcomes has not been conclusive. This study sought to determine the pharmacologic and clinical risk factors of poor TB treatment outcomes in patients with rifampicin-susceptible TB in Ghana.

Methods: A prospective study was conducted in four hospitals in three districts in the Ashanti Region and one hospital in the Bono East Region of Ghana. New and relapse TB patients eligible for management with the first-line anti-TB regimen were recruited and followed up monthly to the end of treatment in 6 months. The sample size was determined to be 164. Eligible participants were recruited by consecutive sampling. The primary outcome variable was treatment outcomes measured on a binary scale, unsuccessful and successful. The main exposure variables of interest were peak plasma concentrations (C_{max}) of rifampicin, isoniazid, pyrazinamide, and ethambutol. C_{max} was dichotomized using universally accepted thresholds. Blood samples were collected for all participants between the 4^{th} and 8^{th} week after treatment initiation. Validated high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used for the determination of the drug concentrations. To determine the association between low C_{max} and treatment outcomes, cross-fit partialing-out lasso for Poisson models was used. The baseline and longitudinal risk factors for bacteriologic failure were determined using generalized estimating equations with

an “independent” working correlation and a logit link.

Results: A total of 164 participants were selected into the study. The proportion of participants with low C_{max} of rifampicin, isoniazid, pyrazinamide, and ethambutol were 94.4%, 87.0%, 31.3% and 52.2%, respectively. Low rifampicin C_{max} was associated with a 19% reduced likelihood of sputum smear conversion at the end of the intensive phase of treatment (IRR = 0.81; 95% CI: 0.73, 0.89; $p < 0.001$). Patients with low pyrazinamide C_{max} had an increased incidence of unsuccessful end-of-treatment outcomes (IRR = 5.92; 95% CI: 2.72, 12.88; $p < 0.001$). When those lost to follow up were excluded from the outcomes category the effect size was 9.11 (IRR = 9.11; 95% CI: 1.66, 50.07; $p = 0.002$). Low ethambutol C_{max} increased the risk of unsuccessful outcomes by 5.74 times (IRR = 5.74; 95% CI: 1.88, 17.53; $p = 0.002$). Normal rifampicin C_{max} perfectly predicted successful treatment outcomes at the end of the continuation phase. Patients with concurrently low C_{max} in all the four anti-TB drugs were 12.40 times more likely to have unsuccessful end-of-treatment outcomes (IRR = 12.40; 95% CI: 2.20, 69.76, $p = 0.004$). Bacteriologic failure over the course of treatment was more likely among participants with low rifampicin C_{max} than those with normal rifampicin C_{max} (AOR = 2.44; 95% CI: p =0.001).

Conclusion: This study showed that low rifampicin, pyrazinamide, and ethambutol plasma concentrations are risk factors for poor TB treatment outcomes. In addition, a concurrent deficiency in the plasma concentration of all the four first-line anti-TB drugs increases the susceptibility to poor treatment outcomes.

Recommendation: Dose adjustment strategies for patients with rifampicin-susceptible TB must be pursued. This includes incorporating therapeutic drug monitoring into clinical practice for TB management and conducting research into the use of higher doses of rifampicin, pyrazinamide, and/or ethambutol. These will help to reduce the likelihood of unsuccessful treatment outcomes.

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To my saviour, the lover of my soul, Jesus Christ, words are not enough.

DEDICATION

I dedicate this work to my dear wife, Mrs. Jemima Opoku-Mireku.

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LIST OF ABBREVIATIONS

AFB	Acid Fast Bacilli
ARVs	Anti-retrovirals
AUC	Area under Concentration-time curve
C_{2h}	2-hour post-dose plasma concentration
C_{4h}	4-hour post-dose plasma concentration
C_{max}	Peak plasma/serum concentration
DM	Diabetes Mellitus
DOTS	Directly Observed Treatment – short course
EPTB	Extra-pulmonary tuberculosis
FDC	Fixed Dose Combination
GBTM	Group-based Trajectory Modeling
KATH	Komfo Anokye Teaching Hospital
LCMS/MS	Liquid chromatography-tandem mass spectrometry
MDR-TB	Multidrug Resistant tuberculosis
MIC	Minimum Inhibitory Concentration
NAT2	N-acetyltransferase 2
NNRTI	Non-Nucleoside Reverse Transcriptase Inhibitor
PD	Pharmacodynamics
PI	Protease Inhibitor
PK	Pharmacokinetics
Pre-XDR	Pre-Extensively Drug Resistant
PTB	Pulmonary tuberculosis
RIF	Rifampicin
RR-TB	Rifampicin Resistant tuberculosis
TB	Tuberculosis
TDM	Therapeutic Drug Monitoring
XDR-TB	Extensively Drug Resistant tuberculosis
WRD	WHO-Approved Rapid Diagnostic

DEFINITION OF TERMS

Term	Definition
Bacteriologic Failure	Sputum smear positive results from microscopy recorded during treatment.
Cured	A pulmonary TB patient with bacteriologically confirmed TB at the beginning of treatment who was smear- or culture-negative in the last month of treatment and on at least one previous occasion.
Died	A TB patient who dies for any reason before starting or during the course of treatment.
Lost to follow-up	A TB patient who did not start treatment or whose treatment was interrupted for 2 consecutive months or more.
Not evaluated	A TB patient for whom no treatment outcome is assigned. This includes cases "transferred out" to another treatment unit as well as cases for whom the treatment outcome is unknown to the reporting unit.
Sputum Smear Conversion	Sputum smear positive converted to sputum smear negative at the end of intensive phase (first 2 months of treatment)
Successful Treatment Outcomes	The sum of *cured* and *treatment completed.*
Treatment completed	A TB patient who completed treatment without evidence of failure BUT with no record to show that sputum smear or culture results in the last month of treatment and on at least one previous occasion were negative, either because tests were not done or because results are unavailable.
Treatment failed	A TB patient whose sputum smear is positive at month 5 or 6 during treatment.
Unsuccessful (Poor) Treatment Outcomes	The sum of *treatment failed, died,* and *lost to follow-up.*

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Tuberculosis (TB) is likely to have existed throughout human existence, initially as isolated events but gradually increased its spread to populations over time (Bates & Stead, 1993; Gagneux, 2018). Currently, TB is a major public health problem globally. It is the leading cause of death from a single infectious agent (WHO, 2023). In 2019, it was responsible for the death of an estimated 1.2 million people without HIV and 208,000 people living with HIV worldwide (WHO, 2020a). Globally, the incidence of TB in 2019 was 130 per 100,000 population (WHO, 2020a). From 2005 to 2015, the average global annual decrease in the rate of TB mortalities was 4.1% among HIV-uninfected patients. The rate of decline in incident and prevalent cases were slower, being 1.6% and 0.7% respectively (Kyu et al., 2018).

The End TB strategy targets that by 2035 TB mortality would be reduced by 95% and incidence by 90% of 2015 rates (WHO, 2014a). The 2025 milestones include reducing TB mortality and incidence rates by 75% and 50% of 2015 rates, respectively. This would be unachievable, much less the targets for 2035, if the rates of reduction recorded between 2005 and 2015 were to remain unchanged. To make the achievement of the milestones a reality, the TB case-fatality rate must reduce from the 17% recorded in 2015 to at least 6.5% and the annual reduction in TB incidence rate decline ought to increase to at least 10% by 2025 (WHO, 2014a). While universal access to health care and socioeconomic development have been shown to be key context factors for accelerated progress, improved testing and treatment regimen are deemed to be at the core of achieving these targets (WHO, 2016c).

Globally, treatment success rates have increased over time. In 1997 when the WHO began its

global report on TB, the treatment success rate for new TB cases in the WHO regions was 77% (WHO, 1997). This increased to 86% in 2013 and decreased to 82% in 2016 (WHO, 2018). Major changes have been observed in the African region, with the treatment success rate increasing from 59% in 1997 to more than 75% in 2016. In Ghana, the rate of successful treatment outcomes for new and relapse cases increased from 43.6% in 1997 to 87.7% in 2010 (Amo-Adjei & Awusabo-Asare, 2013) and dropped to 85% in 2016 (WHO, 2017b).

Several interventions are responsible for the increased rate of treatment success. These include the introduction of the directly observed treatment-short course (DOTS), enabler's package, community treatment care, reduction in treatment duration and standardized treatment (Amo-Adjei & Awusabo-Asare, 2013). Standard TB treatment recommended by the WHO for drug-susceptible TB cases consists of a 2-month initiation phase, where fixed-dose combination (FDC) tablets made up of rifampicin (150mg), isoniazid (75mg), ethambutol (275mg) and pyrazinamide (400mg) are given based on body weight (2 tablets for those within 30 to 39kg; 3 tablets for 40 – 54kg; and 4 tablets for those who weigh 55kg or more). This is followed with a 4 month continuation phase consisting of FDC tablets made of rifampicin (150mg) and isoniazid (75mg) also given based on body weight (2 tablets for those within 30 to 40kg; 3 tablets for 40 – 54kg; 4 tablets for those who weigh 55kg or more) (WHO, 2017a).

In theory, it seems reasonable to blame non-adherence to treatment for poor treatment outcomes. While this is largely true, a study has shown that as high as 98% adherence to the standard treatment is associated with selection of multidrug resistant TB (MDR-TB), an unfavourable treatment outcome (Calver et al., 2010). Interestingly, a study examining the relationship between adherence and poor treatment outcomes found statistical significance only in patients who missed 60% or more of doses (Srivastava, Pasipanodya, Meek, Leff, & Gumbo,

2011). That high levels of adherence do not guarantee the non-emergence of MDR-TB and non-adherence, though above a certain threshold, is associated with favourable treatment response raises questions as to the underlying factors at play. The study by Srivastava et al. (2011) provides a useful answer from the further finding that about 1% of MDR-TB is due solely to the variation between patients' serum concentration of the drugs. If the serum concentrations of the 4 drugs that constitute the standard treatment were adequate from patient to patient such outcomes may not be observed even with reasonably minimal non-adherence.

The varying patient-to-patient plasma concentration of the regimen, also known as pharmacokinetic variability, has been associated with treatment outcomes with conflicting conclusions (Park et al., 2015; Burhan et al., 2013; Pasipanodya, Srivastava, & Gumbo, 2012; Srivastava et al., 2011; Chideya et al., 2009; Weiner et al., 2005). In most of these studies, low serum concentration defined relative to the established thresholds are prevalent but do not necessarily imply poor treatment outcomes. In Indonesia for example, 91% of a cohort of TB patients had low plasma levels of either rifampicin, isoniazid or pyrazinamide yet poor outcomes were recorded in only 18% of them (Burhan et al., 2013a). Another thing noticeable in most of these studies is that the association between treatment outcomes and the plasma concentration of separate drugs in the standard regimen are not evaluated. Very few studies evaluate the combined effect of low serum concentration in any one or more of the drugs on treatment outcomes.

In a systematic review published in 2018, the researchers sought to examine the association between the 4 anti-TB drugs and treatment outcomes. According to the authors, a reasonable conclusion could not be reached on account of the available evidence being limited mainly in methodological design (Sekaggya-Wiltshire, Lamorde, et al., 2018b). Other reviews have

concluded similarly (Mota et al., 2016; Wilby et al., 2014). In more recent systematic reviews which concluded differently, the authors found that low concentrations of pyrazinamide and rifampicin may lead to poor treatment outcomes and in addition suggested a dose-response relationship between rifampicin concentration and treatment outcomes (Perumal et al., 2020; Sileshi et al., 2021). However, both advocate for further investigation into these relationships due to methodological limitations.

Another systematic review describing the timings of TB mortality as a treatment outcome and the associated factors, similarly, determined that there was the need for more prospective clinical studies on the subject. However, they made some useful findings on the determinants of TB mortality including HIV co-infection, advancing immunosuppression, co-morbidity with non-communicable diseases, smear negativity, alcohol and substance abuse (Waitt & Squire, 2011).

Another important treatment outcome is lost to follow up. A study to assess the factors associated with lost to follow up found the following; inadequate knowledge of TB and its treatment, out-migration from the community in which the treatment centre is located, feeling better and untoward effects of drugs (Muture et al., 2011). Another study which found interruption of treatment as a factor for low cure rate also stated adverse drug effects as key reason for treatment interruption (Ai et al., 2010). What remains unclear is the specific side effects in question and the extent to which each one influences the rate of loss to follow up.

A study by Burton et al. (2011) found that the risk factors for unfavourable TB treatment outcomes in Ghana included unknown sputum smear status, HIV-coinfection, disseminated TB, not having a treatment supporter and low body weight. This study was limited in the factors

evaluated as the data collection technique used was medical records review. To end the TB epidemic, it is necessary to determine the factors that predispose patients to unfavourable treatment outcomes in well desgined studies so that specific interventions are designed to address them.

1.2 Problem Statement

Tuberculosis remains a major global health challenge. In spite of improvements in TB treatment success rate, the WHO target of 90% (WHO, 2022) has yet to be achieved. In Ghana, the target for treatment success rate is 91% (Bonsu et al., 2014) yet the rate achieved in 2017 was 85% (WHO, 2017b). Worldwide targets set for 2035 were unachievable at the pre-COVID-19 rates of decline unless they improved from 17% to 6.5% for case-fatality rate and from 2% to 10% for incidence rate per year (WHO, 2018). Reaching the targets is even more bleak now that the COVID-19 pandemic has reversed significant gains made earlier (Kirby, 2021). Although this fact challenges the way forward, the opportunity remains for much to be done considering that significant advances were made in the past over a relatively short time (WHO, 2023). These advances were underpinned by innovations in early diagnosis and appropriate treatment. Specifically, the following were noticeable particularly in sub-Saharan Africa; directly observed treatment-short course (DOTS), enabler's package, community treatment care, reduction in treatment duration and standardized treatment (Amo-Adjei & Awusabo-Asare, 2013).

Despite these, 15% of unsuccessful treatment outcomes are realized in countries like Ghana where the burden is relatively higher (WHO, 2022). It is known through current research aimed at optimizing standardized treatment that even high levels of fidelity to treatment may result in unsuccessful outcomes (Calver et al., 2010). Fortunately, large percentages of patients with

reduced plasma levels of the anti-TB medications get successful outcomes (Park et al., 2015; Burhan et al., 2013). This has led to studies investigating the association| between treatment outcomes and the adequacy or otherwise of the plasma concentration of the anti-TB medication at the current standard doses. However, these studies are few or not designed for this purpose in sub-Saharan African countries where the burden is bigger. In Ghana for example, the studies available in the published literature were limited in not having been designed to assess the relationship between pharmacokinetic variability and treatment outcomes (Antwi et al., 2016; Kwara et al., 2016).

The currently available studies' findings assessing this relationship have led largely to conflicting conclusions. Earlier reviews on the subject could not arrive at acceptable conclusions due to methodological limitations in the studies available at the time (Mota et al., 2016; Sekaggya-Wiltshire, Lamorde, et al., 2018a; Wilby et al., 2014). Recent reviews, however, have suggested that low concentrations of rifampicin, isoniazid and pyrazinamide are associated with poor outcomes (Perumal et al., 2020; T. Sileshi et al., 2021).

Although the recent reviews are more confident in their conclusions the authors advocate further studies in view of specific methodological constraints. Firstly, most studies do not evaluate ethambutol plasma concentration and its link with treatment outcomes. For pyrazinamide and isoniazid, the evidence was synthesized from fewer studies than was the case with rifampicin. It must be noted that even the number of studies on rifampicin were admittedly limited. Secondly, some of the studies were retrospective analysis of data collected for clinical purposes. Thirdly, some of the observational studies did not control for potential confounding in assessing the relationships.

Although it is known that certain time-varying factors such as body weight, adverse drug events, and treatment adherence influence TB treatment outcomes, their time-varying effects are rarely assessed in the literature. A study of the effects of changing body weight showed that the weight at baseline affects treatment outcomes differently than weights at different time points during treatment (Bernabe-Ortiz et al., 2011). This demonstrates the usefulness of assessing the dynamic associations between risk factors and treatment outcomes. As it pertains now, the risk factors associated with TB treatment trajectories remain inadequately characterized in Ghana.

These underscore the need for more well-designed studies to add to the current knowledge of the dynamics between the pharmacokinetics of the anti-TB medication and treatment outcomes. Understanding the association between pharmacokinetic variability and treatment outcomes could shed light on the causes of unfavorable treatment results. This knowledge will contribute to the evidence base needed to identify specific drugs in the regimen that may require dose adjustments. Ensuring doses that achieve optimal serum exposure is crucial to improving patient cure rates, minimizing the risk of relapse and the development of resistant strains, while also considering the potential for adverse drug reactions.

This study seeks to contribute to filling the gap in the understanding of the association between pharmacologic factors and unsuccessful treatment outcomes in rifampicin-susceptible TB patients.

1.3 Justification

Optimization of the anti-TB regimen could help bridge the sub-optimal treatment success rate gap. As has been pointed out using modeling data, optimization of the current first line anti-TB regimen holds the greatest potential for reducing TB incidence and mortality (Kendall et

al., 2017).

Additionally, determining the association between pharmacokinetic variability and treatment outcomes may help to explain the unfavourable treatment outcomes. This will add to the evidence available to help identify the specific drugs in the regimen which may require dose adjustment. It is important that doses that ensure optimal serum exposure are given to increase the chances of every patient being cured, to reduce their risk of relapse and the emergence of resistant strains while not ignoring the incidence of adverse drug reactions.

Identifying other factors related to poor treatment outcomes will provide a guide to help reduce the risk of such outcomes thereby reducing the disease burden. More importantly, identifying patients at risk of such outcomes could help target them appropriately with the adjunctive care that may be required to improve their outcomes. Furthermore, characterizing the longitudinal risk factor trajectories will provide a better understanding of workable interventions and the optimal time points for their implementation to optimize successful treatment outcomes. Altogether, the findings from this study will have value to aid the achievement of a key component of the third target of the Sustainable Development Goal (SDG 3), which is to end epidemics, including TB, by 2030.

1.4 Study Hypothesis and Research Question

The study will test the hypotheses that:

1. There is no difference in treatment outcomes between rifampicin-susceptible TB patients with low serum concentration and those with normal serum concentration of rifampicin, isoniazid, pyrazinamide, and ethambutol.
2. There is no association between TB treatment outcomes and clinical characteristics of

patients with rifampicin-susceptible TB.

Based on these, the main question this study will seek to answer is: "what are the pharmacologic and clinical risk factors of poor TB treatment response in patients with rifampicin-susceptible TB"?

1.5 Objectives

1.5.1 General objective

To determine the pharmacologic and clinical risk factors of poor TB treatment response in patients with rifampicin-susceptible TB

1.5.2 Specific Objectives

1. To characterize pharmacokinetic variability among rifampicin-susceptible TB patients in Ghana.
2. To determine the predictors of low plasma concentration of the first-line anti-TB drugs.
3. To determine the association between low plasma concentration of the first-line anti-TB drugs and treatment outcomes.
4. To determine the baseline and longitudinal risk factors associated with bacteriologic failure over the course of treatment for TB.
5. To identify distinct treatment non-adherence trajectories and the associated factors.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Brief History of Tuberculosis

TB is an infectious disease caused by *Mycobacterium tuberculosis.* It has been hypothesized that the earliest antecedent species of this organism originated about 150 million years ago (Hayman, 1984). Archaeological evidence shows that dating back to ancient Egypt 5,000 years ago, tuberculosis infection among humans had started (Nerlich, Haas, Zink, Szeimies, & Hagedorn, 1997; Cave & Demonstrator, 1939). In the Americas, similar evidence has been found while documented evidence of tuberculosis from early human history has also been found in Asia (Brothwell & Sandison, 1967; Daniel, 2000). The present scientific understanding of TB began to emerge in the 19th century after several centuries of superstitious practices aimed at treating the disease had been unsuccessful at abating morbidity and mortality. Toward the end of the 19th century, however, the increasing trend of TB morbidity and mortality in the Western hemisphere reversed. No conclusive explanations have been found for this reversal (Daniel, 2006). However, a number of theories have been proposed to explain this decline.

McKeown & Record (1962) proposed that the decline may have been as a result of improving socio-economic status of the population with its corresponding influence mainly on improved nutrition. This is reasonable when the observation that from the early 1900s until sometime in the 1990s, the only periods that the TB incidence increased in England and Wales were during the first and second world wars when people's livelihoods were destroyed and proper nutrition could not be guaranteed, is considered (Glaziou et al., 2018). In the 1990s, however, the increase in the disease burden was attributable to the folding up of TB programmes on the pretext that TB was no longer a problem in Europe. Another proposed reason for the decline in

the incidence of tuberculosis in the developed countries was the reduction in the crowding of people (Scofield et al., 1991). Without doubt, this was related to the generally improving socio-economic circumstances, which had started at the time. Another study has shown that one key underlying factor for the reduced TB burden was the reduction in the number of contacts to each case that the disease could be transmitted to over time as a result of behavioural change, reduction in overcrowding and the increased practice of case isolation (Vynnycky & Fine, 1999).

It is worthy of note that the pivotal turnaround in TB care was made in 1882 when Robert Koch, a German physician demonstrated that TB was caused by *Mycobacterium tuberculosis*. Soon thereafter followed the discovery of the X-rays and the tuberculin skin test for the diagnosis of latent TB infection, then later the development of the Bacille Calmette-Guérin (BCG) vaccine for TB prevention in children. Chemotherapy was introduced approximately half a century later (Murray, 2001; Murray, 2004).

Chemotherapy started in 1946 with streptomycin, which demonstrated a significant reduction in deaths and remarkable improvements in clinical manifestations. These improvements were later proven to not last beyond 5 years owing to emergence of resistance. Para-aminosalicylic acid was combined with streptomycin to address the resistance. When isoniazid was discovered, it was added to the regimen after studies of the resistance pattern suggested a 3 drug regimen would address both treating the disease and slowing the emergence of resistance (Mitchison & Davies, 2012). Later, pyrazinamide was proven to destroy mycobacteria that remained after treatment with streptomycin and isoniazid (Mccune et al., 1956) and rifampicin was found to be capable of speeding up the clearance of *M. tuberculosis* (Grumbach, 1970).

With the emergence of resistant strains new challenges included treatment with high doses of up to 7 drugs for up to 20 months. Fortunately, advancements in treatment have led to guidelines including a recommendation for the treatment of multidrug-resistant tuberculosis (MDR-TB) with a novel all-oral 6-month regimen consisting of bedaquiline, pretomanid, linezolid, and moxifloxacin (BPaLM).

The BPaLM regimen has shown promising results and offers several advantages over previous treatment options. It has been found to improve treatment outcomes and to significantly shorten the duration of treatment. This shorter treatment duration has a positive impact on the quality of life for individuals with MDR/RR-TB, as they no longer need to undergo prolonged and complex treatment regimens.

2.2 Tuberculosis

2.2.1 Epidemiology

It is estimated that 2 billion people on the globe are infected with the *M. tuberculosis*, but just about 5 to 10% will progress to active TB disease (WHO, 2020). In 2015, the estimated number of incident cases was 10.4 million (95% CI: 8.8 – 12.2 million); 90% were aged 15 years or more and 10% were people living with HIV (WHO, 2016b). Estimates show that the total number of incident cases in 2017 was 10 million (95% CI: 9.0 – 11.1 million). In absolute numbers, the incidence was highest in men, estimated at 5.8 million and lowest in children, estimated at 1 million. In total, 9 out of 10 cases in 2017 were in people aged 15 years or older. The proportion of incident TB cases in people living with HIV was 9% with about 7 out of 10 of these cases being in Africa. In 2015, the incidence of TB was estimated at 254 per 100,000 population in the African region (WHO, 2016b). It reduced marginally to 237 per 100,000 population in 2017 (WHO, 2018). COVID-19 reversed the gains in TB incidence and mortality

which had been made before the pandemic (WHO, 2023; Kirby, 2021).). A study in Ethiopia found the incidence rate of TB among HIV patients to be highest (95.9 per 100 person-years) in the first year of enrolment into care (Addis Alene, Nega, & Wasie Taye, 2013).

The most recent prevalence study done in Ghana in 2013 estimated the TB burden to be 286 (95% CI: 229 – 343) per 100,000 population, approximately 3 times the WHO estimate for the year (Bonsu et al., 2014). The burden was higher among males (334 per 100,000 males) than females (251 per 100,000 females). Regarding the TB burden distribution by age, it was clear the burden increased with increasing age; increasing from 137 per 100,000 persons aged 15 to 24 years to 657 per 100,000 persons aged 65 years or more.

Globally, the decline in TB incidence is about 2% yearly. Regionally, the fastest rates of decline, 5% and 4%, were in the WHO European and African regions, respectively. In the African region, this was facilitated mainly by significant reductions in Southern Africa when the HIV epidemic reached its peak and TB/HIV prevention and care services were scaled up (WHO, 2018).

The highest burden of the disease summing up to 87% was in 30 countries known as the 30 high TB burden countries. These include Nigeria, Sierra Leone and Liberia in the West African sub-region (WHO, 2018). The region with the least number of cases in 2015 were the WHO Europe (3%) and America (3%) regions. This remained the same through 2017.

Globally, TB was responsible for an estimated 1.6 million deaths in 2017; 1.3 million (95% CI: 1.2 – 1.4 million) in HIV-negative patients and 300,000 (95% CI: 266,000 – 335,000) in HIV-positive ones (WHO, 2018). This makes it the leading cause of death from a single

infectious agent and also one of the top ten causes of death. TB mortality was estimated at 24 per 100,000 population among HIV-positive patients and 40 per 100,000 population among HIV-negative population in Africa in 2017. In absolute terms, mortality among HIV-negative TB patients and that among HIV-positive TB patients has reduced by 29% and 44% respectively, between 2000 and 2017. In Ghana, the TB mortality was 36 per 100,000 population among HIV-uninfected patients and 18 per 100,000 among the HIV- coinfected patients (WHO, 2017b).

A study in Ethiopia found the CFR among TB/HIV patients to be 20.2% (Gesesew et al., 2016). A CFR of 15% was reported by a 9-year longitudinal study in South Africa among MDR-TB cases; 9% among HIV-positive patients and 20% in HIV-negative ones (van der Walt et al., 2016). A 5-year retrospective cohort study in Nigeria estimated the CFR to be 16.6% (Adamu et al., 2017). In Ghana, the case fatality ratio was estimated at 0.39 (95% CI: 0.14 – 0.68) in 2017 (WHO, 2017b).

The new cases of rifampicin-resistant TB (RR-TB) recorded globally in 2017 was 558,000. The highest burden was in India (24%), China (13%) and Russia (10%). It is estimated that about 457,560 people globally had MDR-TB. The estimated number of new cases with MDR-TB or RR-TB was 3.5% while 18% of retreatment cases had MDR-TB or RR-TB (WHO, 2018). In West Africa, researchers found a surprisingly high proportion of MDR-TB cases; 6% in new cases and 35% among previously treated patients (Gehre et al., 2016). They further estimated the burden of MDR among TB patients in Ghana to be 13% among new cases and 28% among retreatment cases. The highest burden of pre-extensively drug resistant (pre-XDR) TB in the West African sub-region was reported among the MDR-TB population in Ghana.

2.2.2 Risk Factors for TB

Significant risk factors for TB have been repeatedly identified as poverty, congested living circumstances, poor access to healthcare, and malnutrition. These elements make people more susceptible to TB infection and prevent prompt detection and treatment (Tulu et al., 2014; Shimeles et al., 2019; WHO, 2022). Immunocompromised states have been well established to be related to TB. The risk of contracting TB is significantly higher in people with damaged immune systems, such as those with HIV/AIDS, diabetes, malignancies and other illnesses that weaken immunity and make people more vulnerable to the infection (Bruchfeld et al., 2015; Cadena et al., 2019). Another factor worth mentioning is close contact with people who have active TB disease. There is a higher chance of transmission when people with active disease are nearby. This is especially relevant in homes, hospitals, prisons, and other healthcare facilities (Aldridge et al., 2016).

People with lower socioeconomic status are more likely to contract tuberculosis. Malnutrition, cramped living quarters, and restricted access to healthcare are all associated with poverty, and these factors raise the risk of tuberculosis. Higher rates of tuberculosis incidence and mortality are also closely linked to low GDP per capita and income inequality (Chung et al., 2021). A higher risk of tuberculosis is correlated with lower educational achievement. Lack of education may make it more difficult to obtain health information, which would lower knowledge of TB treatment and prevention (Osman et al., 2023).

Due to comorbidities and decreased immunity, the aged population is more vulnerable to TB reactivation. Another risk factor for tuberculosis has been found to be male gender, possibly as a result of increased exposure to risky behaviours including smoking and work-related hazards. Compared to rural populations, TB rates are typically greater among urban dwellers in densely

populated, low-income areas. However, access to healthcare is difficult in rural locations, which can result in poor outcomes and delayed diagnoses (Chung et al., 2021; Osman et al., 2023)

People who are homeless or live in overcrowded housing are particularly vulnerable because they are exposed to TB in close-contact settings and face obstacles in getting regular medical care. Multidrug-resistant TB (MDR-TB) and recurrent TB have been associated with homelessness (Osman et al., 2023).

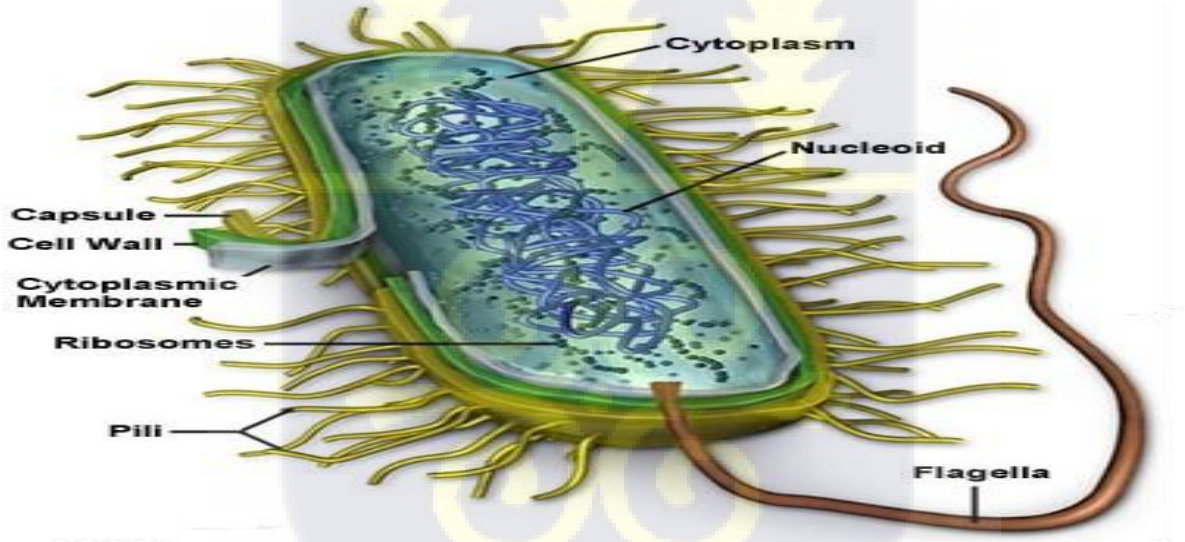
Smoking tobacco has been associated with poor treatment outcomes and higher risk of acquiring TB (Wikurendra et al., 2023). Although alcohol use and the abuse of substances have been linked to TB infection, there are contradictory findings from facility-based studies (Shimeles et al., 2019). The relationship between age and TB infection have followed a pattern that is best explained by the relationship between age and immunity. In childhood (< 5 years) and in old age (> 40 years), the risk of TB infection is higher than in the ages in between (Wikurendra et al., 2023). This also explains contradictory findings of age's association with TB in studies where age is not delineated with such considerations (Jeon & Murray, 2008).

Migration is a risk factor for TB infection for a few reasons. Firstly, individuals who move from LMICs to developed countries often were exposed to the TB bacteria because of the high TB burden in their native countries and are likely to have latent TB at the time of migration. Secondly, during travel, individuals may come into contact with infected people making transmission more likely due to the crowded travel conditions. Once in the country they migrated to, the risk of reactivation of the latent TB has been shown to increase (Dobler et al., 2018; Lillebaek et al., 2002). This is thought to be the consequence of having a poor immune

response and/or the loss of immunity over time due to the sudden low exposure to *M. tuberculosis*. Additionally, they are often confronted with poor access to healthcare, inadequate housing, unemployment and related factors which tend to increase the risk (Lalor et al., 2014; Pareek et al., 2016).

It is also plausible that being in a country with a more robust health system and better diagnostic facilities would lead to a higher rate of diagnosis of TB disease brought in from their countries of origin. On the reverse side, migrants from developed countries to LMIC are prone to TB due to the exposure to a higher burden of the disease. Having a poor immune response as a result increases the risk.

2.2.3 Pathogenesis



Source: Molecular Expressions, 2015
Figure 1: Structure of *M. Tuberculosis*

Mycobacterium tuberculosis, the causative organism of TB, is an aerobic bacterium and is an acid-fast bacillus. It is rod-shaped and non-spore forming (Knechel, 2009). It has a peculiar cell wall structure rich in carbohydrate and lipids which enables it to survive in its host and resist antibiotic action against it (Lee et al., 2004). A schematic representation of

Mycobacterium tuberculosis structure is presented in Figure 1.

M. tuberculosis is spread when droplet nuclei are expectorated by the sneezing, coughing, talking or singing of a person infected with active pulmonary or laryngeal TB disease. When the droplets get airborne they could remain in mid-air for hours, making exposing others to the organism likely if proper precautions are not taken (Lee et al., 2004). The bacteria introduced into the lungs infects the respiratory system and could disseminate to infect other organs of the body, termed extrapulmonary tuberculosis.

In the airways the bacteria are trapped in the upper parts where the goblet cells are located. This induces the secretion of mucus which traps other substances foreign to the body. Once produced, the mucus and the substances caught up in it are repeatedly moved upwards for removal from the body by the cilia on the surface of the goblet cells (Frieden et al., 2003). This primary defense mechanism notwithstanding, some bacteria get through to the alveoli. Such bacteria are immediately surrounded and engulfed by macrophages, providing a second line of defense to get rid of mycobacteria (van Crevel et al., 2002).

Phagocytosis by macrophages is enabled by key mycobacterial cell substance, lipoarabinomannan serving as a major ligand for the phagocytic cell receptor. Thus, mycobacteria may get phagocytized without prior exposure to the macrophages (van Crevel et al., 2002). Subsequent events, influenced mainly by host defense factors and their interaction with invading mycobacteria lead either to successful control of the infection resulting in latent TB or active TB disease also known as primary progressive TB (Centers for Disease Control and Prevention, 2005; Guyot-Revol, Innes, Hackforth, Hinks, & Lalvani, 2006).

Inside the macrophages the mycobacteria continue to divide (Russell et al., 2009). Whether the infection is controlled or not proteolytic enzymes and cytokines are released to destroy the bacteria (Nicod, 2007). T-lymphocytes are attracted to the macrophages as a result of the produced cytokines. Mycobacterial multiplication continues until enough microbes are available to elicit a cell-mediated response which can be detectable by a tuberculin skin test. Cell-mediated immunity, fully elicited results in the formation of lesions from a build-up of T cells and macrophages to reduce the multiplication and spread of the bacteria. The microenvironment thus created to control the mycobacteria is destructive to the macrophages leading to solid necrosis at the middle of the lesions. The mycobacteria on the other hand are able to adapt in that newly created microenvironment with low pH, nutrients and oxygen levels (Dheda et al., 2005). This is how latency is established in immunocompetent individuals.

In persons with poor immune response, cell-mediated immunity is not fully elicited. Granulomas begin to form but are unable to hold the mycobacteria. The fibrous wall loses structural integrity owing to the necrotic tissue undergoing liquefaction. The original site is left with an air-filled cavity after the necrotic material drains into a bronchus or blood vessel. From the bronchus, infected persons can expectorate mycobacteria containing droplets through coughing, sneezing, singing or talking and thereby infect others. Dissemination through the blood stream could lead to extrapulmonary TB. Alternatively, the mycobacteria may spread through the lymphatic system and form cheese-like lesions in the tracheobronchial lymph nodes (Dheda et al., 2005).

The following features of the pathogenesis are relevant to treating the infection. The mycobacteria's slow replication rate allows them to evade the immune system and establish

persistent infection. This makes the treatment duration considerably long (usually 6 to 9 months for drug-susceptible TB), as the drugs need to target both actively replicating bacteria and those in a non-replicating state. The prolonged treatment duration increases the risk of non-adherence and may lead to treatment failure or the development of drug-resistant TB strains. Furthermore, slow replication may lead to delayed or missed diagnosis especially when culture is required. This can result in delayed initiation of treatment, allowing the infection to progress and increase the risk of transmission. Timely and accurate diagnosis is crucial for successful treatment outcomes.

Another point worth noting is, when granulomas are formed to help contain the spread of the infection the bacteria within them are enabled to enter a dormant or non-replicating state, becoming less susceptible to the effects of antibiotics. This dormant state allows the bacteria to survive for prolonged periods and potentially reactivate later. It also contributes to the challenges of eradicating the infection completely, as some bacteria may remain hidden and protected within granulomas even during treatment.

Facilitated by host factors such as malnutrition and immunosuppression, the immune system's ability to control the infection and respond to treatment can be weakened. Weakened immune responses may lead to slower or inadequate clearance of the bacteria, delayed resolution of symptoms, and increased risk of treatment failure or relapse.

2.3 Clinical Characteristics and TB Diagnosis

2.3.1 Latent TB

In immunocompetent people infected with the *M. tuberculosis*, the mycobacteria remain enclosed in the necrotic lung tissues. This causes persistent stimulation by the antigens that

elicits an immune response which though unable to completely get rid of the bacteria suppresses them (Guyot-Revol et al., 2006). In latent TB no symptoms of disease are manifested, and patients are non-infectious. A reactive tuberculin skin test or TB blood test is the only sign of latent TB (Centers for Disease Control and Prevention, 2011b).

2.3.2 Primary Disease

Primary pulmonary disease occurs in people not previously exposed to *M. tuberculosis.* It was considered a disease of children owing to this but a study by McAdams, Erasmus, & Winter (1995) showed that its incidence in adults in low TB burden countries is consistently increasing. The proportion of primary TB cases who are adults was estimated at 23 – 34%. While largely asymptomatic, four main features are identifiable upon radiological assessment; parenchymal disease, lymphadenopathy, miliary disease and pleural effusion (Burrill et al., 2007). Primary TB is self-limiting.

2.3.3 Primary Progressive Disease

Primary progressive TB is also termed post-primary disease by some authors ((Burrill et al., 2007). It occurs in patients with prior *M. tuberculosis* infection. Active TB disease refers to reinfection or reactivation of the bacteria and it occurs in about 5 to 10% of previously exposed people (WHO, 2016b). Due to the cavitation that characterizes it, it is associated with the spreading of the disease through the blood and throughout the lungs (Burrill et al., 2007). Early on-set signs and symptoms include weight loss, fatigue, fever, chills and night sweats (Centers for Disease Control and Prevention, 2011b). As the disease progresses, other characteristics feature more predominantly such as wasting due to long-standing loss of appetite and poorer metabolism resulting from the immune response, cough which produces purulent sputum which could progress to being blood-stained, difficulty in breathing and chest pains (Centers

for Disease Control and Prevention, 2011; Paton, Chua, Earnest, & Chee, 2004).

2.3.4 Extrapulmonary TB

Extrapulmonary TB is TB disease found at any part of the body outside the lungs. Its incidence is 20% among immunosuppressed persons (Knechel, 2009). The frequently affected locations are the central nervous system, musculoskeletal system, head and neck, the spine, bones, joints, the blood stream (Burrill et al., 2007). In the case of infection involving the blood stream, also known as disseminated TB, there is the possibility of the infection affecting several organs at the same time. The characteristics are dependent of which location the mycobacteria infect but generally include fever, fatigue, malaise and weight loss (Knechel, 2009).

2.3.5 Diagnosing Tuberculosis

The gold standard of TB diagnosis is culture (P. R. Murray et al., 2007). The mycobacteria when cultured can be identified. It takes 3 to 6 weeks for growth to be detected on solid media. Predominantly, sputum samples are used for the culture. Not only is culture good for diagnosing TB, it is also the least unbiased means of assessing treatment progress after drug administration is started. When a negative culture result follows a succession of positive culture results for a patient on treatment, there has been culture conversion and it is the ultimate output for determining treatment cure.

Sputum microscopy is another means of TB diagnosis (Knechel, 2009). Though not recommended as initial diagnostic test for TB, it is still used because of resource constraints. For monitoring progress of treatment on the anti-TB medication, culture, while being the gold standard, is not recommended due to the long duration required for growth of the culture (1 to 8 weeks). GeneXpert MTB/RIF on the other hand functions by nucleic acid amplification

(WHO, 2016a). Thus, its tendency for detecting nucleic acid of dead *M. tuberculosis* makes it prone to high false positive results if used for treatment monitoring as the nucleic acid of dead bacteria could be picked up. Done at first, second, third, fifth and sixth months of treatment, sputum smear microscopy is recommended by the WHO for monitoring treatment progress (WHO, 2010b). Two (2) months after treatment initiation, sputum samples are to be collected for sputum smear microscopy. If a positive outcome is recorded, the microscopy is required to be repeated at the end of the fifth month. If positivity persists, culture testing and drug sensitivity testing is required for isoniazid.

The GeneXpert MTB/RIF (for *Mycobacterium tuberculosis* (MTB) and rifampicin (RIF) resistance) is the only WHO-Approved Rapid Diagnostic (WRD) test that simultaneously detects *Mycobacterium tuberculosis* (MTB) and rifampicin (RIF) resistance (WHO, 2016a). It detects MTB and rifampicin in 2 hours, has superior sensitivity for TB detection than sputum smear microscopy and is comparable to solid culture with regards to sensitivity and specificity. The WHO recommends the GeneXpert MTB/RIF as the initial diagnostic test for detecting MTB and rifampicin resistance for all cases of TB including TB meningitis and testing tissues for suspected extrapulmonary TB ahead of microscopy, culture and drug sensitivity testing.

TB disease may also be diagnosed on the premise of abnormal chest radiograph findings. Patients being assessed based on respiratory symptoms who test negative for the GeneXpert MTB/RIF or sputum smear with the distinguishing radiological findings of infiltrates with cavitation in the lung lobes may be considered as having X-ray suggestive TB and put on the full TB treatment course (Knechel, 2009).

2.3.6 GeneXpert MTB/RIF

GeneXpert MTB/RIF is the recommended first-line test for diagnosing TB (WHO, 2014b). Sputum smear and culture remain critical for monitoring treatment progress and outcomes. GeneXpert MTB/RIF has been shown to have a sensitivity of 88% (95% CI: 84-92) and a specificity of 99% (95% CI: 98-99) when used to replace smear microscopy for initial diagnosis of TB. When used as an additional test to diagnose smear negative patients it had a sensitivity of 68% (95% CI: 61-74) and a specificity of 99% (95% CI: 98-99).

2.3.7 Sputum Smear Microscopy

Microscopy is the least sensitive and specific of all the recommended tests used in the diagnoses of TB (WHO, 2014b). It detects AFB and not *M. tuberculosis* in particular. This makes it less specific particularly in areas with low TB burden or high non-tuberculous mycobacteria (NTMs). It is, however, used for monitoring patient response because it is relatively more affordable and does not require as much biosafety measures. Also, it is unable to differentially identify drug sensitive bacilli from non-sensitive ones and viable from non-viable ones.

There are two microscopy procedures for identifying AFB; the Ziehl-Neelson and light-emitting diode fluorescence microscopy. Fluorescence microscopy is recommended because it allows for more fields to be examined without much effort and in a shorter time span (WHO, 2014b). Furthermore, it is more likely to detect AFB in sputum samples with fewer bacilli. However, it requires stable electrical power, superior know-how in microscope adjustment and reading. Also, its maintenance cost is higher making it challenging to sustain its use in resource-poor settings. As such, the Ziehl-Neelson method of AFB detection is still used in a Ghana.

2.3.8 Culture

M. tuberculosis is grown either in liquid or solid media. While the WHO recommends both, liquid culture is considered the gold standard for TB detection as it provides results more rapidly (WHO, 2014b). Culture results, irrespective of medium used, takes between 1 to 8 weeks due to the slow growth of the bacilli (WHO, 2014b). Also, handling the culture requires the highest biosafety conditions. Culture is therefore suited for national or regional levels of the health system. Programmatically, it is used mainly for monitoring treatment response in patients with MDR-TB or XDR-TB.

2.4 TB Treatment

2.4.1 Treatment progress and challenges so far

The objectives of TB treatment are to cure the patient and restore quality of life and productivity. This is achieved through the rapid reduction of the bacterial population thereby reducing duration of therapy, and the prevention of the emergence and spread of resistant strains.

One major challenge is the long duration of treatment, 6 months for drug-susceptible TB patients, up to 9 or 12 months for TB of the meninges, bones or joints and up to 20 months for drug-resistant TB with even shorter novel regimen lasting 6 to 9 months (WHO, 2016; WHO, 2017). This creates a burden for patients, their families and for the health system. Further, there is a higher risk of the emergence of resistance with poor adherence due to the long duration of treatment. Another treatment challenge is the increasing rate of resistance to the medicines, mainly rifampicin, isoniazid (Mitchison & Davies, 2012).

2.4.2 First-line anti-TB regimen

The WHO recommends treating drug-susceptible TB with rifampicin, isoniazid, pyrazinamide and ethambutol for the first 2 months, known as the initiation or initial phase, followed with rifampicin and isoniazid for the next 4 months, the continuation phase.

To make less cumbersome the process of procurement, distribution and administration of the medication by patients and reduce the likelihood of exclusion of some drugs from administration by patients, the WHO recommends fixed dose combinations of the drugs and their administration based on 3 or 4 weight bands. The daily recommended doses and their respective ranges for patients aged 15 years or above and with minimum weight of 30kg are: isoniazid, 5mg/kg body weight (range: 4 – 6) up to a maximum of 300mg; rifampicin, 10mg/kg body weight (range: 8 – 12) up to a maximum of 600mg; pyrazinamide, 25 mg/kg body weight (range: 20 – 30); and ethambutol, 15mg/kg body weight (range: 15 – 20) (WHO, 2010a).

In Ghana, patients doses are determined thus: in the initiation phase, FDC tablet consisting of rifampicin (150mg), isoniazid (75mg), ethambutol (275mg) and pyrazinamide (400mg) is given based on body weight (2 tablets for those who weigh 30 to 39kg; 3 tablets for 40 – 54kg; and 4 tablets for ≥55); in the continuation phase, FDC tablet consisting of rifampicin (150mg) and isoniazid (75mg) also given based on body weight (2 tablets for those within 30 to 40kg; 3 tablets for 40 – 54kg; and 4 tablets for ≥55) (Ministry of Health, Ghana, 2017).

Regarding the administration of the regimen, it is recommended that all doses are taken by DOTS. Dose administration by DOTS may be done by a health worker, trained lay worker or a family member (WHO, 2017a). Doses are recommended for daily administration from initiation to completion. However, for newly diagnosed patients, 3 times weekly ingestion is

allowed from the continuation phase or the entire duration of treatment provided patient is not HIV co-infected. In Ghana, however, only the daily administration of the medication is recommended for all TB patients (Ministry of Health, Ghana, 2017).

For all HIV-coinfected patients, the WHO recommends prompt start of cotrimoxazole prophylaxis and its continuation throughout the treatment period (WHO, 2010b). Also, ARVs are recommended to be given as soon as possible within 8 weeks after starting TB treatment.

2.4.3 Rifampicin

Rifampicin is derived semi-synthetically from rifamycin (WHO, 2010b). It has been shown to act in a concentration-dependent fashion (van Ingen et al., 2011). It exerts its action by inhibiting the synthesis of ribonucleic acid in the mycobacteria (Sturkenboom, 2016). Rifampicin is the key sterilizing agent in the regimen; much of the bactericidal activity against persisting mycobacteria in the intensive phase of treatment is done by rifampicin. When administered orally, it reaches maximum plasma concentration in 2 to 4 hours and has a half-life of 2 to 3 hours (WHO, 2010b). Rifampicin is a liver enzymes inducer and as such leads to lower than supposed concentrations of several medicines when taken concomitantly with them. Though well tolerated, the side effects include gastrointestinal disturbances, fever, flu-like syndrome, low platelet count, exfoliative dermatitis, oliguria, dyspnoea, haemolytic anaemia, and hepatitis which is dose-dependent (WHO, 2010b).

2.4.4 Isoniazid

Isoniazid is a prodrug. It is activated by the bacterial enzyme, KatG. It has a disproportionately higher bactericidal action against multiplying bacilli relative to dormant ones and this is observed principally in the first 2 days of treatment (Sturkenboom, 2016). It compromises the

quality of the bacterial cell wall by inhibiting the synthesis of mycolic acid. Its half-life varies from 1 to 3 hours as determined by the individual's N-acetyltransferase (NAT2) enzyme genotype (WHO, 2010b). Isoniazid is an inhibitor of liver enzymes and as such increases the plasma concentration of most drugs taken concomitantly with it. It is well tolerated but the following adverse effects have been reported: hypersensitivity reactions, lethargy, peripheral neuropathy, optic neuritis, toxic psychosis, convulsions, hepatitis, lupus-like syndrome, anaemia, pellagra and arthralgia.

2.4.5 Ethambutol

Ethambutol inhibits the synthesis of a key component of the mycobacterial cell wall, arabinogalactan (Sturkenboom et al., 2015). It is the least potent of all 4 drugs that make up the first-line regimen. It functions to protect against the emergence of rifampicin resistance. Readily absorbed from the gut, it reaches peak serum concentration in 2 to 4 hours. Its half-life is 3 to 4 hours (WHO, 2010b). Notable untoward effects are ocular neuritis which is dose-dependent and reversible if stopped before blindness develops, peripheral neuritis, arthralgia, and hepatitis.

2.4.6 Pyrazinamide

Pyrazinamide is a prodrug activated by the mycobacterial enzyme, pyrazinamidase (Sturkenboom, 2016). It has a relatively weak bactericidal action but is effective against persisting bacilli in the low pH macrophage environment and areas acutely inflamed. This explains its sterilizing effect in the first 2 months of treatment when acute inflammations remain (WHO, 2010b). The proposed modes of action are that pyrazinamide acts by depleting adenosine triphosphate in the bacterial cell, or by inhibition of either fatty acid synthase I or protein translation (Egelund et al., 2015). Peak plasma concentration is achieved in 2 hours and

the half-life is approximately 10 hours. Its role has been critical in shortening treatment duration. Adverse effects include gastrointestinal disturbances, hypersensitivity reactions, hyperuriceamia and occasionally gout, and arthralgia.

2.5 Pharmacokinetics (PK) and Pharmacodynamics (PD) of first-line anti-TB drugs

The efficacy of first-line anti-tuberculosis (TB) drugs is influenced by their plasma concentrations, which can be influenced by various factors. Understanding the factors that predict plasma concentrations of these drugs is crucial for ensuring therapeutic efficacy and preventing treatment failure.

2.5.1 Therapeutic Drug Monitoring (TDM)

Therapeutic drug monitoring (TDM) is a clinical technique which involves measuring the serum concentration of drugs and interpreting the pharmacokinetics and pharmacodynamics to inform the adjustment of doses of the drug for optimal treatment outcomes (Walker & Whittlesea, 2012). In TB treatment, it has been recommended for patients with poor treatment response. The toxicity profile of the first-line drugs vis-à-vis their wide pharmacokinetic variability, risk of treatment failure, relapse and emergence of resistance are reasons for which TDM may be valuable in the management of TB (Magis-Escurra et al., 2012). Indeed, TDM has been shown to be of value for the treatment of TB (Magis-Escurra et al., 2012; Heysell, Moore, Keller, & Houpt, 2010), but is not routinely recommended considering the resource-constraints of the settings where the burden is highest.

The thresholds which serve as reference values for the determination of adequate exposure of the drugs have been defined and are widely accepted (Peloquin, 2002). In a recent review, Alsultan & Peloquin (2014b) defined the normal peak plasma concentration of the first-line

anti-TB drugs as follows: 600mg of rifampicin, 3 to 6 µg/ml; 300mg of isoniazid, 8 to 24 µg/ml; 25 to 35 mg/kg body weight of pyrazinamide, 20 to 60 µg/ml; and 25mg/kg body weight of ethambutol, 2 to 6 µg/ml. A number of studies have used the lower limit of the ranges as the threshold for defining low and normal serum concentrations (Prahl, Johansen, Cohen, Frimodt-Moller, & Andersen, 2014; Burhan et al., 2013; Tostmann et al., 2013). Serum concentrations below the threshold are categorized as low and those equal to or above as high.

Others have categorized low or high concentrations using 90% of the thresholds as the cut-off instead (Heysell et al., 2011; Holland et al., 2009). This is reasonable considering that the actual C_{max} values were not obtained, instead the modal time to reach C_{max} (2 hours) was used as a guide to determine the time point for blood sample collection. Since concentrations at 2 hours post-dose were used as a proxy for peak concentrations it has been argued that the 90% of the threshold cut-off may be better representative.

2.5.2 Assessment of Acetylator Status

The wide person-to-person pharmacokinetic variability observed in patients on isoniazid could possibly be explained by the NAT2 metabolizing enzyme genetic variation (Aklillu et al., 2018). The acetylator status of patients may be assessed using genotyping or phenotyping methods. Isoniazid acetylator genotyping has been shown to be well concordant with its elimination phenotyping (Parkin et al., 2012). Phenotype approaches are usually found in the literature for acetylator status determination. Tostmann et al. (2013) used 3 different approaches to categorize patients' acetylator status. One approach was the assessment of the half-life of the drugs to determine the acetylator status of study participants. Study participants with a half-life of isoniazid greater than 130 minutes were categorized as slow metabolizers while those whose were less than 130 minutes were categorized as fast metabolizers.

2.5.3 Pharmacokinetics of First-line Anti-TB Drugs

The plasma concentration of all the first-line drugs have been shown to vary significantly from patient to patient (Egelund et al., 2015; Pasipanodya et al., 2013; Burhan et al. (2013). Mota et al. (2016) did a systematic review of the prevalence of low plasma concentration for the first-line anti-TB medication in which they found that a pooled proportion of 67% of study participants had low rifampicin concentration, 43% for isoniazid, 27% for ethambutol and 12% for pyrazinamide. From the studies used in their analysis, the prevalence of low plasma concentration was as high as 89%, 88%, 39% and 50% respectively for rifampicin, isoniazid, ethambutol and pyrazinamide. The wide-ranging variability makes predicting plasma concentrations for any patient seem impracticable. Several studies have sought to determine patient factors associated with plasma concentrations of the first-line anti-TB drugs and found none ((Fahimi et al., 2013; Hiruy et al., 2015). It appears direct measurement is always required when concentrations of these drugs are needed (Pasipanodya et al., 2013).

Despite the above, some studies have found that older people and those with lower body weight may have greater plasma concentrations because of decreased clearance and distribution volume. Ethambutol concentration was found to increase with increasing age (Denti et al., 2015). Other patient factors include sex, nutritional status, dose/kg body weight (Kwara et al., 2016). Severe TB disease impacts the absorption, distribution, metabolism and excretion of the medication and thereby influences plasma concentrations. Comorbidities like HIV and diabetes have also been linked to variations in the pharmacokinetics of the anti-TB regimen (Fonseca et al., 2020). This effect is elicited through the impact of these diseases on the digestive system and the body's metabolism.

Rifampicin is a known inducer of drug-metabolizing enzymes and isoniazid is a known

inhibitor including cytochrome P450 enzymes. This induction or inhibition can influence the metabolism and plasma concentration of other drugs. Concurrent administration of medications that are substrates or inhibitors of these enzymes may lead to changes in the anti-TB drug concentrations in the plasma (Regazzi et al., 2014). Poor medication adherence, characterized by missed doses or irregular usage, can compromise the desired drug exposure and potentially impact treatment outcomes. Some variations in genes have been found to be responsible for differences in plasma concentration of the drugs. Examples include genetic polymorphisms in organic anion transporting polypeptides, genotypic variations in cytochrome P450 2E1 and N-acetyltransferase (Cai et al., 2012).

Alcohol use and tobacco smoking have been linked to changes in the plasma concentration of rifampicin, isoniazid and pyrazinamide with conflicting conclusions (Aït Moussa et al., 2016; Myers et al., 2018; Rodríguez-Fernández et al., 2021). Due to alcohol use and smoking being associated with TB treatment outcomes and also having effects on cytochrome P-450 enzymes it was thought that the latter was the mechanism by which the former occurred. However, emerging evidence suggests that smoking may not necessarily affect the plasma concentrations of the anti-TB medication, rather, its effect on treatment outcomes may be due to its impairment of the host's immunity (Rodríguez-Fernández et al., 2021; Gómez et al., 2020). Again, these findings must be considered in the context of the methodological limitations the studies were fraught with.

Indeed, the findings on the factors which predict plasma concentrations of the anti-TB medication have been hardly consistent for most of the factors discussed. Most of the studies had small sample sizes, some as low as 32, because of the high expenditure that comes with plasma concentration quantification. This underscores the need for more studies to provide better insights into the relationships and their effect on treatment outcomes.

2.5.4 Area under the concentration-time curve (AUC)

The AUC is a pharmacokinetic measure of the patient's exposure to the drug. Several studies have identified it as a better pharmacokinetic predictor of drug efficacy than the peak plasma concentration (Jayaram et al., 2003; Jayaram et al., 2004; Gumbo et al., 2007). Much worse, using the C_{2h} as an estimate for the C_{max} may be even more limited as delayed absorption may lead to gross underestimation (Akkerman et al., 2014). It is, therefore, imperative that the AUC is used in the assessment of the relationship between the drugs and treatment response.

Given the high expenditure associated with determining the AUC using full PK monitoring requiring sampling several times post-dose, PK models have been determined to enable using 2 to 3 samples for optimal estimation of the AUC (Akkerman et al., 2014; Sturkenboom et al., 2015). In another study, the AUC and C_{max} were determined for a sub-sample (n=9) of the study participants (n=181) and associated with the C_{2h} to ensure the latter was a good estimate of the C_{max} and AUC (Burhan et al., 2013a). While this approach provides a more confident use of the C_{2h}, the stated limitation is hardly addressed by it.

2.6 TB Treatment Outcomes

Worldwide, the treatment success rate dropped from 83% in 2014 to 82% in 2016 among TB patients but increased among MDR-TB and XDR-TB patients from 52% in 2013 to 55% in 2013 and 28% in 2013 to 34% in 2016, respectively (WHO, 2015; WHO, 2018). The increase in treatment success rates for MDR and XDR patients may have been due to the use of new medicines, bedaquiline and delamanid in a number of countries (WHO, 2018).

Prahl et al. (2014) found that 38.5% of patients on treatment had low plasma concentration in both rifampicin and isoniazid. Out of 28 patients, 5 (17.9%) had treatment failure, 3 (10.7%)

died and 2 (7.1%) relapsed a year after ending treatment. In the study by Chideya and colleagues, they defined poor treatment outcomes as having sputum smear or culture positivity at the fifth month after treatment initiation or death during treatment. In total, 16% had poor treatment outcomes.

Previously, the WHO defined treatment failure to include cases in which treatment had been initiated but were found at any time during treatment to have resistant strains of the bacilli, but this has been excluded in the most recent review (WHO, 2010b). In an Indonesian study, 56 out of 167 (34%), 11 out of 155 (7.1%) and 2 out of 131 (1.5%) were culture positive at the 4th, 8th and 24th weeks of treatment, respectively (Burhan et al., 2013a). Further, 82% were cured by the 5th or 6th month of treatment while 17% dropped out and 1.2% had treatment failure. A study in Burkina Faso found weight loss between the initiation of treatment and the 2-month sputum-smear microscopy and high bacillary load during the 2-month sputum smear microscopy as risk factors for bacteriologic failure (Diallo et al., 2018). Studies in Africa with bacteriologic failure as an outcome are rare to find in the literature.

The standard definitions of the treatment outcomes for patients with drug-susceptible TB are presented in Table 1 (WHO, 2013).

Table 1: Standard definitions for TB Treatment outcomes (excluding patients treated for RR-TB or MDR-TB)

Outcome	Definition
Cured	A pulmonary TB patient with bacteriologically confirmed TB at the beginning of treatment who was smear- or culture-negative in the last month of treatment and on at least one previous occasion.
Treatment completed	A TB patient who completed treatment without evidence of failure BUT with no record to show that sputum smear or culture results in the last month of treatment and on at least one previous occasion were negative, either because tests were not done or because results are unavailable.
Treatment failed	A TB patient whose sputum smear or culture is positive at month 5 or later during treatment.
Died	A TB patient who dies for any reason before starting or during the course of treatment.
Lost to follow-up	A TB patient who did not start treatment or whose treatment was interrupted for 2 consecutive months or more.
Not evaluated	A TB patient for whom no treatment outcome is assigned. This includes cases "transferred out" to another treatment unit as well as cases for whom the treatment outcome is unknown to the reporting unit.
Treatment success	The sum of *cured* and *treatment completed.*

Source: WHO, Definitions and Reporting Framework for Tuberculosis, 2013

2.7 Risk Factors for Poor Treatment outcomes

2.7.1 Pharmacokinetic variability as a risk factor for poor treatment outcomes

Some studies have reported high incidence of poor outcomes in patients with low serum concentrations of the first-line anti-TB drugs. Mah and colleagues reported an incidence of poor outcomes of 42.5% in those with low isoniazid concentration compared to 18.3% in those with normal isoniazid concentrations (Mah et al., 2015). Several other studies have found evidence relating isoniazid concentration to treatment outcomes (Pasipanodya et al., 2013b; Prahl et al., 2014; Sekaggya-Wiltshire, von Braun, et al., 2018) with others not finding enough evidence for similar conclusions (Burhan et al., 2013; Requena-Méndez et al., 2012; Park et

al., 2015; Rockwood et al., 2017).

Low pyrazinamide concentration tends to be linked to end-of-treatment bacteriological failure and relapse (Burhan et al., 2013a; Pasipanodya et al., 2013a; Ramachandran et al., 2020). Similarly, low rifampicin concentration showed association with poor outcomes (Burhan et al., 2013a; Perumal et al., 2020). In fact, rifampicin concentration in comparison with the other drugs in the regimen, has been found most often to be predictive of poor outcomes (Aarnoutse et al., 2017; Pasipanodya et al., 2013a; Ramachandran et al., 2017, 2020; Sekaggya-Wiltshire, von Braun, et al., 2018; Svensson et al., 2018). More to that, Svensson et al. (2018) and Velásquez et al. (2018) have demonstrated a dose-response relationship between rifampicin plasma concentration and treatment outcomes. Be that as it may, others have found otherwise for both pyrazinamide and rifampicin (Park et al., 2015; Sekaggya-Wiltshire, Lamorde, et al., 2018a; Wilby et al., 2014).

The relationship between ethambutol pharmacokinetics and treatment response is rarely evaluated in the literature owing to expected role of forestalling selection of resistant strains to rifampicin in case of isoniazid failure in the intensive phase of treatment. Of the few available, Chigutsa et al. (2015) determined that low ethambutol concentration predicted poor outcomes while Park et al.(2015) found nothing. These studies were limited in several ways which are later discussed.

2.7.2 Adverse Drug Events

Adverse drug reactions have been reported among TB patients as occurring with wide variations in frequencies. A concurrent cohort study in China to determine the occurrence of adverse drug reactions among patients on DOT therapy had 15.1% of the patients showing at

least one (1) adverse effects (Lv et al., 2013). The adverse effects were distributed such that 6.3% of patients were with liver dysfunction, 3.7% with gastrointestinal disorders, 2.5% with arthralgia, 2.4% with allergic reactions, 2.0% with neurological disorders, 0.07% with renal impairment and 0.05% were with non-specified and less reported adverse effects (Lv et al., 2013).

In Brazil, a retrospective analysis of 297 TB patient records yielded an adverse events incidence of 49.1% (Vieira & Gomes, 2008). The authors dichotomized the adverse events into minor and major adverse effects, major events being those which were life threatening, caused death or required hospitalization. The proportion of minor events was 81% of all events. The adverse events reported included epigastric pain (20.4%), arthralgia (16.4%), drug-induced liver disease (10.6%), exanthema (8.4%), nausea/vomiting (9.3%), pruritus (8.0%), peripheral neuropathy (5.8%), acne (4.9%), dizziness (3.1%), myalgia (2.7%), swelling/arthritis (2.2%), weakness/asthenia (1.8%), decreased visual acuity (1.3%), headache (1.3%), motor deficit (0.9%), insomnia (0.9%) and other less incident ones including decreased libido, tachycardia/palpitation, alopecia, skin hyperpigmentation.

2.7.3 Treatment Adherence

The WHO has not recommended a standard definition for measuring treatment adherence. A study in Ethiopia to assess the factors associated with non-adherence to anti-TB regimen measured treatment adherence on a binary scale, adherent and non-adherent (Woimo et al., 2017). Adherent patients were defined as a new or retreatment patient not missing any dose or missing less than 10% of total doses prescribed and non-adherence defined as missing 10% or more of total doses prescribed. Based on this categorization, the prevalence of non-adherence was 24.5%. In China, Xu and colleagues (2009) defined treatment adherence much the same

way Woimo and colleagues did. However, the prevalence of non-adherence was 12.2%.

Hu et al. (2008) termed non-adherence as "interruption of treatment" and defined it as missing last 3 doses or not having medicines for more than a week or stopping treatment 6 days before treatment ends, for those completing the recommended treatment. Among the 4 counties where the study was conducted, the rate of non-adherence ranged from 6.2 to 21.7%.

Regarding the measurement of treatment adherence, the approach used by Hu and colleagues allows for the measurement to be done prior to treatment completion. It assesses treatment adherence for only a short period within the treatment duration. Using the proportion of total doses missed is prone to recall bias but provides an estimate that represents the entire treatment duration and could also be used to estimate adherence for any specified duration. About selecting the cut-off at 10%, the basis is unclear. A study by Srivastava et al. (2011) established the cut-off for enhancing the emergence of resistant strains at 60% or less. Since the association of other treatment outcomes with this cut-off was not assessed, the 10% cut-off seems reasonable.

In an Ethiopian study assessing the factors associated with treatment adherence, the factors identified were comparable with most of the risk factors for lost to follow-up, found by other studies discussed below (Woimo, Yimer, Bati, & Gesesew, 2017). Patients with poor knowledge on treatment were 4.6 times more likely to be non-adherent. Related to that, those not provided with health information on every visit were 3 times more likely to be non-adherent. Patients who had high pill burden had 6.1 times higher odds of non-adherence. Other factors included staying 10 km or more away from the treatment centre (AOR=5.7) and high cost of non-TB medication (AOR=4.7). Experiencing medication side effects was not significantly associated with treatment adherence.

A qualitative study in Ethiopia identified poor patient and health worker interactions, health seeking behaviour that leans towards the traditional healing system, availability of TB care in distant facilities, adverse effects of medication, high pill burden, stigma and discrimination as factors contributing to treatment non-adherence (Boru, et al., 2017). Findings from this study were largely corroborated by a similar study in India (Bhattacharya et al., 2018).

From Xu and colleagues' work, illiteracy was associated with 2.5 times higher odds of treatment non-adherence while being directly observed by a village doctor reduced the odds of non-adherence by 77% (Xu et al., 2009). According to a study by Nahid et al. (2011) non-adherence to treatment during the initiation phase is significantly associated with mortality. Those who interrupted treatment during the intensive phase were 3 times more likely to die than otherwise.

2.7.4 Risk Factors for Mortality, Loss to Follow Up, and Treatment Failure.

Age is a commonly reported risk factor for mortality in tuberculosis (TB) patients. Several studies have found an increased risk of mortality among older age groups. For example, a study conducted in Ethiopia showed that individuals between the ages of 35 to 44 had a 2.9 times higher likelihood of mortality compared to those under 25 years old (Gesesew et al., 2016). Similarly, a study in Nigeria found that individuals aged 45 to 55 were at an increased risk of mortality compared to other age groups groups (Adamu et al., 2017). Older age at the time of TB diagnosis has also been associated with an increased risk of TB mortality, with the risk of death increasing by 52% for every 10-year increase in age (Rodrigo et al., 2016).

HIV co-infection is another known risk factor for mortality in TB patients. Studies have

consistently shown that TB/HIV co-infected patients have a higher risk of mortality compared to those without HIV patients (Gupta, Wood, Kaplan, Bekker, & Lawn, 2013). It is relatively more common to find a higher risk of mortality among those in WHO clinical stages III and IV since they have advanced disease (Addis Alene, Nega, & Wasie Taye, 2013a; Refera & Wencheko, 2013). Being on antiretroviral treatment (ARV) has been shown to improve survival among HIV-positive TB patients, with a reduced risk of death observed in patients on ARVs compared to those not on ARV treatment (Adamu et al., 2017).

Functional status of patients has also been identified as a predisposing factor for TB mortality (Refera & Wencheko, 2013). Patients who were bed-ridden (not capable of performing activities of daily living) had a three-fold higher risk of death compared to those who were of a working functional status (Gesesew et al., 2016). In these studies, although severity of disease was associated with functional status and mortality, its confounding effect was not adjusted for. Ambulatory patients (capable of performing activities required for daily living only) were not significantly different in terms of mortality risk compared to those who were of a working functional status.

Other risk factors for mortality in TB patients include sputum smear positivity, previous exposure to TB treatment, comorbidities such as diabetes and low hemoglobin levels, poor nutrition, smoking, alcohol consumption, referral from a health facility not linked to the TB programme, residence outside the city, and female sex work (Alavi-Naini et al., 2013; Bhargava et al., 2013; B. Sileshi et al., 2013; Kazempour DIzaji et al., 2018; Adamu et al., 2017; Gesesew et al. (2016).

Regarding loss to follow-up during TB treatment, risk factors include illicit drug use, alcohol

consumption, lack of HIV status checking, underweight, and lower education level (Lackey et al., 2015). Factors associated with treatment loss to follow up include low income, previous defaulting of TB treatment, stigma perception, being a relapse case, smoking, alcohol consumption, residing far from the treatment center, and inadequate information about TB (Park et al., 2016). Males, younger age, retreatment cases, and HIV-coinfected patients not on antiretroviral therapy (ART) are more likely to be lost to follow-up (Slama et al., 2013; Kigozi et al., 2017).

Risk factors for treatment failure in TB patients include alcohol consumption, lower body weight, low plasma concentrations of TB drugs, sputum smear positivity at 2 months, extensive disease on chest radiograph, retreatment, and slow isoniazid acetylator status (Burhan et al., 2013a; Pasipanodya et al., 2012; Prahl et al., 2014).

These risk factors provide valuable insights for the identification of potential confounders of the relationship between the pharmacokinetics of the anti-TB drugs and treatment outcomes.

2.7.5 Sputum Smear Conversion

Sputum smear conversion is a crucial marker indicating treatment response and decreased infectivity in pulmonary tuberculosis patients. It signifies the transition from positive to negative results for acid-fast bacilli (AFB) upon microscopic examination of sputum samples, typically occurring within the initial months of anti-tuberculosis treatment (WHO, 2013). A meta-analysis of 24 studies explored the dynamics of sputum conversion during effective anti-tuberculosis treatment and revealed that sputum smear conversion occurred in 9% of patients at 2 weeks (95% CI 3%-24%) and 82% of patients at 2 months of treatment (95% CI 78%-86%). On the other hand, solid culture conversion occurred in 5% of patients at 2 weeks (95% CI 0%-14%) and 88% of patients at 2 months (95% CI 84%-92%) (Calderwood et al., 2021).

These findings emphasize the relatively swift nature of sputum smear conversion compared to the more prolonged process of solid culture conversion. This underscores the correlation between the two approaches to monitoring progress during treatment.

Some factors that have been found to influence smear conversion include baseline bacterial load, adherence to treatment and patient factors such as nutritional status, comorbidities, and immune status. Patients with higher initial bacterial loads tend to experience slower conversion rates. Those adhering to their regimen are more likely to achieve prompt conversion (WHO, 2020; WHO 2013).

Delayed sputum smear conversion (non-conversion to smear-negative PTB at the end of the intensive treatment phase) is linked to adverse outcomes, including treatment failure, drug resistance and mortality (WHO, 2020).

2.8 Limitations of Studies

The limitations of the studies reviewed are summarized from Tables 2 to 5. Most of the pharmacokinetic studies had small sample sizes (Table 2). Some of the studies with large sample sizes retrospectively analyzed data of patients who had undergone therapeutic drug monitoring. These were usually patients who were not responding to treatment. There is, therefore, a selection bias that must be noted in interpreting the results of such studies.

Table 2: Summary of limitations of pharmacokinetic studies reviewed.

No.	Study authors	Study design	Limitations
1.	Chigutsa et al. (2015)	Pharmacokinetic study	- Small sample size (54) - Long term outcomes (the standard treatment outcomes) were not assessed
2.	Tostmann et al. (2013)	Pharmacokinetic study	- Small sample size (20)
3.	Denti et al. (2015)	Randomized controlled trial	- Limited number of pharmacokinetic sampling, limiting the description of the PK curve - Assessment of the effect of time on treatment constrained by large missing data
4.	Pasipanodya et al. (2013)	Pharmacokinetic study	- A high proportion of participants were categorized as "retreatment", limiting generalizability to new patients - The analytical method used for determining the pharmacokinetic thresholds of the medicines is prone to overfitting - Outcomes were not ascertained by sputum microscopy for all participants - Quantitative sputum bacillary burden was not included in the design though it is crucial for assessment of bacteriologic outcomes
5.	Kumar et al. (2017)	Open-label cross-over design	- Small sample size (25) - Non-randomization of the sequence of food intake and drug administration - PK sampling was done once (2-hours post dose) and used as a proxy for $C_{max,}$ though semi-intensive sampling was done for a sub-sample of participants
6.	Sturkenboom et al. (2015)	Pharmacokinetic study	- Selection bias owing to the inclusion of only in-patients. This may limit generalization to outpatients
7.	Lalande et al. (2015)	Population pharmacokinetic modelling and simulation study	- Majority of study participants were not infected with TB (93.3%). The PK data may vary in the absence of lung lesions
8.	Mota et al. (2016)	Systematic review and meta-analysis	- Included studies had PK sampling done only 2-hours post-dose; C_{max} may have been grossly underestimated - Studies were largely heterogeneous

Regarding study designs assessing pharmacokinetic variability and its association with treatment outcomes, different studies defined treatment outcomes differently (Table 3). This presents a challenge as patient classifications will vary in ways that have implications for comparing findings across different settings. Again, most of the studies had small sample sizes. This implies the possibility of not finding meaningful differences even where they truly exist. Several studies only used samples taken after 2 hours of drug ingestion. Taking the samples 2 hours post dose alone also precludes the assessment of those with malabsorption and delayed absorption. Despite this limitation, a number of studies measured C_{max} using only the 2-hour post-dose samples. Another limitation was the exclusion of some features such as clinical characteristics identifiable on x-rays.

Table 3: Summary of limitations of studies which assessed the association between serum concentrations of anti-TB medication and treatment outcomes.

No.	Study authors	Study design	Limitations
1.	Prahl et al. (2014)	Prospective cohort study	- Small sample size (32) - Timepoint after treatment initiation when PK sampling was commenced was not standardized as it ranged from 6 to 206 days - Some patients may have eaten before ingesting medication. Given the interaction with food this may have led to lower than optimal serum concentrations - In about 40% of participants adherence to the medication was not assessed
2.	Burhan et al. (2013)	Prospective cohort study	- Only the 2-hour post dose PK sample was taken and presumed to be the $C_{max,}$ although semi-intensive sampling was done for a sub-sample of participants. C_{max} may have been underestimated for that matter. -
3.	Heysell et al. (2011)	Prospective cohort study	- Small sample size (16) - Some patients may have eaten before ingesting medication. Given the interaction with food this may have led to lower than optimal serum concentrations - Only the 2-hour post dose PK sample was taken and presumed to be the C_{max}. C_{max} may have been underestimated for that matter.
4.	Holland et al. (2009)	Retrospective cohort study	- Small sample size (21) - Only TB/HIV co-infected patients were recruited limiting generalization for TB patients without HIV co-infection.
5.	Chideya et al. (2009)	Prospective cohort study	- TB Treatment outcomes may have been misclassified since there were no autopsies to ascertain the cause of death. This was particularly important because some TB/HIV co-infected patients were not antiretrovirals. - PK sampling was done once (2-hours post dose) and used as a proxy for C_{max}.
6.	Pasipanodya et al. (2012)	Meta-analysis	- Misclassification bias may have resulted due to the lack of a standard quality assurance means of defining bacteriologic outcomes across studies - Some studies categorized as treatment failure patients with certain adverse drug reactions. This was not standard across studies

No.	Study authors	Study design	Limitations
7.	Ahmed et al. (2012)	Cross-sectional analysis	- Small sample size (30) - Only TB/HIV co-infected patients were recruited limiting generalization for TB patients without HIV co-infection. - The timing of food intake and the administration of the medication were not documented. Some may have eaten prior to taking the medication which could lead to sub-optimal serum concentrations - Doses were adjusted in instances of low serum concentration thereby precluding assessment of low serum concentration on treatment outcomes
8.	Heysell et al. (2010)	Retrospective cohort study	- PK sampling was done once (2-hours post dose) and used as a proxy for C_{max} - Clinical and demographic characteristics included for analysis were limited by the design
9.	Park et al. (2015)	Retrospective analysis of TDM data	- PK sampling was done once (2-hours post dose) and used as a proxy for C_{max} - Clinical and demographic characteristics included for analysis were limited by the design - Doses of the medications were adjusted following TDM as such association of serum concentration with treatment response was not generalizable
10.	Tongeren et al. (2013)	Retrospective analysis of TDM data	- Doses of the medications were adjusted following TDM as such association of serum concentration with treatment response was not generalizable
11.	Perumal et al. (2020)	Systematic review and meta-analysis	- Combined effect of low concentration of 2 or more of the drugs not assessed - Definition of treatment outcome not standard across studies

Summaries of the limitations of studies on the first-line anti-TB treatment adherence and adverse drug reactions are presented in Table 4. Adverse drug reactions and adherence to the medication are usually assessed using self-reported measures. Causality between treatment adherence and treatment outcomes are limited mainly due to methodological design of the studies.

Table 4: Summary of limitations of studies which assessed the incidence of adverse drug reactions or adherence to the first-line anti-TB medication.

No.	Study authors	Study design	Limitations
1.	Lv et al. (2013)	Prospective cohort study	- The incidence of ADRs may have been underestimated since laboratory assessments were not done - The confounding effect of known confounders on the association between ADRs and treatment outcomes were not adjusted for
2.	Vieira & Gomes (2008)	Retrospective cohort study	- Three drugs (rifampicin, isoniazid, and pyrazinamide) were used in the intensive phase of treatment as is required in Brazil. This is different than the 4 used in most countries. This limits generalization beyond Brazil - The dose of isoniazid was higher than is recommended in the standard treatment.
3.	Woimo et al. (2017)	Cross-sectional study	- Adherence was self-reported and as such was prone to recall and social desirability bias - Assessment of causality between adherence and treatment outcomes was limited owing to the lack of temporality - Recruitment of participants from a single higher-level referral facility limits generalization of findings
4.	Hu et al. (2008)	Cross-sectional study	- Assessment of treatment adherence was subject to recall bias since it depended solely on the respondent
5.	Srivastava et al. (2011)	Hollow fiber study	- The hollow fibre system while a reasonable approximation of reality does not have an immune system and it achieves higher bacillary load than will be usually expected in a human system
6.	Boru, et al. (2017)	Qualitative study	- Findings are not generalizable - The opinion of patients who completed treatment and health workers managing the patients were not explored
7.	Bhattacharya et al. (2018)	Qualitative study	- Study participants were selected from a small geographic area providing a narrow scope of the experiences shared
8.	Xu et al. (2009)	Mixed-methods design	- Some selected participants were untraceable and as such excluded from the analysis. This may have introduced selection bias

Most of the studies reviewed for risk factors of mortality, loss to follow up and treatment failure were retrospective cohort studies (Table 5). As such researchers were limited as to the variables they could include. Typically, confounding variables such as co-morbidities, drug resistance and treatment adherence were usually left out in most of the retrospective studies. Also, most had to make up for a lot of missing data during analysis. Another limitation of most of the studies was that they were conducted at single treatment centres.

Specifically, for factors associated with mortality, most of the studies assessed all-cause mortality. This introduces a misclassification bias and is a limitation on the inference that factors identified were associated with TB mortality, as some may have died due to other causes. In resource-constrained settings autopsies may not always be done to ascertain the cause of death as the cost implications may be unbearable for families. Yet, that the WHO definition of mortality during TB treatment is consistent with the all-cause mortality as used in most of these studies (WHO, 2013).

Table 5: Summary of limitations of studies which assessed risk factors for poor treatment outcomes

No.	Study authors	Study design	Limitations
1.	Gesesew et al. (2016)	Retrospective cohort study	- High rate of missingness in the dataset - Potential confounders such as alcohol use, smoking etc were not included - Incidence of death may have been underestimated since some participants who were transferred out or defaulted on treatment were not traced.
2.	Adamu et al. (2017)	Retrospective cohort study	- Some important clinical and socio-economic data such as nutritional status and income were not available for analysis - As a facility-based study, the findings are only representative of similar tertiary level facilities. - Mortalities may have been underestimated since follow-up ended at treatment completion
3.	Rodrigo et al. (2016)	Prospective cohort study	- There were missing data on dates of deaths for some participants - A key confounding factor, diabetes mellitus, was not included
4.	Bhargava et al. (2013)	Retrospective cohort study	- High rate of missingness in the dataset - The severity of TB disease as measured by radiological means was not adjusted for - Incidence of death may have been underestimated since some participants who were transferred out or defaulted on treatment were not traced.
5.	Sileshi et al. (2013)	Retrospective cohort study	- TB/HIV co-infected patients who started ART treatment before TB treatment initiation and those who started while on TB treatment were categorized together. This may have introduced bias - Potential confounders including severity of immunosuppression, adherence to treatment and co-morbidities were not adjusted for
6.	Nahid et al. (2011)	Retrospective cohort study	- Not all deaths had autopsies done as such the identified factors may not necessarily be causal - Majority of participants were ARV treatment-naïve limiting the assessment of the role of ARVs in preventing deaths
7.	Bigna et al. (2015)	Retrospective cohort study	- During the 7-year study duration ARV treatment initiation policy changed. This differential was unaccounted for in the analysis
8.	Refera & Wencheko (2013)	Retrospective cohort study	- Without a clear basis all deaths were assumed to have been caused by TB/HIV co-infection
9.	Alavi-Naini et al. (2013)	Retrospective cohort study	- Not all deaths had autopsies done as such the identified factors may not necessarily be causal

No.	Study authors	Study design	Limitations
10.	Lackey et al. (2015)	Prospective cohort study	- Several variables were self-reported and as such prone to social desirability bias - Only smear positive patients were recruited. Some TB cases may have been unduly excluded while MDR-TB cases may have been wrongly included as a result - High rate of missing data
11.	Park et al. (2016)	Case-control study	- Sample selected was prone to selection bias. Only 46 out 102 defaulters were available for interviews
12.	Slama et al. (2013)	Case-control study	- The questionnaire was administered by health workers and was therefore subject to interviewer bias and social desirability bias - Some participants were required to provide information from over 2 years prior. This was a source of recall bias.
13.	Kigozi et al. (2017)	Retrospective cohort study	- Incidence of death may have been underestimated since some participants who were transferred out or defaulted on treatment were not traced. - Participant data were pulled from a database which required that patients had pre-treatment smear results. This limits the generalization only to those who were smear tested prior to treatment initiation.

2.9 Knowledge Gaps

For pharmacokinetic variability as a risk factor for poor treatment response, very few studies have assessed the combined effect of the low plasma concentration of all the drugs on treatment outcomes. Most of the studies used only plasma concentration at one time point (2-hour post-dose) and this may grossly underestimate the peak concentration. Prospective studies assessing the relationship between plasma concentration of anti-TB drugs and treatment outcomes are few and most of those available used a small sample size. Data on the pharmacokinetics of the TB drugs are limited in the African population, particularly West Africa.

Regarding factors associated with treatment outcomes, most studies have not investigated the dynamic relationship between patient characteristics and outcomes over the course of treatment. Bacteriologic failure as an outcome is understudied in Africa.

2.10 Conceptual Framework

The literature review has been used to design a framework which describes the relationships between the main variables in the study (Figure 2). Socio-demographic variables are associated with behavioural factors and with clinical characteristics of patients. These characteristics could influence the plasma concentration of the first-line drugs which in turn determine treatment outcomes. For example, the severity of TB disease manifested in the extent of lesions in the lungs could make patients want to adhere to treatment in hopes of getting better, yet, the incidence of adverse events from taking the drugs could reduce adherence after they begin to feel well (symptoms improve). This could ultimately result in poor treatment outcomes. Patients in the reproductive age may have a higher rate of HIV infection.

The TB/HIV co-infection may result in lower-than-expected levels of plasma concentration of the anti-TB drugs compared to patients above the reproductive age. The higher levels of plasma concentration of the older age group compounded by slower metabolism may be associated with higher incidence of adverse drug events. These might influence treatment outcomes indirectly through treatment non-adherence or higher lost to follow-up rates.

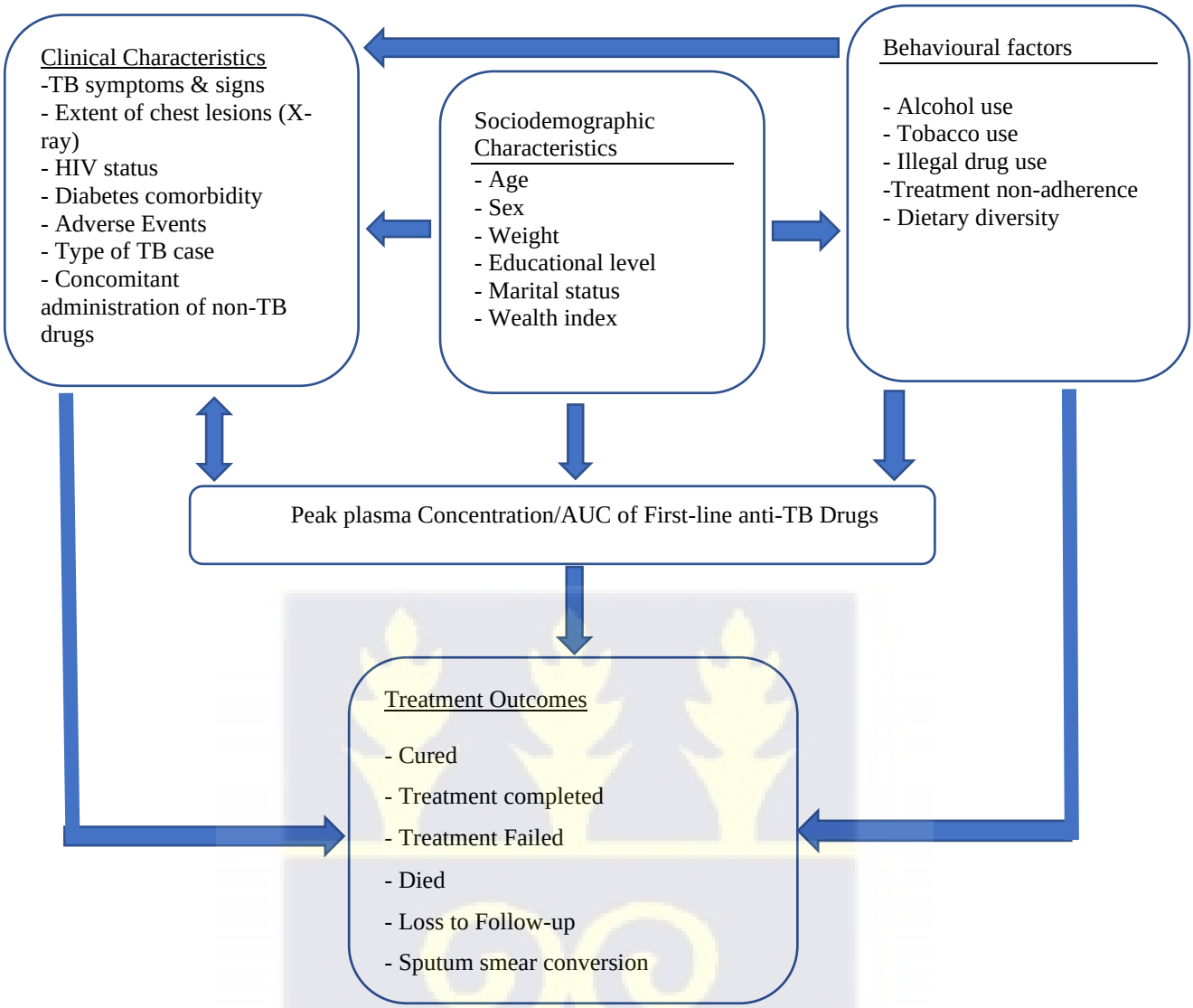


Figure 2: Conceptual framework showing the relationships amongst patient's clinical, pharmacologic, behavioural and socio-demographic characteristics, and treatment outcomes.

CHAPTER THREE

3.0 METHODS

3.1 Study location

The study was conducted in the Ashanti, Bono, and Bono East regions of Ghana. The Ashanti region lies in the country's middle belt, somewhat located in the southern half. It covers 10.2% of the land area of Ghana and is critical to transportation and commerce to the north of the country and countries beyond the north, being centrally located. In 2021, it was estimated to have the second highest proportion of the Ghanaian population, 17.6 (Ghana Statistical Service, 2021). As regards TB, the regional case notification rate was marginally lower (58.7 per 100,000 population) than the average national rate of 62.2 per 100,000 population in 2013 (Bonsu et al., 2014).

Within the Ashanti region, 5 study sites in 3 out of the 43 administrative units (districts, municipalities or metropolis) were intended for inclusion. These administrative units were the Kumasi metropolis, Asokwa and Old Tafo municipalities. They were chosen because of the high outpatient department (OPD) attendance at the major public health facilities located within them. Being near each other allowed for easy coordination of study sites, blood sampling and storage of the samples. The 5 facilities were Komfo Anokye Teaching Hospital, Kumasi South Hospital, Suntreso Government Hospital, Manhyia Government Hospital and Tafo Government Hospital.

Three (3) months after beginning participant enrollment a review suggested that the rate of enrollment (10 participants per month) might unduly delay the study. As a result, three new study sites were included, the Holy Family Hospital, Techiman in the Bono East region, and Sunyani Municipal Hospital and Bono Regional Hospital, Sunyani both in the Bono region

(Appendix G). These sites were chosen with consideration for their proximity of at most 1-hour motorized access to ultra-low temperature freezers.

Three (3) out of the 8 facilities did not enroll any participants. These 3 facilities were Manhyia Government Hospital in the Ashanti region, Sunyani Municipal Hospital and Bono Regional Hospital, Sunyani in the Bono region.

3.1.1 Kumasi Metropolitan Area

The Kumasi Metropolis is located between Latitude 6.35ºN and 6.40ºS and Longitude 1.30ºW. It is approximately 270km north of the national capital, Accra. It covers an area of 214.3 square km and is in the middle belt of the country (Figure 3) (Kumasi Metropolitan Health Directorate, 2023).

3.1.1.1 Komfo Anokye Teaching Hospital (KATH)

The Komfo Anokye Teaching Hospital (KATH) is a tertiary hospital in the Kumasi metropolis with a bed capacity of 1,200. A total of 210 TB cases were diagnosed between January and June 2019. Of this number, 43 (20.5%) were bacteriologically confirmed, 132 (62.9%) were clinically diagnosed and the rest were Extrapulmonary cases diagnosed bacteriologically or clinically.

3.1.1.2 Suntreso Government Hospital

The Suntreso Government hospital is a 119-bed capacity hospital in the Kumasi metropolis. From January to June 2019, 52 cases of TB were diagnosed; 27 (51.9%) were new bacteriologically confirmed cases and 2 (3.8%) were relapse cases also bacteriologically confirmed (DHIMS 2 Tech Team, 2024).

3.1.2 Asokwa Municipal

The Asokwa Municipality was carved out of the Kumasi Metropolis in 2018. It covers an area of 24 square km. It is at the centre of the region and is located between Latitude 6.35°N and 6.40°S and Longitude 1.30°W and 1.35°E (Figure 3). There are 18 towns in the municipality grouped into 3 sub-municipalities. The total population in 2018 was estimated to be 173,457, 54.0% of which were aged 15 years or above. The male to female ratio was 0.92. As regards health facilities, the municipality has one (1) government-owned hospital and 14 non-governmental facilities; 6 hospitals, 8 clinics, maternity homes and child health facilities (Asokwa Municipal Health Directorate, 2023).

3.1.2.1 Kumasi South Hospital

The number of TB cases diagnosed at the Kumasi South Hospital in the first half of 2019 (January to June 2019) was 85. Fifty-three (53; 62.4%) were bacteriologically confirmed. Of this number, 47 were new and 6 were relapse cases. Males formed 68.2% of the total cases diagnosed and TB/HIV co-infected patients were 25 (29.4%) (DHIMS 2 Tech Team, 2024).

3.1.3 Old Tafo Municipal

The Old Tafo Municipality was originally part of the Kumasi metropolis until 2018. It borders Kwabre East municipality to the north, Kumasi Metropolis to the south and east, and Suame municipality to the west. It has a projected population of 288,549, representing 5.8% of the population of the Ashanti region. The municipality covers a surface area of 31.13 square kilometres (Old Tafo Municipal Health Directorate, 2023).

3.1.3.1 Old Tafo Government Hospital

The Tafo Government Hospital is a primary hospital in the Old Tafo Municipality. From

January to June 2019, a total of 62 TB cases were diagnosed at the hospital. Of the 46 (74.2%) bacteriologically confirmed cases, 45 (97.8%) were new and 1 was relapse. The proportion of males diagnosed within the period was 69.8%. TB/HIV co-infected patients were 11, representing 17.7% of all TB cases diagnosed within the period (DHIMS 2 Tech Team, 2024).

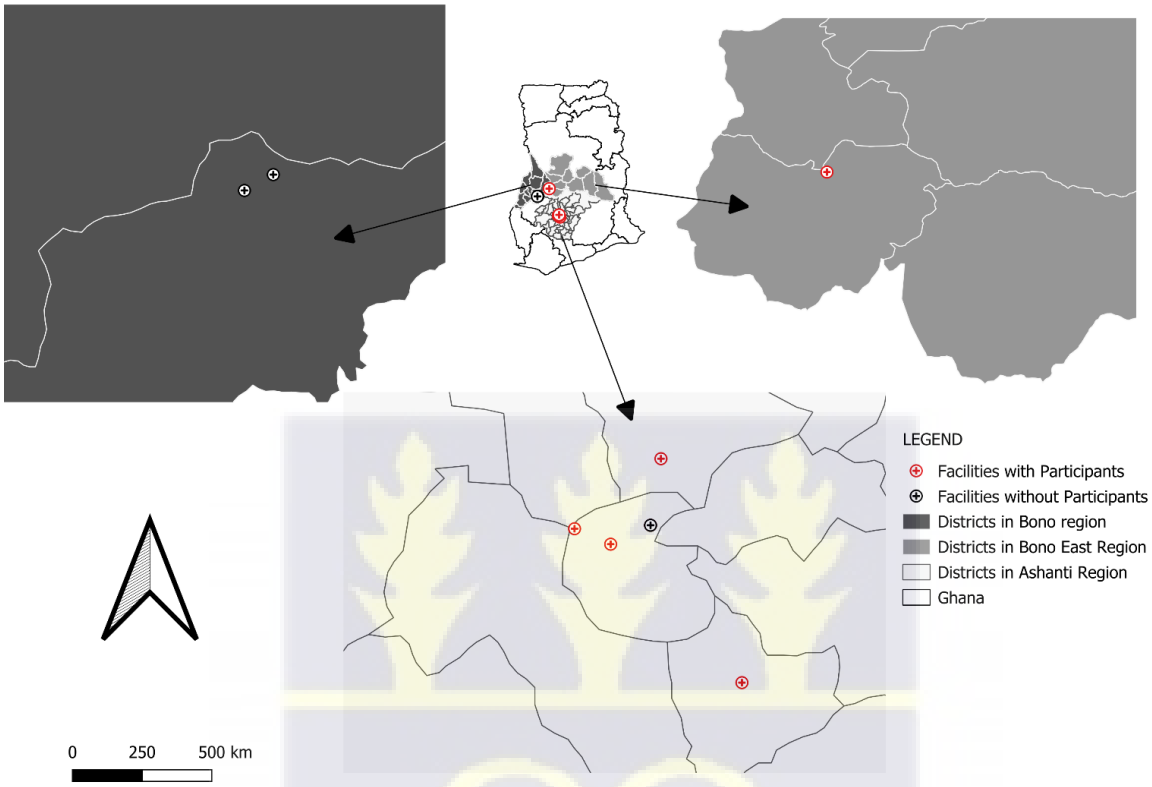


Figure 3: Map of the Ghana showing the regions and districts in which the study was conducted.

3.1.4 Techiman Municipality

The Techiman Municipality lies between longitude 10 49'E and 20 30'W and latitude 80 00'N and 70 35'S. It covers a land surface area of 649.07 sq. km. It has a population density of 227.7 persons per kilometer with an estimated population of 147,788 (Figure 3) (Techiman Municipal Health Directorate, 2023).

3.1.4.1 Holy Family Hospital, Techiman

The Holy Family Hospital is a secondary referral hospital in the Bono East Region. It has a bed

capacity of 330. The number of TB cases managed at the facility was 114 in 2020 and 143 in 2021 (DHIMS 2 Tech Team, 2024).

3.2 Study Design

A prospective cohort study design was used. A cohort of patients diagnosed with rifampicin-susceptible TB was selected and followed from treatment initiation to completion. Plasma concentrations of the first-line anti-TB drugs were determined one to two months after baseline and treatment outcomes were compared between those with low pharmacokinetic exposure and those who had normal exposure. Study participants were recruited consecutively from all 5 treatment centres beginning from 1st August, 2021. Study participants were followed up to treatment completion 6 months after treatment initiation. Each month was made of 28 days and each week of 7 days. Data was collected at treatment inception (baseline – month 0), 1, 2, 3, 4, 5 and 6 months (end point) after the start of anti-TB treatment.

3.3 Study population

The target population for the study was patients with rifampicin-susceptible TB who were to begin treatment with the WHO recommended first-line anti-TB regimen. Previously treated and new TB patients with bacteriologically confirmed TB disease at the treatment centres were targeted for recruitment into the study.

3.4 Eligibility Criteria

3.4.1 Inclusion criteria

All TB patients with the following characteristics were eligible for inclusion in the study:

- Patients aged 15 years or more with a minimum body weight of 30kg; the doses of the recommended first-line regimen are defined differently for patients who are less than 15

years or weigh less than 30kg. The dosing differentials based on these parameters will make the serum concentrations incomparable among participants.

- Patients who had been bacteriologically confirmed using GeneXpert MTB/Rif test. This was to reduce the likelihood of misclassifying rifampicin-susceptibility and ensure a verified premise for treatment with the first-line anti-TB medication.
- Previously treated patients whose treatment outcome was "cured" or "treatment completed" at the end of the most recent treatment, newly diagnosed with rifampicin-susceptible TB using GeneXpert MTB/Rif test (Relapse patients). These patients are categorized as incident cases (WHO, 2013).

3.4.2 Exclusion criteria

Patients with the following characteristics were excluded from the study:

- Those who were bacteriologically diagnosed using smear positivity without GeneXpert or clinically diagnosed using radiological evidence or clinical judgement of a clinician. This is because of the high risk of information bias associated with including them.
- Those diagnosed with MDR-TB or XDR-TB were also excluded. Such patients were treated with different regimen than the recommended first-line anti-TB medication making them incomparable to the target population.
- Those diagnosed with extra-pulmonary TB. The treatment outcomes for patients with extrapulmonary TB are determined based on the clinical judgement of the clinician owing to resource-constraints limiting the use of culture for the purpose. Including these will increase the risk of information bias. Furthermore, the treatment duration could vary based on the part of the body infected (WHO, 2010b). This could distort the study timelines.
- Those previously treated whose treatment failed at the end of the most recent course of treatment (treatment after failure patients (WHO, 2013)).
- Those previously treated and categorized as lost to follow-up during the most recent course

of treatment (treatment after lost to follow-up patients (WHO, 2013)).

- Those previously treated for TB but whose treatment outcomes at the most recent course of treatment is not known or recorded (Other previously treated patients).

3.5 Variables Definition

3.5.1 Exposure variables

The exposure variables of interest were peak plasma concentration levels of each of the 4 first-line anti-TB drugs. Each was ‘low’ for concentrations below the threshold for each drug (low concentration) and ‘normal’ for concentrations equal to or above these thresholds (normal concentration) (Table 6).

Table 6: Operational Definitions of Main Exposure Variables of Interest

Category	Variable	Scale of measurement	Operational definition
Main exposure variables	Peak plasma concentration level of ethambutol (C_{max})	Numeric (continuous)	Maximum likelihood estimation using C_{2hr} and C_{4hr} modelled with Monolix 2023 (Lixoft SAS, a Simulations Plus company, 2022)
	Peak plasma concentration level of rifampicin (C_{max} Level)	Binary (Low or Normal)	**Low** – rifampicin C_{max} value of <8µg/ml **Normal** – rifampicin C_{max} value of ≥8µg/ml
	Peak plasma concentration level of isoniazid (C_{max} Level)	Binary (Low or Normal)	**Low** – isoniazid C_{max} value of <3µg/ml **Normal** – isoniazid C_{max} value of ≥3µg/ml
	Peak plasma concentration level of pyrazinamide (C_{max} Level)	Binary (Low or Normal)	**Low** – pyrazinamide C_{max} value of <20µg/ml **Normal** – pyrazinamide C_{max} value of ≥20µg/ml
	Peak plasma concentration level of ethambutol (C_{max} Level)	Binary (Low or Normal)	**Low** – ethambutol C_{max} value of <2µg/ml **Normal** – ethambutol C_{max} value of ≥2µg/ml
	Area under the concentration-time curve 0 to 4 hours after drug ingestion ($AUC_{(0-4)}$)	Numeric (continuous)	Estimate from the C_{2h} and C_{4h} of each of the 4 drugs using non-compartmental analysis Monolix 2023

AUC: Area under the drug concentration-time curve 0 to 4 hours after drug ingestion; C_{max}: Peak plasma concentration level.

3.5.2 Outcome Variables

The primary and secondary study outcomes are defined in Table 7. The primary outcome for this study was poor treatment outcomes. It was coded "1" for poor outcomes and it included clinical outcome categories "Treatment failed", "Died" and "lost to follow up", and "0" for

successful outcomes and it included the categories “Cured” and “Treatment Completed”. The outcomes “not evaluated” was excluded because there is no theoretically valid link between the exposures of interest in this study and the outcomes.

Table 7: Operational Definitions of Primary and Secondary Outcomes

Category	Variable	Scale of measurement	Operational definition
Primary Outcome	Treatment outcomes	Binary (Poor or Successful)	**Poor Outcomes** – Participants with clinical outcomes “treatment failed” or “Died” or “lost to follow up”. **Successful Outcomes** – Participants with clinical outcomes “Cured” or “Treatment Completed”
Secondary Outcomes	Sputum smear conversion	Binary (Positive or Negative)	**Positive** – Sputum microscopy result of scanty, +, ++, or +++ at the end of the intensive phase of treatment. **Negative** – Sputum microscopy result of negative at the end of the intensive phase of treatment.
	Bacteriologic failure	Binary (Positive or Negative)	**Positive** – Sputum microscopy result of scanty, +, ++, or +++ at the end of the continuation phase of treatment. **Negative** – Sputum microscopy result of negative at the end of the continuation phase of treatment.
	Treatment non-adherence	Count	Number of days treatment was missed within a month

The clinical outcomes are as defined by the WHO (WHO, 2013). These definitions are adopted for use by the National TB Control Programme in Ghana (Bonsu et al., 2014):

Treatment failed - A TB patient whose sputum smear or culture is positive at month 5 or later during treatment.

Died - A TB patient who dies for any reason before starting or during the course of treatment.

Lost to follow-up - A TB patient who did not start treatment or whose treatment was interrupted for 2 consecutive months or more.

Not evaluated- A TB patient for whom no treatment outcome is assigned. This includes cases "transferred out" to another treatment unit as well as cases for whom the treatment outcome is unknown to the reporting unit.

Cured - A pulmonary TB patient with bacteriologically confirmed TB at the beginning of treatment who was smear- or culture-negative in the last month of treatment and on at least one previous occasion.

Treatment Completed - A TB patient who completed treatment without evidence of failure BUT with no record to show that sputum smear or culture results in the last month of treatment and on at least one previous occasion were negative, either because tests were not done or because results are unavailable.

Not Evaluated - A TB patient for whom no treatment outcome is assigned. This includes cases "transferred out" to another treatment unit as well as cases for whom the treatment outcome is unknown to the reporting unit.

3.5.3 Other Variables

Data was collected on date of recruitment into study, date of treatment commencement and date treatment outcome was observed. This was done to enable the assessment of time effects. The other covariates included age at last birthday (in years), sex (male or female), weight (kg),

height (metres), religion (Christian, Muslim, Others), marital status (married or unmarried), household ownership of selected assets, types of sanitation facilities, employment status (employed, unemployed, student, other), clinical symptoms at the start of treatment (fever, cough, haemoptysis, duration of cough, loss of appetite, dyspnoea, orthopnoea, night sweats, finger clubbing, chest pains, anaemia, white cell count), radiological extent of lesions (lung cavitation or no lung cavitation), HIV status (HIV positive or negative) , DM status (Diabetic or not diabetic), alcohol use (drinker or non-drinker), current tobacco use (smoker or non-smoker), other medicines in concurrent use at treatment inception, functional status (working, ambulatory or bedridden), treatment category (new case or relapse), total daily dose of drugs, side effects of drugs (defined as such if effect was not present prior to or at the inception of treatment).

All participants were offered HIV testing services at baseline to determine their HIV status. In addition, random blood glucose test was done for all participants who were not known diabetics at baseline. All those with a glucose concentration above 11.1mmol/l were referred to a clinician for further assessment and testing to determine their diabetes status.

For participants with TB/HIV co-infection, other variables measured were clinical stage of HIV (stages I, II, III and IV), CD4 cell count (if available), viral load (if available), cotrimoxazole initiation status (started or not started), type of ART (none, non-nucleoside reverse transcriptase inhibitor (NNRTI) based or protease inhibitor (PI) based).

Dietary diversity score was determined as the sum of the score for each of 10 food groups eaten in the last 24 hours. A score of "1" was given if food from a food group was taken within the last 24 hours and "0" if not. Scores for the 10 food groups were then summed to obtain the

dietary diversity score. Adherence as an independent variable was measured as a binary variable; adherent was for those who missed less than 20% of the monthly doses assigned and non-adherent was for those who missed 20% or more of the doses assigned.

3.5.4 Standardization of Measurements

To reduce the risk of bias, only patients who had been bacteriologically confirmed with active TB were recruited. Sputum smear microscopy was used only for monitoring treatment progress during follow up, specifically at baseline and months 1, 2, 3, 4, 5 and 6. Participants who missed a scheduled visit were contacted directly by phone. Where they were unreachable by phone, their treatment supporter was contacted by phone. The HIV or DM status of the participants were not considered during recruitment into the study since these may predispose patients to a higher risk of poor treatment outcomes and make the study prone to bias (Babatunde et al., 2013).

Non-adherence to TB treatment was determined using records provided by DOTS provider. To measure alcohol use, the standard guideline recommended by the WHO was adapted (WHO, 2000; Nugawela, Langley, Szatkowski, & Lewis, 2016). Tobacco use was assessed using standard survey questions recommended by the CDC (Centers for Disease Control and Prevention, 2011a).

3.6 Sample size determination

The following assumptions were made:

- the probability of incorrectly detecting a difference when none actually exists will be 0.05 (α)
- the probability of failing to detect a difference when one actually exists will be 0.20

(β)

- that the incidence of poor outcomes in the reference group (patients who have normal plasma concentration for isoniazid and rifampicin) will be 5% based on the estimate in the study by Pasipanodya et al. (2013).
- that a minimum difference of 25% will be detected in treatment outcomes between those with low and normal plasma concentration of the drugs.

Based on these assumptions, a figure of 34 per group was estimated using a two-sample proportions likelihood ratio test with a two-sided significance of 5%. To account for independence of data resulting from the multiple study sites and repeated measures taken on each subject, a design effect of 2.0 was assumed because of the likelihood of facility-level differences in treatment outcomes. The figure was adjusted by a 20% non-response rate to account for incomplete responses by study participants. Thus, the total sample size was 164. Participants were recruited from all 5 facilities consecutively until the sample size was reached. In the end, 92 participants were recruited from KATH, 21 from the Kumasi South Hospital, 37 from the Holy Family Hospital, Techiman, 7 from the Suntreso Government Hospital, and 7 from the Tafo Government Hospital.

3.7 Specimen collection and Laboratory Methods

3.7.1 Blood Specimen Collection and Handling

During the intensive phase of treatment, the fixed dose combination of all 4 first-line anti-TB drugs was given to the patients by DOTS as recommended by the WHO. The dosing was based on body weight bands. Study participants who weighed 30 to 39kg were given 300mg of rifampicin, 150mg of isoniazid, 550mg of ethambutol and 800mg of pyrazinamide. Those who weighed 40 to 54kg took 450mg of rifampicin, 225mg of isoniazid, 825mg of ethambutol and

1200mg of pyrazinamide. Participants with a body weight of 55kg or more were given 600mg of rifampicin, 300mg of isoniazid, 1,100mg of ethambutol and 1,600mg of pyrazinamide.

Blood samples were collected on the 4th week of treatment initiation. Those who were not available for the blood sampling in the 4th week were re-scheduled for the 5th to 8th week but not after. This was to ensure the steady state concentration was achieved prior to specimen collection. The contacts of each participant and one (1) family member were taken to reduce the likelihood of specimen collection being missed.

The medication was administered after a minimum fast of 8 hours. A light meal was provided for participants 30 minutes after dosing. Two hours and 4 hours after the administration of the medication, blood samples were obtained through an intravenous catheter, using a syringe. All participants had their blood specimen taken at the treatment centres where they were recruited. The volume of blood drawn per participant per sampling time for drug concentration determination was 4 ml for both the 2-hour and 4-hour post-dose samples.

Blood samples were transferred from syringes to heparin containing tubes immediately after drawing. Tubes containing the blood samples were put on ice. Within 30 minutes of post collection, the samples were centrifuged at 3000g for 10 minutes at a temperature of 4^{o}C. The plasma was then aliquoted into Eppendorf tubes. The plasma samples taken from sites in Kumasi were then transported on ice to the KATH serology unit, while those from Techiman were sent to the Kintampo Health Research Centre for storage. They were stored at -70 to -80^{o}C, as the drugs are stable for long only under such temperature conditions. After all the plasma samples for the study had been collected, they were shipped to the Infectious Disease Pharmacokinetic laboratory (IDPL) at the University of Florida for the drug concentrations to

be determined. A validated high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used for the quantification of the drug concentrations.

3.7.2 Sputum Specimen Collection and Handling

All processes for sputum collection and handling, and performance of Xpert MTB/RIF, sputum smear microscopy followed the programmatic protocol. Two sputum samples were required at 1-hour intervals by each participant at each of the follow up visits. Two (2) samples were collected, the second one was used if an indeterminate or negative result was recorded for the first. For those unable to produce enough sputum, sputum containers were supplied to them for the collection to be done at dawn. Otherwise, all the sputum samples were taken at the TB treatment centres.

For GeneXpert testing to bacteriologically confirm active TB, each participant was required to produce one (1) spot sample (Table 8). Sputum samples for microscopy were sent to the laboratories of the study site facilities to be processed. They were received, checked, and documented. All samples were processed within 48 hours after collection. If the sputum samples could not be processed due to weekend breaks or for any other reason the sputum samples were kept in the refrigerator at 2 to 8^{o}C.

Table 8: Laboratory tests, specimen required and Laboratory where analyses was done.

Test	Specimen required	Laboratory where analyses was done
GeneXpert MTB/RIF	1 container of sputum	KATH or Kumasi South Hospital or Holy Family Hospital
Smear Microscopy	2 containers of sputum	Study site
LC-MS/MS	2 plasma samples	IDPL, University of Florida

IDPL, Infectious Disease Pharmacokinetic Laboratory; KATH, Komfo Anokye Teaching Hospital; LC-MS/MS, Liquid chromatography-tandem mass spectrometer; MTB, Mycobacterium tuberculosis; RIF, Rifampicin

3. 8 Laboratory Procedures

3.8.1 Procedure for GeneXpert MTB/RIF

The procedure used was based on the WHO (2014) recommendation. The sputum samples were processed to get rid of bacteria other than *M. tuberculosis* and to liquefy the sputum. A decontamination mixture consisting of 1% N-Acetyl-L-Cysteine (NALC), 2% Sodium Hydroxide (NaOH) and 1.45% sodium citrate was prepared and used. Sodium hydroxide (4%) was used for samples with a lot of blood. Volumes of the mixture equivalent to the sputum sample was mixed and phosphate buffered saline with pH 6.8 added up to 50ml. This was centrifuged at 3000g for 10 minutes. One to two millilitres (ml) of phosphate buffered saline (pH 6.8) was then added to the sediment.

A small portion of the liquefied and decontaminated sputum thus produced was mixed with the Xpert MTB/RIF sample reagent, processed, and transferred to the GeneXpert cartridge for testing. The report was interpreted as follows: "detected" for both *M. tuberculosis* and rifampicin resistance if the respective target DNA is detected; "not detected" if the target DNA is not detected for both *M. tuberculosis* and rifampicin resistance; "invalid" for *M. tuberculosis* if sample was not properly processed; "error" for *M. tuberculosis* if geneXpert quality control system fails; "indeterminate" for rifampicin resistance if *M. tuberculosis* concentration was so low resistance could not be determined; "no result" for both *M. tuberculosis* and rifampicin resistance if insufficient data was collected before the machine was stopped. All tests with "invalid", "error", "no result" and "indeterminate" results were repeated with a new cartridge.

3.8.2 Procedure for Sputum Smear Microscopy

The florescence microscopy (Auramine staining) was used for smear microscopy at the study site facility laboratories. The Standard Operating Procedure developed by the NTP was

followed (Ghana Health Service, 2014).

3.8.2.1 Florescence Microscopy

Using a loop, a small portion of sputum was transferred to a properly labelled slide and evenly spread on the slide over an approximate area of 1-2cm by 2-3cm to form an ellipse shape. After allowing to air-dry, the underside of the slides was passed over flame to heat slightly. Placed on a staining rack, the slide was covered with 0.1% Auramine solution poured over it through a filter paper in a funnel held over it. The covered slide was allowed to stand for 20 minutes. After draining Auramine solution and rinsing with distilled water, the slide was covered completely in 0.5% acid-alcohol decolorizing solution for 3 minutes. The acid solution was drained, and the slides rinsed gently with water. The slides were then flooded with 0.5% potassium permanganate for 1 minute to counterstain and was mounted on a fluorescent microscope for examination. Results were reported based on the microscopy system used as shown in Table 9.

Table 9: Number of AFBs seen and the corresponding results based on microscopy system used.

Result	Number of AFB	
	Using a microscope with fluorescence: 200-250x magnification, 1 length=30 fields=300 HPF	Using a microscope with fluorescence: 400x magnification, 1 length =40 fields=200 HPF
Negative	0 per length	0 per length
Scanty	0 per length	1-19 per length
+	1-29 per length	20-199 per length
++	30-299 per length	5-50 field
+++	10-100 per 1 field	>50 per field

AFB, Acid Fast Bacilli; HPF, High Performance Field.

3.8.3 Quantification of Plasma Concentration of Drugs

3.8.3.1 Principle

The method uses LC-MS/MS to analyze rifampicin, isoniazid, ethambutol, and pyrazinamide. Specifically, it uses internal standardization to monitor conditions during the assay and to quantify the drugs in the specimen. A series of equal-volume solutions of known concentrations of the reference standards (rifampicin, isoniazid, pyrazinamide, and ethambutol) were prepared. These were to be used to plot a standard curve for calibration. For internal standardization, specified concentrations of the chosen internal standards (deuterated-rifampicin, deuterated-isoniazid, deuterated-pyrazinamide, and deuterated-ethambutol) were prepared, and specific volumes added to the reference standard solutions and to all the samples. The ratios of the concentration of the reference standard to the concentration of the internal standard were determined for each drug. When equal volumes of these solutions were injected in the high-performance liquid chromatography tandem mass spectrometry (LC-MS/MS), chromatograms with two peaks resulted. For the solutions with the reference standard, one chromatogram was the response for the internal standard and the other, the response for the reference standard. The ratio of the area of the chromatograph for the internal standard to that of the reference standard was determined.

A calibration plot (standard curve) of the known concentration ratios on the y-axis and the corresponding area ratios from the chromatograms on the x-axis were drawn. These plots were considered acceptable if the two variables charted were correlated by a coefficient of determination of at least 0.98. For the samples to be determined, the concentration ratios corresponding to the area ratios could be determined by charting on the standard curve drawn above. Since the concentrations of the internal standards added were known, the unknown concentrations of the drugs in the samples could be calculated. All calculations were done by

the Xcalibur computer system, the software that operates the LC-MS/MS. These were according to validated practice standards by the IDPL.

3.8.3.2 Instrumentation

The mobile phase of the LC consisted of formic acid in LCMS-grade water, formic acid in LCMS-grade methanol and formic acid in LCMS-grade acetonitrile. The solid phase consisted of a column with Thermo, Hypersil GOLD CN. The components of the LC unit were the pump which supplied the mobile phase at a defined rate, the autosampler which injected the samples for separation, the column which separated the components of the samples and the detector which analysed the separated components.

3.8.3.3 Procedure

Each set of samples analysed consisted of an 8-point standard curve, 3 quality controls, the samples to be analysed (40 per batch), a blank plasma sample, a water standard sample and one rinse. All the samples were extracted using a uniform means except for the blank which did not include the internal standard. The blank, being plasma from healthy people who were not on any medication was added to give an indication of how well the procedure reports the absence of the drugs. The water standard did not include plasma and it helped to assess interference, if any, that owed to the plasma proteins extracted from the other samples. The rinse cleaned out the stationary phase, sample-after-sample, to ensure that there was no carry-over from one sample to the next.

3.9 Data Collection and Management

3.9.1 Recruitment of Study Team

One (1) medical officer with more than 5 years' experience in TB treatment was recruited as the study clinician, two (2) health workers at each TB treatment centre were engaged as

research assistants and one (1) Medical Laboratory Scientist was engaged to handle blood specimen, run the sputum microscopy tests, and the random blood glucose tests. The TB focal persons for each of the study sites were supervisors at their sites. In addition, a radiologist with 2 years post-qualification experience was engaged to read chest x-rays.

3.9.2 Training of Study Team Members

All study team members were trained before enrollment started. The training covered general knowledge in clinical research, study background, justification, study objectives, data collection tool and standard operating procedure (SOP) for laboratory tests and other relevant activities. The responsibilities of each team member were discussed. The primary objective of the training was to ensure that each team member was familiar with the study protocol and the processes they were required to be involved in as relates to the study. The facilitators were the principal investigator, a clinician who had been a principal investigator or co-investigator in at least one clinical research and a medical laboratory scientist with at least 3 years' experience in TB microscopy and GeneXpert.

3.10 Pretesting of Data Collection Tools

The questionnaire/data abstraction tool (Appendix B) was pretested at the KNUST hospital on 10 participants. The sequencing, participants' acceptability and clarity of the questions were assessed. Their willingness to participate in the study based on the above was also evaluated. All variables in the tool were measured during the pretesting except for those requiring the taking of blood specimen. This is because the results of the pretest samples were not to be reported though running those few samples was going to be more expensive per unit compared to those of the actual study participants owing to the small number. The time taken to complete the tool for each participant was noted and averaged to properly inform the consenting process.

Changes were made to the data collection tool, after the proposed changes were discussed and agreed on by the study team.

3.11 Assuring Data Quality

To reduce the risk of bias during the data collection, a standard operating procedure manual was used to guide all data collectors. All filled questionnaires were double entered into KoboCollect. Discrepancies provided the opportunity to address data entry errors. Prior to the data entry, all filled questionnaires were checked for completeness and accuracy. Incomplete questionnaires were sent back to the study sites for completion, where possible. The principal investigator randomly selected 10% of completed questionnaires, re-administered the unfilled questionnaire to the participants whose data were filled in the completed questionnaires and compared the two for discrepancies. Discrepancies identified were corrected. This validation process helped reduce data errors to the barest minimum. A meeting with the research team was held to discuss the observations made. All the completed questionnaires were kept under lock and key by the principal investigator. A soft copy of the data was kept on a password-protected laptop computer with the principal investigator alone having access to the data. A copy is also stored remotely in a cloud account.

3.12 Statistical Analysis

All analyses were done using Stata 16. Bar charts for categorical variables and box and whisker plots for continuous variables were used to assess the variables in exploratory analysis. The socio-demographic, characteristics of participants were described using frequency tables. Continuous variables were summarized using means and standard deviations.

3.12.1 Characterization of Pharmacokinetic variability

The C_{max} and $AUC_{(0-4)}$ were determined by maximum likelihood estimation from the C_{2h} and C_{4h} with the Monolix software (Lixoft SAS, a Simulations Plus company, 2022). The $AUC_{(0-4)}$ was estimated by non-compartmental analysis also using the Monolix software. To show the distribution of the C_{max} and $AUC_{(0-4)}$ box and whisker plots were presented for each of the drugs. The C_{max} and $AUC_{(0-4)}$ of the participants in whose samples the drugs were undetectable during the LC-MS/MS were treated as missing. They were 7 for rifampicin, 5 for isoniazid, 7 for pyrazinamide and 15 for ethambutol. To further characterize the pharmacokinetic variability, bivariate analyses between the C_{max} and $AUC_{(0-4)}$ of the drugs and selected variables were done.

Specifically, spearman rank correlation was used to determine the correlation for continuous variables (age, weight, and dietary diversity score). Wilcoxon rank sum test was used to determine dependence with the binary variables (sex, alcohol use, current smoking status, diabetes comorbidity, HIV co-infection, co-administration of other medication, and adherence). The variables chosen for the bivariate analyses were selected from the literature. These non-parametric tests were necessary because the distribution of the C_{max} and $AUC_{(0-4)}$ were non-normal. None of the plausible variable transformation (inverse, squaring, natural logarithm) methods explored made the distribution normal. The outputs of the tests were presented in tables.

To provide a detailed characterization of pharmacokinetic variability, regression analyses were done. Due to the high correlation between C_{max} and $AUC_{(0-4)}$, and the limitation of being able to estimate the AUC only for the earliest period post dose ingestion, the $AUC_{(0-4)}$ was not further analysed. For the following reasons quantile regression was used rather than ordinary

least square (OLS) regression:

1. C_{max} was not normally distributed. Additionally, there were outliers in the distribution.
2. No appropriate transformation was found to normalize the distribution.

Consequently, diagnostics of the OLS regression showed a heteroskedastic pattern in the residuals versus fitted plot. The plotted points increased in dispersion with increasing fitted values contrary to the patternlessness expected for a well specified linear model. Also, the distribution of the residuals was non-normal. The residuals vs fitted plot and histogram of residuals from the OLS regression for rifampicin were presented. They were not presented for the other drugs since they were similar to that of rifampicin.

Quantile regression presents some useful advantages over linear regression. While linear regression models the mean of the distribution, quantile regression provides an opportunity to quantify relationships across the whole distribution. All the conditional distribution of C_{max} can be modelled (Huang et al., 2017; Lê Cook & Manning, 2013; Yu et al., 2003). It allows the slopes of the regression line to change across the quantiles of the distribution providing insights into relationships which may be non-linear. In addition, it is useful for distributions which may have heavy tails as is the case here, given the outliers in the distribution. Therefore, quantile regression was used to characterize C_{max}.

Multivariable sequential quantile regression was undertaken at the 10^{th} (q0.10), 25^{th} (q0.25), 50^{th} (q0.50), 75^{th} (q0.75), and 90^{th} 9q0.90) percentiles. These quantiles were chosen to provide reasonable representation of the whole distribution. Pseudo R2 was used to assess goodness of fit. If removing a variable from the model did not change the Pseudo R2 by ±1.0, it was removed. This was done to prevent overfitting since there were several variables vis-à-vis a relatively small sample size. For visual assessment of the relationships, plots of the

multivariable sequential quantile regression fitted to the quantiles of q0.025 to q0.95 at intervals of 0.025 were presented in addition. The OLS regression output was added to demonstrate the advantage of using quantile regression under the prohibitive circumstances discussed earlier.

3.12.2 Predictors of Low Plasma Concentration

C_{max} was dichotomized to determine the proportion of participants with low or normal C_{max} using cut-offs set by Peloquin (2002). The cut-offs for low C_{max} were <8µg/ml for rifampicin, <3µg/ml for isoniazid, <20µg/ml for pyrazinamide, and <2µg/ml for ethambutol. Two-tailed independent sample t-test was used to assess the association between low C_{max} and the continuous variables. Fisher's exact test was used for the binary variables as in each case there was a cell count with a frequency of 5 or less.

Logistic regression was used to fit models to determine the strength of the association between low C_{max} and the variables of interest. For the univariable models, each outcome and a predictor were modelled. Predictors with $p<0.20$ were put together to fit the adjusted models.

3.12.3 Association between Low C_{max} and Treatment Outcomes

3.12.3.1 Low C_{max} and Sputum Smear Status at the End of the Intensive Phase

The association between low C_{max} and sputum smear conversion at the end of the intensive phase of treatment were determined using chi square test or Fisher's exact test (for variables with at least 1 cell with a frequency that had fewer than 5 cell counts). Sputum smear conversion was coded "0" if the smear microscopy was positive for acid-fast bacilli (AFBs) and "1" if negative for AFBs. To estimate the strength of the association between low C_{max} and sputum smear conversion, a machine learning algorithm, Lasso for inference was used.

Lasso was originally an acronym for “least absolute shrinkage and selection operator” but is now a word (StataCorp LLC, 2021). It was developed as an unbiased method for selecting variables in a regression model. In high dimensional data analysis, even when the number of predictors exceed the sample size lasso is able to determine the most important predictors while ensuring model interpretability and avoiding multicollinearity (Hastie et al., 2015; Tibshirani, 1996). It achieves these by penalizing regression coefficients associated with less important predictors, promoting sparsity in the coefficient vector, and identifying the strongest relationship with the target variable.

To adapt lasso regression for causal inference, newer approaches have been developed (Belloni et al., 2014a; Belloni & Chernozhukov, 2011). They include double selection solution, partialing-out solution, and cross-fit partialing-out solution (Belloni et al., 2012, 2014b; Chernozhukov et al., 2018). They are optimized solutions for estimating with accuracy the true model that generate the data that they are used to analyse. The cross-fit partialing-out is deemed superior to the others because of the more robust mechanism it uses to estimate coefficients (StataCorp LLC, 2021; Chernozhukov et al., 2018).

The cross-fit partialing-out lasso model was fitted with a poisson link. Robust standard errors were estimated using variance covariate estimator. The choice of the robust poisson model was based on findings that even for binary outcomes, it provides more accurate estimates than the logistic alternative provided it is interpretable given the design of the study (Chen et al., 2018; Gallis & Turner, 2019; Janani et al., 2015). The sputum smear negativity was specified as the outcome and low C_{max} as the exposure for each drug separately. The potential confounders specified as controls (no parameters were estimated for them in the model) were: age, weight,

dietary diversity score, sex, facility where treatment was received, current smoking status, alcohol use status, household size, adherence (<20% of doses missed or ≥20% missed), co-administration of medicines other than the anti-TB drugs, HIV co-infection and diabetes comorbidity. This method was chosen so that all these variables could be adjusted without overfitting the model as would be the case in a regular regression model, given the sample size.

3.12.3.2 Low C_{max} and Treatment outcomes at the End of the Continuation Phase

Treatment outcomes were summarized as proportions for treatment completed, cured, treatment failure, loss to follow up, and died. These were then dichotomized as successful (treatment completed and cured) and unsuccessful (treatment failure, loss to follow up, and death). It was coded "0" for successful and "1" for unsuccessful. The association between treatment outcomes as a binary variable and the potential confounders were tested using Wilcoxon rank sum test for the continuous variables and chi square test or Fisher's exact test for the binary variables. Cross-fit partialing-out lasso regression was used to determine the incidence rate risk of unsuccessful treatment outcomes given low C_{max}, controlling for all the potential confounders. The potential confounders included as controls (no parameters were estimated for them in the model) were: age, weight, dietary diversity score, sex, facility where treatment was received, current smoking status, alcohol use status, household size, adherence (<20% of doses missed or ≥20% missed), co-administration of medicines other than the anti-TB drugs, HIV co-infection and diabetes comorbidity.

3.12.4 Baseline and Longitudinal Risk Factors for Bacteriologic Failure

A bar chart was used to present the number of participants and their sputum smear microscopy results at the end of each month. Bivariate analysis between sputum smear status and the factors of interest were presented for months 0 (baseline), 3 (midline), and 6 (endline).

Given the hierarchical structure of the data, generalized linear mixed effects model was preferred for the regression model to determine the baseline and longitudinal risk factors for bacteriologic failure (Li et al., 2022). The hierarchical structure refers to patients being nested within 5 different hospitals and also having had repeated measurements taken on each at multiple time points. However, fitting the null model yielded very small within-group variability relative to between-group variability, indicating independence between the observations (Schoot, 2017). As such, generalized estimating equations (GEE) using the logit link was used to determine the risk factors. Furthermore, it was a good model to choose because the outcome was binary as opposed to being normally distributed. Also, 7 responses were expected from each subject, and some covariates were time dependent. GEE is an analytical approach that extends the generalized linear models for application in controlling dependence in outcome variables that are collected repeatedly on the same study subjects at different times (Ballinger, 2004; M. Wang, 2014).

In Stata 16, the dataset was declared to be panel data using the "xtset" command. Study participants' unique identifier which was repeated for each observation on a participant was the panel ID variable (clustering variable) and time coded from "0" to "6" was also specified for each of the visits from baseline to endline. The working correlation structure specified for the model was 'independent'. It was chosen for reasons discussed earlier about the lack of dependence determined from the generalized linear mixed effects model. The binomial family was specified for the distribution of the outcome variable. Although a log link was preferred to estimate relative risks, the logit link was chosen because the former could not converge. The variance covariate estimator was used to estimate robust standard errors.

Bacteriologic failure was measured as sputum smear microscopy result of negative coded "0"

and positive coded "1". The variables used to fit the model were time in months measured from "0" at baseline to "6" at endline, weight, age, dietary diversity score, current smoking status, alcohol use, HIV co-infection, diabetes comorbidity, adherence, facility where treatment was received, and low C_{max}. An interaction term of time and body weight was added to determine how bacteriologic failure varies over time with body weight. Another interaction included was between smoking status and alcohol use. Other interactions explored were diabetes comorbidity and coadministration of drugs not related to TB, and HIV co-infection and coadministration of drugs not related to TB. These were to enable an assessment of how medication use may influence the relationship between known risk factors and the outcome. Due to the known correlation between the C_{max} for the different drugs, separate models were fitted for each. However, only rifampicin and isoniazid C_{max} were used since only those two were ingested from baseline to endline. It was assumed that the level of C_{max}, low or normal, remained unchanged for each participant at every observed time point.

3.12.5 Treatment non-adherence trajectories and factors

Group-based trajectory modeling (GBTM) was chosen to identify the developmental trajectories of treatment non-adherence and to determine their risk factors. GBTM is a statistical technique that helps to identify latent subgroups or trajectories within a population based on their developmental patterns over time. It is a powerful approach for understanding heterogeneity in longitudinal data and capturing different patterns, such as stable, increasing, decreasing, or fluctuating trajectories (Nagin, 2005). GBTM can handle missing data and accommodate different repeated measurements but requires a minimum of 3 repeated measurements per study participant. It provides more robust and flexible modeling than traditional methods such as hierarchical modeling and latent curve analysis which often depend on having a qualitative dimension for grouping individuals (Nagin, 2005, 2014).

GBTM makes no assumptions about pre-existing subgroups being homogenous but determines for each participant how the outcome changes over time (the developmental trajectory) and then groups them based on similarity of their developmental trajectories. Having defined the groups the factors that determine membership of the group and those which influence each trajectory can be identified. This helps to identify risk factors associated with specific trajectories and to characterize how changes in these risk factors influence outcomes over time (Nagin & Odgers, 2010). However, GBTM has limitations, such as sensitivity to the number of repeated measurements, potential model misspecification and need for larger sample sizes to detect smaller trajectory groups. It was chosen for the advantages it offers over the others and the possibility to address the limitations.

A stata plugin was used to undertake the GBTM analyses (Jones & Nagin, 2012). This plugin provides estimates of parameters which allow for the determination of the probability of group membership, the predicted trajectory for each group, and the posterior probabilities of group membership.

Fundamental to GBTM is the model specification, which requires determining the number of groups and the shapes of the trajectories. The Bayesian information criterion (BIC) was used for selecting the optimal model. Smaller negative BIC values lower by a difference of 10 or more were considered better. To determine the optimal number of groups and their shapes, 1-group models through to 4-group models were fitted, testing zero-order, linear (first order), quadratic (second order), cubic (third order), and quartic orders iteratively. Based on the DOTS approach to treatment it was expected that non-adherence will be low for a high proportion of study participants. As such, all models fitted included at least one zero-order. The choice of the

best model from models with comparable BIC was made based on the following considerations recommended by Nagin & Odgers, (2010):

(a) preference for a parsimonious model that effectively captured the data patterns;

(b) ensuring a close correspondence between the estimated probabilities of each group and the actual proportions of study members classified to those groups using the maximum posterior probability assignment rule;

(c) Requiring an average posterior probability value greater than 0.7 for each group, indicating a high level of confidence in the assigned group membership;

(d) Ensuring an adequate number of samples in each group to support reliable estimates;

(e) Expecting reasonably narrow confidence intervals, indicating precision in the estimated parameters and

(f) Requiring odds of correct classification, based on the posterior probabilities of group membership, to be greater than 5 for each group, indicating a strong likelihood of accurate classification.

Furthermore, a candidate model was rejected if any of the groups had a membership proportion less than 10%. This was necessary to forestall having groups with small numbers of people.

3.13 Ethical Considerations

A copy of the study protocol was submitted to the Ghana Health Service Ethics Review Committee (GHS-ERC 002/02/21) and another to the KATH Ethics Committee (KATH IRB/AP/023/21 and KATH IRB/CR01/023/22) for ethical clearance before data collection began (Appendix C and E). The ethical approval was renewed a year later since data collection was still ongoing at the time (Appendix D and F). Administrative permission to conduct the study was sought from the Ashanti Regional Health Directorate, Bono Regional Health

Directorate, Bono East Regional Health Directorate, the Kumasi Metropolitan Health directorate, the Asokwa Municipal Health Directorate, the Old Tafo Municipal Health Directorate, the Sunyani Municipal Health Directorate, and Techiman Municipal Health Directorate. Also, the heads of the KATH, Kumasi South Hospital, Manhyia Hospital, Old Tafo Government hospital, South Suntreso Hospital, Sunyani Municipal Hospital, Bono Regional Hospital, Sunyani and Holy Family Hospital, Techiman were informed of the study and their permissions sought.

Recruitment of study participants was done at the chest clinic of each facility. The study background, objectives and what was required of them was explained to eligible participants and their guardians in the case of those less than 18 years. It was further explained to them that participation in the study was voluntary and that they could withdraw at any time without any consequences whatsoever. Those above 18 years who agreed to voluntarily participate in the study were required to sign or thumb print the informed consent forms. The guardians of those less than 18 years were required to sign or thumb print the informed consent form (Appendix A) and the participants themselves required to give their assent. A standard breakfast was provided to the participants on the day blood specimen was obtained. Although there was no direct benefit to them, they were compensated with GHS90 for their time and transportation on the day of blood sampling.

3.13.1 Compliance with COVID-19 Protocols

To reduce the risk of infection with the COVID-19 virus, participants were given face masks and alcohol hand rub. They were required to always maintain a minimum distance of 6 feet with the data collectors. Data collectors were trained on infection prevention and control as part of preparations for the exercise.

3.17 Funding Information

The study was jointly funded by the principal investigator and the Fogarty International Centre of the National Institutes of Health, US under the UG-Florida Academic Partnership project (D43 TW010055).

CHAPTER FOUR

4.0 RESULTS

4.1 Background Characteristics of Participants

A total of 164 participants were recruited into the study. Their background characteristics are described in Table 10. Participants' age ranged from 17 to 75 years. The mean age was 41.3 years (SD = 13.4). Majority of the participants were male (70.1%, 115/164), had up to high school education (66.5%, 109/164), were Christian (72.2%, 117/162), employed (67.5%, 110/164), and married (55.8%, 91/163). More than half of the participants received treatment at the Komfo Anokye Teaching Hospital (56.1%, 92/164), about a quarter at the Holy Family Hospital, Techiman (22.6%, 37/164), and the rest at Kumasi South Hospital (12.8%, 21/164), Suntreso Government Hospital (4.3%, 7/164) and Tafo Government Hospital (4.3%, 7/164). The number of observations reported for each variable varies depending on the presence of missing data. Missing data occurred due to the inability to quantify plasma concentrations, either because the levels were typically too low to measure or because participants were unavailable for sampling due to death or loss to follow-up before the scheduled sampling time.

Table 10: Background characteristics of participants at baseline

Characteristic	Mean (sd)	Frequency	Percent (%)
Age in years (n = 164)	41.3 (13.4)		
Sex (n = 164)			
Female		49	29.9
Male		115	70.1
Highest Educational Level (n = 164)			
No formal Education		27	16.5
Primary School		17	10.4
High School		109	66.5
Tertiary		11	6.7
Religion (n = 162)			
Christian		117	72.2
Muslim		42	25.9
Other*		3	1.9
Employment status (n = 163)			
Unemployed		40	24.5
Employed		110	67.5
Student		10	6.1
Other#		3	1.8
Marital Status (n = 163)			
Unmarried		91	55.8
Married		72	44.2
Facility (n = 164)			
Komfo Anokye Teaching Hospital		92	56.1
Suntreso Government Hospital		7	4.3
Kumasi South Hospital		21	12.8
Tafo Government Hospital		7	4.3
Holy Family Hospital, Techiman		37	22.6

*Includes atheists and another who chose not to disclose; #Includes retirees and apprentices; sd, standard deviation.

4.2 Characterization of Pharmacokinetic Variability

Figure 5 presents the box and whisker plots of the peak plasma concentration and area under the concentration-time curve from time 0 to 4 hours after drug administration ($AUC_{(0-4)}$) of the first-line anti-TB drugs. The median rifampicin C_{max} and $AUC_{(0-4)}$ were 3.93µg/ml (IQR: 1.70 – 5.58) and 6.76µg.h/ml (IQR: 2.84 – 9.36), respectively. For isoniazid, the median C_{max} was 1.54µg/ml (IQR: 0.63 – 2.38) and the median $AUC_{(0-4)}$ was 2.59µg.h/ml (IQR: 1.06 – 3.94). At

least 50% of study participants had a pyrazinamide C_{max} of 24.18µg/ml (IQR: 7.61 – 31.79) and $AUC_{(0-4)}$ of 44.76µg.h/ml (IQR: 13.94 – 58.70), and ethambutol C_{max} of 1.81µg/ml (IQR: 0.56 – 2.50) and $AUC_{(0-4)}$ of 2.91µg.h/ml (IQR: 0.98 – 4.11). All the distributions showed skewness to the right with outliers observed in rifampicin and ethambutol C_{max} and $AUC_{(0-4)}$ and isoniazid C_{max}.

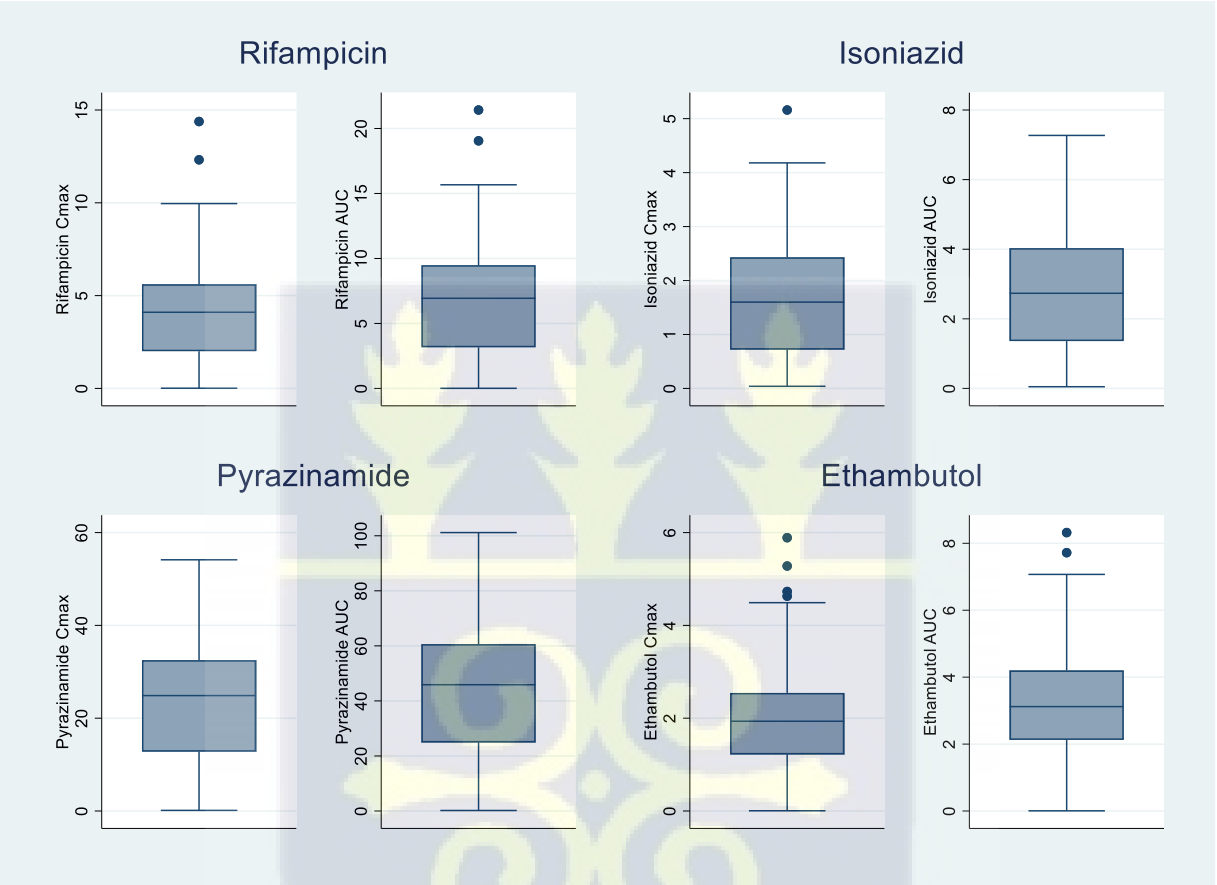


Figure 4: Boxplot distribution of the peak plasma concentration and area under the drug concentration time curve ($AUC_{(0-4)}$) of the first-line anti-TB drugs

The C_{max} levels of the first-line anti-TB drugs are presented in Table 11. The proportion of study participants with low rifampicin C_{max} was 94.4% (136/144) and those with low pyrazinamide C_{max} was 31.3% (45/144). Participants with low C_{max} for exactly one of the drugs were 15 out of the 128 (11.7%) with known C_{max} level for all 4 drugs. Those with low C_{max} for

two of the drugs formed 38.3% (49/128) whereas those with low C_{max} in any three of the drugs formed 24.2% (31/128). Those with low C_{max} in all four drugs formed 22.7% (29/128).

Table 11: Peak Plasma Concentrations of first-line Anti-TB drugs

Drug C_{max}	**Frequency**	**Percent (%)**
Rifampicin (n = 144)		
Low	136	94.4
Normal	8	5.6
Isoniazid (n = 146)		
Low	127	87.0
Normal	19	13.0
Pyrazinamide (n = 144)		
Low	45	31.3
Normal	99	68.7
Ethambutol (n = 136)		
Low	71	52.2
Normal	65	47.8
Number of drugs with low C_{max} (n = 128)		
No drug with low C_{max}	4	3.1
Exactly one drug with low C_{max}	15	11.7
Exactly two drugs with Low C_{max}	49	38.3
Exactly three drugs with Low C_{max}	31	24.2
All four with Low C_{max}	29	22.7

4.2.1 Assessment of the Appropriateness of the Linear Regression Model

The residuals vs fitted plot and histogram of residuals (for rifampicin) are presented in Figure 6 to depict the violation of key assumptions underlying the linear regression for which reason quantile regression was used instead. The plot shows that the spread of the residuals increases with increasing fitted values. This pattern illustrates unequal variance, a violation of the assumption of homoskedasticity. Furthermore, the histogram of residuals shows truncation of the left tail indicating a violation of the normality of residuals assumption.

The non-normality of the distribution of the residuals was confirmed using the Shapiro-Wilk test ($W = 0.97$, $p = 0.009$). None of the transformations explored adequately addressed these violations. Based on the foregoing quantile regression was used.

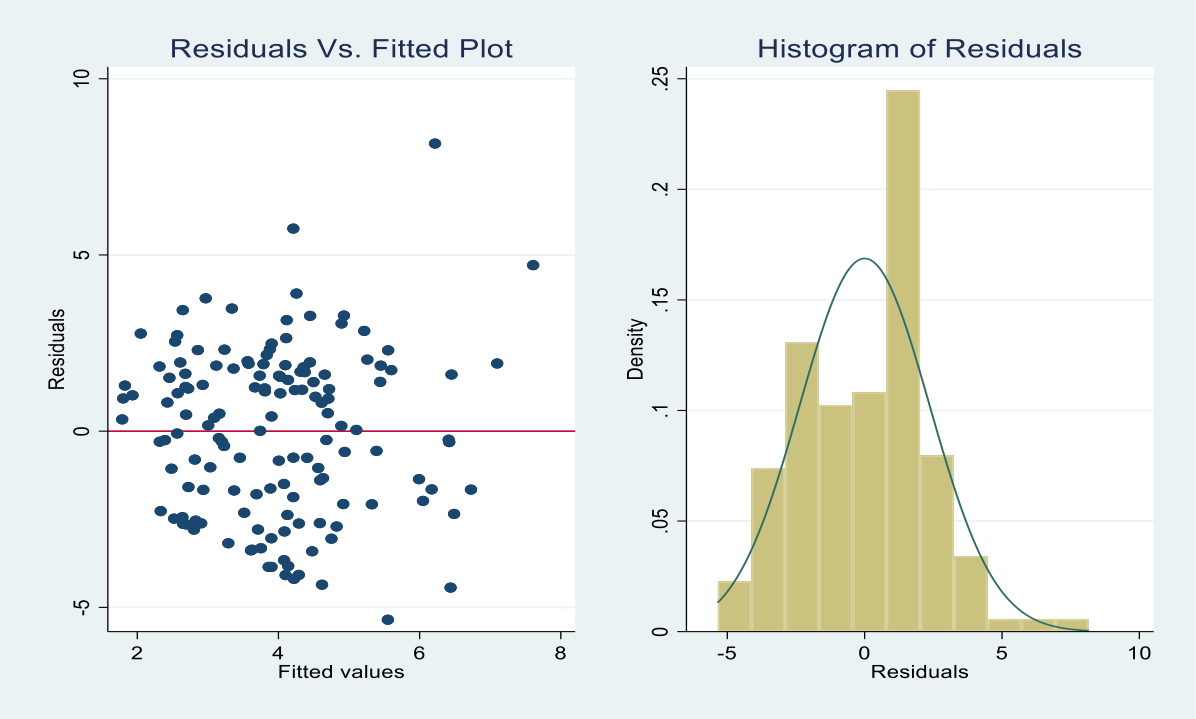


Figure 5: Residuals Vs fitted plot and histogram of residuals from the multivariable linear regression of rifampicin C_{max} on covariates of interest.

4.2.2 Characterization of Rifampicin C_{max} and $AUC_{(0-4)}$

Table 12 presents the results of the bivariate analysis between rifampicin C_{max} $AUC_{(0-4)}$, and covariates of interest. A mildly positive correlation was identified between rifampicin C_{max} and age (spearman correlation coefficient (degrees of freedom) (r(142)) = 0.21, p = 0.010) , as well as with weight (r(142) = 0.18, p = 0.033). For the $AUC_{(0-4)}$, age (r (142) = 0.26, p = 0.002) was significantly correlated but not weight (r(142) = 0.14, p = 0.086). The median C_{max} for females was higher than the median C_{max} for males (5.06 Vs 3.66, p = 0.014). Participants with diabetes had a higher median C_{max} than those without diabetes but this difference was not statistically significant (5.18 vs 3.94, p = 0.054). The difference in median $AUC_{(0-4)}$ between diabetics and non-diabetics was significant (10.07 Vs 6.76, p = 0.014).

Table 12: Results of bivariate analysis of Rifampicin C_{max} and $AUC_{(0-4)}$ and covariates of interest

Characteristic		C_{max}		$AUC_{(0-4)}$	
	n (%)	r / Median (IQR)	p-value	r / Median (IQR)	p-value
Age (years) $^{\phi}$	144 (100.0)	0.21	0.010*	0.26	0.002*
Weight $^{\phi}$	144 (100.0)	0.18	0.033*	0.14	0.086
Dietary diversity $^{\phi}$	143 (100.0)	0.03	0.748	-0.02	0.779
Sex			0.014*		0.015*
Female	40 (27.8)	5.06 (2.77 - 6.33)		8.50 (4.15 - 11.63)	
Male	104 (72.2)	3.66 (1.80 - 5.45)		6.30 (2.91 - 9.02)	
Total	144 (100.0)	4.11 (2.01 - 5.61)		6.94 (3.17 - 9.48)	
Current smoker			0.789		0.657
Non-smoker	133 (95.0)	4.07 (2.02 - 5.59)		6.91 (3.19 - 9.39)	
Smoker	7 (5.0)	5.02 (1.14 - 5.51)		7.80 (1.80 - 8.99)	
Total	140 (100.0)	4.11 (1.99 - 5.59)		6.94 (3.12 - 9.39)	
Alcohol drinker	116		0.180		0.214
Non-drinker	116 (81.1)	4.20 (2.07 - 5.79)		7.07 (3.44 - 9.89)	
Drinker	27 (18.9)	3.12 (0.92 - 5.51)		5.60 (1.37 - 9.08)	
Total	143 (100.0)	4.07 (2.00 - 5.59)		6.91 (3.14 - 9.39)	
Diabetes Co-morbidity			0.054		0.014*
Non-diabetic	134 (93.1)	3.94 (1.90 - 5.58)		6.76 (3.10 - 9.16)	
Diabetic	10 (6.9)	5.18 (4.63 - 6.17)		10.07 (8.18 - 11.75)	
Total	144 (100.0)	4.11 (2.01 - 5.61)		6.94 (3.17 - 9.48)	
HIV co-infection			0.870		0.684
Negative	117 (81.3)	3.94 (2.02 - 5.58)		6.76 (3.40 - 9.75)	
Positive	27 (19.3)	4.35 (1.67 - 5.91)		7.03 (2.82 - 9.06)	
Total	144 (100.0)	4.11 (2.01 - 5.61)		6.94 (3.17 - 9.48)	
Concomitant drug intake			0.159		0.245
No	41 (28.5)	4.91 (2.85 - 6.00)		7.76 (4.52 - 9.89)	
Yes	103 (71.5)	3.65 (1.90 - 5.57)		6.76 (2.93 - 9.33)	
Total	144 (100.0)	4.11 (2.01 - 5.61)		6.94 (3.17 - 9.48)	
Adherence			0.210		0.183
Adherent	135 (93.8)	4.15 (2.01 - 5.69)		7.10 (3.29 - 9.74)	
Non-adherent	9 (6.2)	3.12 - 0.43 - 4.31)		4.72 (0.83 - 7.03)	
Total	144 (100.0)	4.11 (2.01 - 5.61)		6.94 (3.17 - 9.48)	

*p < 0.05; $^{\phi}$spearman rank correlation constant reported; $AUC_{(0-4)}$: Area under concentration-time curve from time 0 to 4 hours after drug administration; C_{max}: peak plasma concentration; IQR, interquartile range, r: spearman's rank correlation coefficient

Table 13 presents the coefficients from the multivariable sequential quantile regression of rifampicin C_{max} on covariates of interest. None of the covariates was significantly associated with rifampicin C_{max} at q0.10. At q0.25, male sex was associated with a 1.39µg/ml (95% CI: -2.74, -0.05) reduction in rifampicin C_{max} and having diabetes was associated with an increase

of 2.84µg/ml (95% CI: 0.15, 5.54). At q0.90 where the effect is significant, the estimates appeared to be stronger compared to that at lower quantiles. They were 2.84µg/ml (95% CI: 3.92, 1.05) for sex and 3.08µg/ml (95% CI: 0.16, 5.99) for diabetes. Age (β = 0.04µg/ml; 95% CI: 0.004, 0.17), weight (β = 0.05µg/ml; 95% CI: 0.02, 0.09), and male sex (β = -1.59µg/ml; 95% CI: -2.78, -0.40) were associated with rifampicin C_{max} at the q0.75. Participants concurrently using medication other than the anti-TB drugs had lower q0.90 rifampicin C_{max} by 1.55 µg/ml (95% CI: -2.76, -0.35) compared to those who were taking only the anti-TB drugs. The multivariable linear regression model predicted significant estimates for age (β = 0.4µg/ml; 95% CI: 0.01, 0.07), weight (β = 0.05µg/ml; 95% CI: 0.01, 0.08) and male sex (β = -1.44µg/ml; 95% CI: -2.48, -0.40) which were all comparable with their corresponding estimates from the quantile regression model.

Table 13: Results of multivariable ordinary least square regression and multivariable sequential quantile regression of rifampicin C_{max} on covariates

	OLS	q0.10	q0.25	q0.50	q0.75	q0.90
Characteristic	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Age (years)	0.04 (0.01, 0.07) *	0.01 (-0.04, 0.05)	-0.002 (-0.07, 0.06)	0.05 (0.002, 0.10) *	0.04 (0.004, 0.07) *	0.05 (0.01, 0.10) *
Weight	0.05 (0.01, 0.08) *	0.04 (-0.03, 0.10)	0.06 (-0.004, 0.12)	0.03 (-0.03, 0.10)	0.05 (0.02, 0.09) *	0.03 (-0.03, 0.09)
Dietary diversity	-0.05 (-0.23, 0.14)	-0.05 (-0.24, 0.14)	-0.12 (-0.48, 0.23)	0.02 (-0.32, 0.35)	-0.10 (-0.32, 0.12)	-0.09 (-0.37, 0.19)
Sex						
Female	Ref	Ref	Ref	Ref	Ref	Ref
Male	-1.44 (-2.48, -0.40) *	-1.29 (-2.96, 0.37)	-1.39 (-2.74, -0.05) *	-0.97 (-2.66, 0.72)	-1.59 (-2.78, -0.40) *	-2.48 (-3.92, -1.05) *
Diabetes Co-morbidity						
Non-diabetic	Ref	Ref	Ref	Ref	Ref	Ref
Diabetic	1.44 (-0.29, 3.16)	1.01 (-1.92, 3.94)	2.84 (0.15, 5.54) *	1.33 (-0.90, 3.56)	1.65 (-0.91, 4.21)	3.08 (0.16, 5.99) *
HIV co-infection						
Negative	Ref	Ref	Ref	Ref	Ref	Ref
Positive	0.39 (-0.92, 1.70)	-0.11 (-1.37, 1.14)	-0.21 (-2.32, 1.90)	1.04 (-1.08, 3.17)	0.28 (-1.25, 1.80)	1.18 (-1.11, 3.48)
Concomitant drug intake						
No	Ref	Ref	Ref	Ref	Ref	Ref
Yes	-1.01 (-2.06, 0.05)	0.50 (-1.81, 0.81)	-0.72 (-2.67, 1.22)	-1.58 (-3.39, 0.23)	-1.17 (-2.37, 0.03)	-1.55 (-2.76, -0.35) *
Adherence						
Adherent	Ref	Ref	Ref	Ref	Ref	Ref
Non-adherent	-1.08 (-2.99, 0.84)	-1.54 (-4.08, 0.99)	-2.74 (-6.15, 0.68)	0.01 (-2.87, 2.88)	-0.94 (-2.68, 0.80)	-1.05 (-3.03, 0.94)
Pseudo R2	0.20^	0.06	0.09	0.10	0.16	0.24

*p<0.05; ^R-squared estimate; β: parameter coefficient; R2: R-squared; CI, confidence interval; OLS, ordinary least square; Ref, reference category.

Notes: The quantiles used for the quantile regression are 0.10, 0.25, 0.50, 0.75, and 0.90, and are denoted by q0.10, q0.25, q0.50, q0.75, and q0.90, respectively.

Figure 7 presents plots of the coefficients of covariates from the multivariable sequential quantile regression (solid blue lines) and their 95% confidence interval (CI) (shaded gray regions) by their respective quantiles with an overlay of the plot of the coefficient of the ordinary least squares (black solid lines) and their 95% CI (red dotted lines). These plots demonstrate how the covariates influence concentrations differently at different quantiles of the C_{max} distribution whereas their effect on the mean C_{max} remains unchanged at the different quantiles. The charts show minimal effects of age at lower quantiles and greater effects at higher quantiles whereas the effect of sex appeared to decrease from lower to higher quantiles. The effects of the other covariates did not vary much from lower to higher quantiles although the reducing effect of the concomitant use of other medication was observable at certain intermediary quantiles.

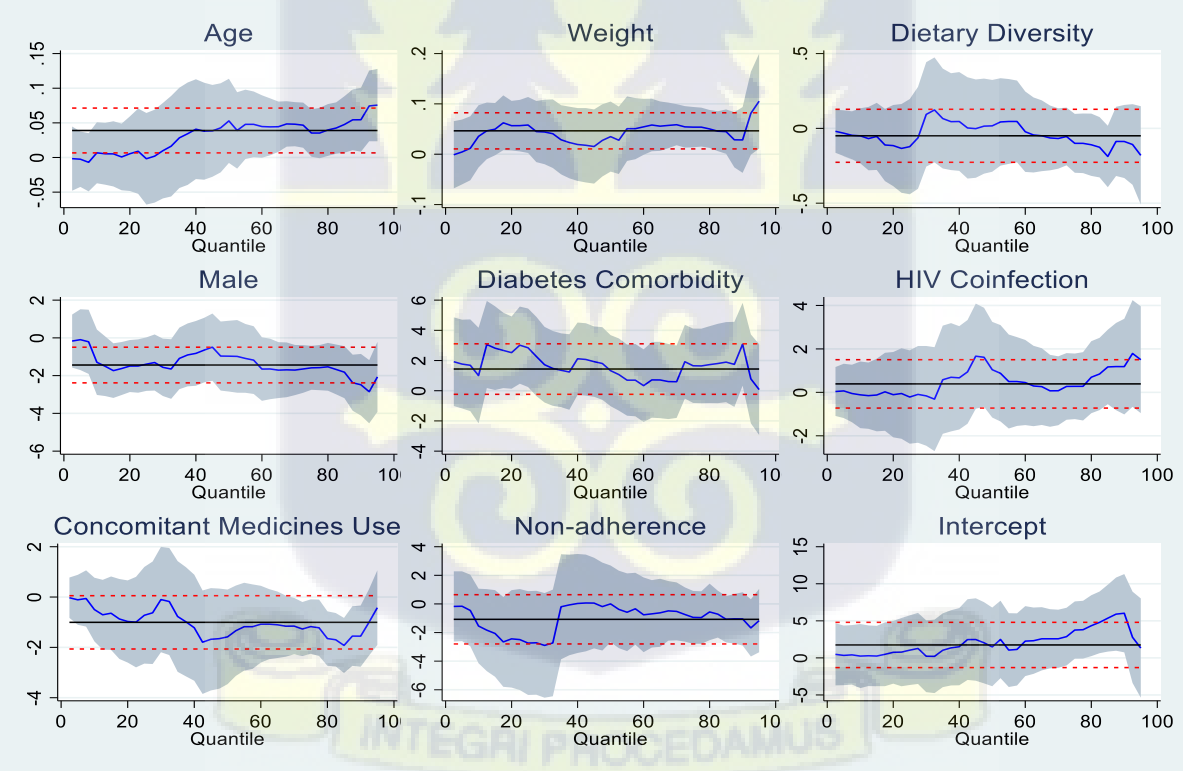


Figure 6: Plot of the sequential quantile regression coefficients and ordinary least squares coefficients of Rifampicin C_{max} on covariates of interest

Notes: sequential quantile regression coefficients (solid blue lines) and their 95% CI (shaded gray regions) by their respective quantiles with an overlay of the plot of the coefficient of the ordinary least squares (black solid lines) and their 95% CI (red dotted lines).

4.2.3 Characterization of Isoniazid C_{max} and $AUC_{(0-4)}$

Table 14 summarizes the results of the bivariate analyses between isoniazid C_{max} and the covariates of interest. The correlation that was found between body weight and isoniazid C_{max} ($r(144) = 0.19$, $p = 0.020$) and $AUC_{(0-4)}$ ($r(144) = 0.16$, $p = 0.047$) were mild. Non-drinkers of alcohol had a higher median C_{max} compared to drinkers (1.78µg/ml Vs 1.19µg/ml, $p = 0.015$). The median isoniazid C_{max} was higher for diabetics than non-diabetics (2.60µg/ml vs 1.55µg/ml, $p = 0.021$). The conditional distribution of the $AUC_{(0-4)}$ had associations with the covariates of interest comparable with covariates' associations with C_{max}.

Table 14: Results of bivariate analysis of Isoniazid C_{max} and $AUC_{(0-4)}$ and covariates of interest

		C_{max}		$AUC_{(0-4)}$	
Characteristic	**n (%)**	**r / Median (IQR)**	**p-value**	**r / Median (IQR)**	**p-value**
Age (years) $^{\phi}$	146 (100.0)	0.10	0.251	0.09	0.297
Weight $^{\phi}$	146 (100.0)	0.19	0.02*	0.16	0.047*
Dietary diversity $^{\phi}$	145 (100.0)	-0.02	0.849	-0.07	0.512
Sex			0.670		0.784
Female	41 (28.1)	1.65 (0.76 - 2.71)		2.90 (1.46 - 4.76)	
Male	105 (61.9)	1.55 (0.72 - 2.36)		2.59 (1.36 - 3.97)	
Total	146 (100.0)	1.60 (0.72 - 2.43)		2.74 (4.03)	
Current smoker			0.734		0.608
Non-smoker	135 (95.1)	1.63 (0.71 - 2.43)		2.76 (1.09 - 4.05)	
Smoker	7 (4.9)	1.44 (1.35 - 1.89)		2.43 (1.92 - 3.15)	
Total	142 (100.0)	1.56 (0.72 - 12.38)		2.65 (1.36 - 3.97)	
Alcohol drinker			0.015*		0.024*
Non-drinker	116 (80.0)	1.78 (0.78 - 2.60)		2.92 (1.47 - 4.26)	
Drinker	29 (20.0)	1.19 (0.56 - 1.80)		1.93 (0.77 - 3.15)	
Total	145 (100.0)	1.57 (0.72 - 2.38)		2.71 (1.36 - 3.97)	
Diabetes Co-morbidity			0.021*		0.020*
Non-diabetic	136 (93.2)	1.55 (0.64 - 2.36)		2.54 (1.08 - 3.79)	
Diabetic	10 (6.8)	2.60 (1.52 - 3.18)		4.93 (2.59 - 5.24)	
Total	146 (100.0)	1.60 (0.72 - 2.43)		2.74 (1.36 - 4.03)	
HIV co-infection			0.263		0.498
Negative	120 (82.2)	1.51 (0.68 - 2.47)		2.59 (1.09 - 4.04)	
Positive	26 (17.8)	1.93 (1.19 - 2.38)		3.01 (1.93 - 3.76)	
Total	146 (100.0)	1.60 (0.72 - 2.43)		2.74 (1.36 - 4.03)	
Concomitant drug intake			0.721		0.979
No	42 (28.8)	1.82 (0.79 - 2.36)		2.91 (1.07 - 3.90)	
Yes	104 (71.2)	1.55 (0.72 - 2.49)		2.59 (1.38 - 4.13)	
Total	146 (100.0)	1.60 (0.72 - 2.43)		2.74 (1.36 - 4.03)	
Adherence			0.358		0.397
Adherent	137 (93.8)	1.57 (0.76 - 2.50)		2.76 (1.39 - 4.05)	
Non-adherent	9 (6.2)	1.68 (0.30 - 1.95)		2.43 (0.56 - 3.32)	
Total	146 (100.0)	1.60 (0.72 - 2.43)		2.74 (1.36 - 4.03)	

*p < 0.05; $^{\phi}$spearman rank correlation constant reported; $AUC_{(0-4)}$: Area under concentration-time curve from time 0 to 4 hours after drug administration; C_{max}: peak plasma concentration; IQR: interquartile range, r: spearman's rank correlation coefficient

From the multivariable sequential quantile regression (Table 15), the median isoniazid C_{max} increased by 0.63µg/ml (95% CI: 0.01, 1.26) among HIV coinfected participants compared to those not coinfected. For every kilogram increase in body weight the 75th percentile isoniazid C_{max} increased by 0.03µg/ml (95% CI: 0.001, 0.05) and alcohol use reduced it by 0.85µg/ml

(95% CI: -1.58, -0.11). The OLS regression findings identified the 3 covariates significant in the sequential quantile regression as being independently associated with the mean isoniazid C_{max}. Body weight increased the mean isoniazid C_{max} by 0.02µg/ml (95% CI: 0.003, 0.03) for each kilogram increase while current alcohol drinkers had a lower mean isoniazid C_{max} by 0.52µg/ml (95% CI: 0.09, 1.05) compared to non-drinkers. For HIV coinfected patients, the OLS estimated a 0.57µg/ml (95% CI: 0.001, 0.05) higher mean isoniazid C_{max} compared to those without HIV.

Table 15: Results of multivariable ordinary least square regression and multivariable sequential quantile regression of isoniazid C_{max}

	OLS	q0.10	q0.25	q0.50	q0.75	q0.90
Characteristic	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Age (years)	0.01 (-0.01, 0.02)	-0.002 (-0.02, 0.01)	-0.01 (-0.02, 0.01)	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.03)	0.01 (-0.01, 0.04)
Weight	0.02 (0.003, 0.03)*	0.0002 (-0.02, 0.02)	0.03 (0.004, 0.05)	0.02 (-0.01, 0.04)	0.03 (0.001, 0.05)*	0.01 (-0.02, 0.04)
Sex						
Female	Ref	Ref	Ref	Ref	Ref	Ref
Male	0.08 (-0.36, 0.52)	0.37 (-0.16, 0.89)	0.17 (-0.43, 0.78)	-0.02 (-0.63, 0.60)	-0.03 (-0.71, 0.66)	001 (-0.72, 0.74)
Current smoker						
Non-smoker	Ref	Ref	Ref	Ref	Ref	Ref
Smoker	0.10 (-0.36, 0.55)	0.15 (-0.89, 1.19)	0.41 (-0.53, 1.35)	0.31 (-0.41, 1.02)	-0.26 (-0.95, 0.44)	-0.66 (-1.60, 0.29)
Alcohol drinker						
Non-drinker	Ref	Ref	Ref	Ref	Ref	Ref
Drinker	-0.52 (-1.00, -0.04)*	-0.47 (-1.01, 0.08)	-0.30 (-0.84, 0.25)	-0.43 (-0.96, 0.09)	-0.85 (-1.58, -0.11)*	-0.99 (-2.12, 0.14)
Diabetes Co-morbidity						
Non-diabetic	Ref	Ref	Ref	Ref	Ref	Ref
Diabetic	0.57 (-0.20, 1.33)	0.61 (-0.14, 1.36)	0.69 (-0.15, 1.52)	0.93 (-0.33, 2.19)	0.24 (-1.15, 1.64)	0.61 (-0.47, 1.69)
HIV co-infection						
Negative	Ref	Ref	Ref	Ref	Ref	Ref
Positive	0.57 (0.09, 1.05)*	0.36 (-0.27, 0.99)	0.50 (-0.20, 1.19)	0.63 (0.01, 1.26)*	0.39 (-0.57, 1.34)	0.46 (-0.33, 1.26)
Concomitant drug intake						
No	Ref	Ref	Ref	Ref	Ref	Ref
Yes	-0.12 (-0.55, 0.30)	0.20 (-0.29, 0.69)	0.07 (-0.50, 0.64)	-0.37 (-1.10, 0.35)	0.06 (-0.51, 0.64)	0.11 (-0.49, 0.72)
Adherence						
Adherent	Ref	Ref	Ref	Ref	Ref	Ref
Non-adherent	-0.29 (-0.99, 0.40)	-0.43 (-1.44, 0.57)	-0.11 (-1.36, 1.15)	0.07 (-0.94, 1.08)	-0.31 (-1.38, 0.75)	0.75 (-1.68, 0.18)
Pseudo R2	^0.14	0.08	0.09	0.09	0.11	0.16

*p<0.05; ^R-squared estimate; β: parameter coefficient; R2: R-squared; CI: confidence interval; OLS: ordinary least square; Ref: reference category.
Notes: The quantiles used for the quantile regression are 0.10, 0.25, 0.50, 0.75, and 0.90, and are denoted by q0.10, q0.25, q0.50, q0.75, and q0.90, respectively

Although age was not significantly associated with the isoniazid C_{max} at any of the quantiles, it showed a mildly increasing effect from lower to higher quantiles whereas male sex had an opposite effect (Figure 8). Sex showed a statistically significant effect at the higher ends of the quantiles suggesting that the OLS could not fit the data well at the extreme highs of the data. Conversely, the effect of alcohol use on isoniazid was observed only at the extreme lows of the quantiles. The plot for smokers showed intermittently significant reducing effects at some intermediate quantiles.

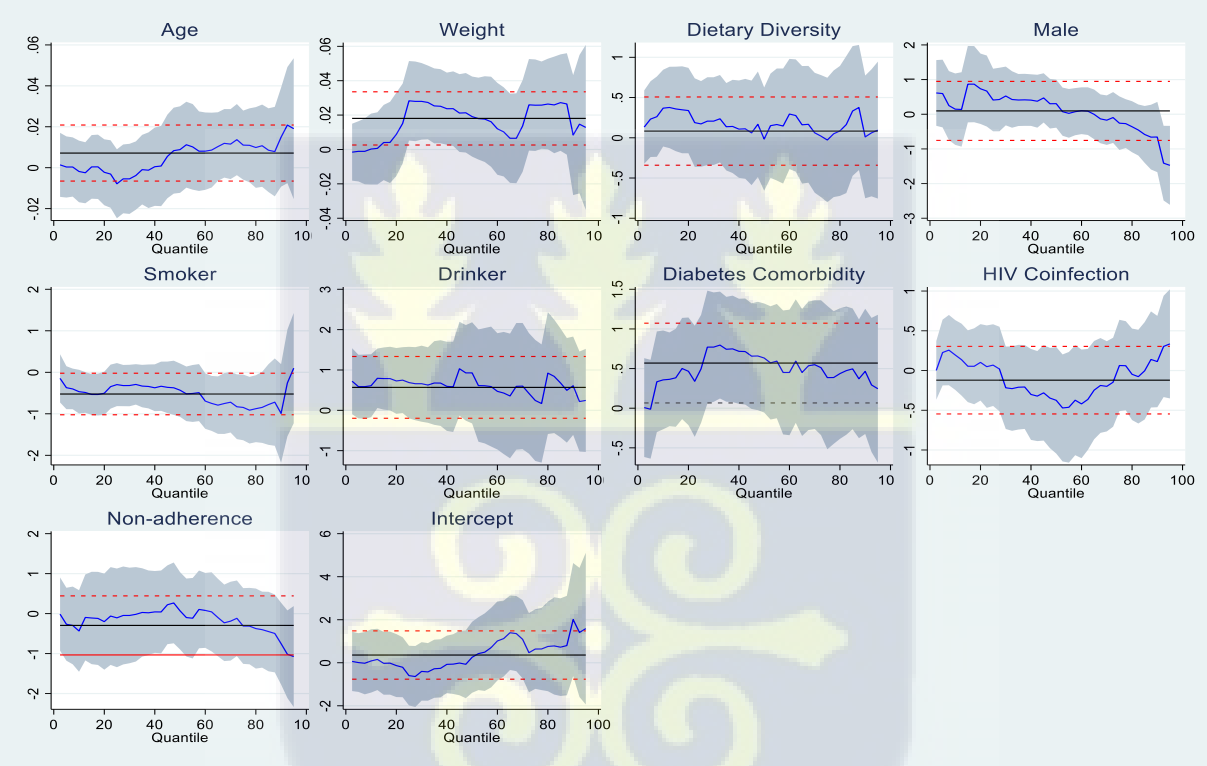


Figure 7: Plot of the sequential quantile regression coefficients and ordinary least squares coefficients of Isoniazid C_{max} on covariates of interest

Notes: sequential quantile regression coefficients (solid blue lines) and their 95% CI (shaded gray regions) by their respective quantiles with an overlay of the plot of the coefficient of the ordinary least squares (black solid lines) and their 95% CI (red dotted lines).

4.2.4 Characterization of Pyrazinamide C_{max} and $AUC_{(0-4)}$

The results of the bivariate analysis of pyrazinamide plasma concentration and covariates of interest are presented in Table 16. There was a statistically significant but mild correlation between pyrazinamide C_{max} and body weight ($r(142) = 0.20$, $p = 0.014$) and also between $AUC_{(0-4)}$ and age ($r(142) = 0.17$, $p = 0.037$) as well as weight ($r(142) = 0.17$, $p = 0.034$). The median C_{max} for females was 28.75µg.h/ml (median = 28.75; 95% CI: 20.16, 37.77) and that for males was 23.92µg.h/ml (median = 23.92; 95% CI: 9.72, 30.71) and this difference was statistically significant.

Table 16: Bivariate analysis of Pyrazinamide C_{max} and $AUC_{(0-4)}$ and covariates of interest

		C_{max}		$AUC_{(0-4)}$	
Characteristic	**n (%)**	**r / Median (IQR)**	**p-value**	**r / Median (IQR)**	**p-value**
Age (years) $^{\phi}$	144 (100.0)	0.15	0.064	0.17	0.037*
Weight $^{\phi}$	144 (100.0)	0.20	0.014*	0.17	0.034*
Dietary diversity $^{\phi}$	143 (100.0)	-0.05	0.550	-0.082	0.333
Sex			0.038*		0.041*
Female	39 (27.1)	28.75 (20.16 - 37.77)		51.66 (37.98 - 66.78)	
Male	105 (62.9)	23.92 (9.72 - 30.71)		44.38 (19.36 - 54.26)	
Total	144 (100.0)	24.87 (12.8 - 32.50)		45.89 (24.93 - 60.64)	
Current smoker			0.479		0.414
Non-smoker	133 (95.0)	24.73 (12.82 - 31.93)		45.35 (25.22 - 59.62)	
Smoker	7 (5.0)	26.78 (22.35 - 34.13)		52.32 (44.21 - 64.42)	
Total	140 (100.0)	24.87 (14.45 - 32.17)		45.89 (25.29 - 60.64)	
Alcohol drinker			0.834		0.888
Non-drinker	117 (81.8)	24.96 (16.08 - 32.40)		46.14 (25.36 - 61.66)	
Drinker	26 (18.2)	24.11 (12.78 - 29.65)		44.88 (24.63 - 53.16)	
Total	143 (100.0)	24.78 (12.78 - 32.40)		44.64 (24.63 - 61.66)	
Diabetes Co-morbidity			0.178		0.200
Non-diabetic	134 (93.1)	24.27 (9.94 - 31.93)		44.88 (19.70 - 59.62)	
Diabetic	10 (6.9)	30.39 (25.59 - 32.65)		55.59 (45.35 - 62.22)	
Total	144 (100.0)	24.87 (12.8 - 32.5)		45.89 (24.93 - 60.64)	
HIV co-infection			0.634		0.961
Negative	117 (81.3)	24.73 (12.78 - 31.64)		46.14 (24.63 - 58.87)	
Positive	27 (18.7)	26.42 (12.82 - 35.01)		45.35 (25.22 - 64.90)	
Total	144 (100.0)	24.87 (12.8 - 32.50)		45.89 (24.93 - 60.64)	
Concomitant drug intake			0.475		0.500
No	42 (29.2)	26.03 (16.60 - 31.64)		46.85 (29.37 - 61.66)	
Yes	102 (70.8)	24.54 (7.40 - 32.65)		45.17 (13.23 - 59.62)	
Total	144 (100.0)	24.87 (12.80 - 32.50)		45.89 (24.93 - 60.64)	
Adherence			0.336		0.355
Adherent	135 (93.8)	25.06 (12.78 - 32.79)		46.35 (24.63 - 62.35)	
Non-adherent	9 (6.2)	22.34 (20.86 - 25.33)		41.39 (25.36 - 48.70)	
Total	144 (100.0)	24.87 (12.80 - 32.50)		45.89 (24.93 - 60.64)	

*: $p < 0.05$; $^{\phi}$ spearman rank correlation constant reported; $AUC_{(0-4)}$: Area under con centration-time curve from time 0 to 4 hours after drug administration; C_{max}: peak plasma concentration; IQR, interquartile range; r: spearman's rank correlation coefficient

Table 17 presents a summary of the coefficients of the multivariable sequential quantile regression and of the multivariable linear regression of the pyrazinamide C_{max} on covariates of interest. None of the covariates of interest was significant in the multivariable linear regression model. Participants taking medicines other than the anti-TB drugs had a lower tenth C_{max} percentile by 5.68µg/ml (95% CI: -11.64, -0.09) compared to those who were not on any other

medication. The q0.25 C_{max} was higher by 13.12µg/ml (95% CI: 0.68, 25.57) among diabetics compared to non-diabetics. It decreased by 7.93µg/ml (95% CI: -15.70, -0.17) among those on medications other than the anti-TB drugs compared to those not on any other medicines. Like the mean C_{max} modeled by the linear regression model, the median C_{max} was unaffected by any of the covariates included in the model. A 1-year increase in age increased the q0.75 C_{max} by 0.15µg/ml (95% CI: 0.003, 0.29) and the q0.90 C_{max} by 0.20µg/ml (95% CI: 0.05, 0.36) whereas non-adherence to the anti-TB regimen accounted for a reduction of 9.70µg/ml (95% CI: -15.89, -3.51) in the q0.90 C_{max}.

Table 17: Results of multivariable ordinary least square regression and multivariable sequential quantile regression of pyrazinamide C_{max}

	OLS	q0.10	q0.25	q0.50	q0.75	q0.90
Characteristic	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Age (years)	0.16 (-0.01, 0.33)	0.20 (-0.08, 0.47)	0.29 (-0.11, 0.68)	0.07 (-0.17, 0.30)	0.15 (0.003, 0.29) *	0.20 (0.05, 0.36) *
Weight	0.16 (-0.02, 0.34)	0.07 (-0.17, 0.32)	0.24 (-0.08, 0.55)	0.16 (-0.14, 0.46)	0.24 (-0.07, 0.56)	0.08 (-0.28, 0.44)
Sex						
Female	Ref	Ref	Ref	Ref	Ref	Ref
Male	-5.24 (-10.52, 0.04)	-4.20 (-12.21, 3.81)	-10.61 (-21.35, 0.13)	-2.46 (-7.74, 2.81)	-4.69 (-11.53, 2.16)	-3.69 (-10.74, 3.36)
Current smoker						
Non-smoker	Ref	Ref	Ref	Ref	Ref	Ref
Smoker	7.18 (-4.30, 18.66)	3.04 (-16.28, 22.35)	10.03 (-9.60, 29.67)	2.50 (-12.21, 17.20)	4.67 (-15.30, 24.64)	17.67 (-5.23, 40.58)
Alcohol drinker						
Non-drinker	Ref	Ref	Ref	Ref	Ref	Ref
Drinker	0.73 (-5.59, 7.06)	1.28 (-6.47, 9.03)	0.87 (-11.13, 12.87)	-1.03 (-9.31, 7.24)	-0.56 (-6.56, 5.45)	-0.43 (-11.16, 10.29)
Diabetes Co-morbidity						
Non-diabetic	Ref	Ref	Ref	Ref	Ref	Ref
Diabetic	3.63 (-4.05, 11.31)	-0.73 (-24.46, 23.01)	13.12 (0.68, 25.57) *	3.09 (-3.06, 9.24)	-2.93 -9.47, 3.62)	1.63 (-8.62, 11.90)
HIV co-infection						
Negative	Ref	Ref	Ref	Ref	Ref	Ref
Positive	0.97 (-5.86, 7.81)	-1.48 (-10.09, 7.12)	4.67 (-5.35, 14.68)	1.25 (-7.52, 10.02)	2.35 (-5.03, 9.73)	2.22 (-5.34, 9.78)
Concomitant drug intake						
No	Ref	Ref	Ref	Ref	Ref	Ref
Yes	3.24 (-8.27, 1.80)	-5.86 (-11.64, -0.09) *	-7.93 (-15.70, -0.17) *	-0.89 (-8.13, 6.34)	0.21 (-5.03, 5.45)	-3.83 (-12.49, 4.82)
Adherence						
Adherent	Ref	Ref	Ref	Ref	Ref	Ref
Non-adherent	-2.86 (-11.66, 5.94)	-4.81 (-26.70, 17.08)	5.01 (-16.65, 26.68)	-0.53 (-9.69, 8.62)	-2.97 (-10.20, 4.25)	-9.70 (-15.89, -3.51) *
Pseudo R2	**0.10**	**0.03**	**0.11**	**0.05**	**0.08**	**0.11**

*p<0.05; ^R: squared estimate; β: parameter coefficient; R2: R-squared; CI, confidence interval; OLS, ordinary least square; Ref, reference category.

Notes: The quantiles used for the quantile regression are 0.10, 0.25, 0.50, 0.75, and 0.90, and are denoted by q0.10, q0.25, q0.50, q0.75, and q0.90, respectively.

Figure 9 presents a plot of the coefficients of the covariates from the multivariable sequential quantile regression against the quantiles of the distribution of the pyrazinamide C_{max} with an overlay of the coefficients from the ordinary least square regression. Age, diabetes comorbidity, HIV coinfection, and non-adherence appeared to have more precise estimates at higher quantiles although only age and non-adherence showed some significant effects at those quantiles. The effect of diabetes comorbidity seemed to reduce from lower to higher quantiles. A similar observation pertained for non-adherence to the anti-TB regimen.

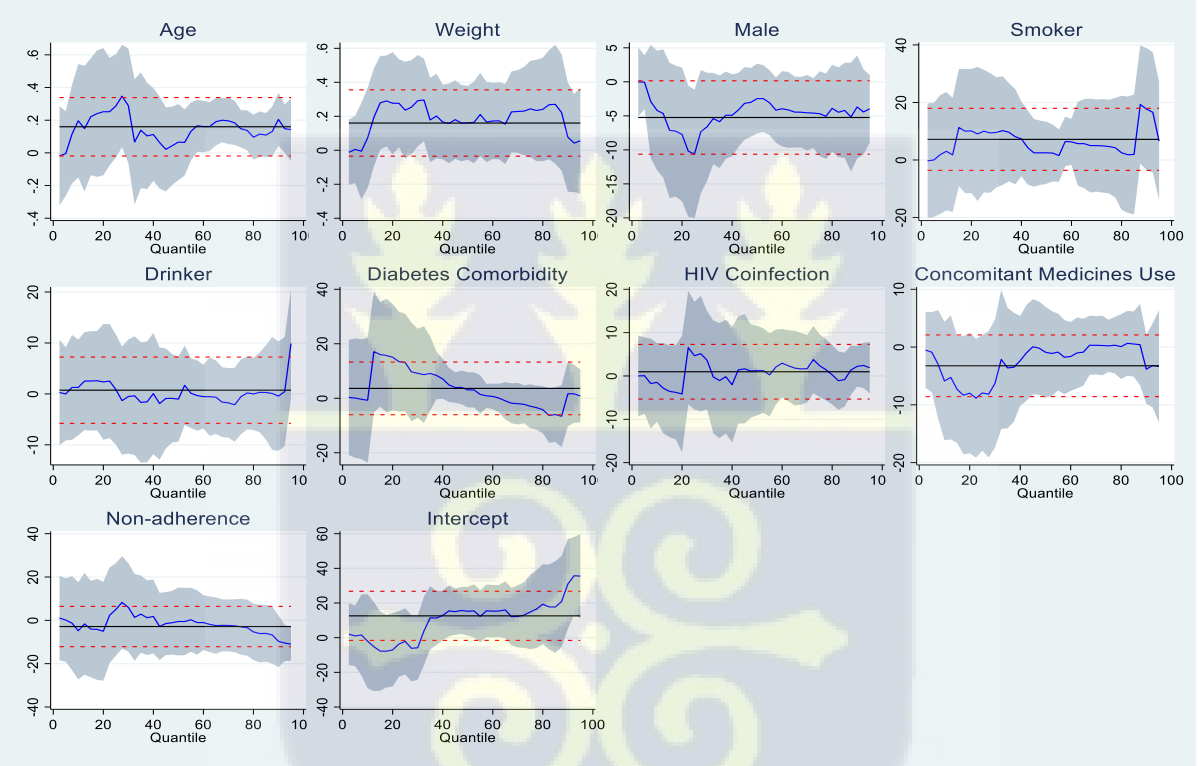


Figure 8: Plot of the sequential quantile regression coefficients and ordinary least squares coefficients of Pyrazinamide C_{max} on covariates of interest

Notes: sequential quantile regression coefficients (solid blue lines) and their 95% CI (shaded gray regions) by their respective quantiles with an overlay of the plot of the coefficient of the ordinary least squares (black solid lines) and their 95% CI (red dotted lines).

4.2.5 Characterization of Ethambutol C_{max} and $AUC_{(0-4)}$

The association between the median C_{max} and $AUC_{(0-4)}$ of ethambutol and covariates of interest are presented in Table 18. Age was mildly correlated with ethambutol C_{max} ($r(134) = 0.30$, $p < 0.001$) and also with $AUC_{(0-4)}$ ($r(134) = 0.31$, $p < 0.001$). Neither the apparently mild association between weight and C_{max} ($r(134) = 0.15$, $p = 0.090$) nor $AUC_{(0-4)}$ ($r(134) = 0.11$, $p = 0.218$) was significant. However, those with diabetes (3.97µg.h/ml (95% CI: 3.60, 4.85)) had a higher median $AUC_{(0-4)}$ than those without diabetes (3.05µg.h/ml (95% CI: 1.98, 4.19)).

Table 18: Bivariate analysis of ethambutol C_{max} and $AUC_{(0-4)}$ and covariates of interest

		C_{max}		AUC(0-4)	
Characteristic	**n (%)**	**r / Median (IQR)**	**p-value**	**r / Median (IQR)**	**p-value**
Age (years) $^{\phi}$	136 (100.0)	0.30	<0.001 *	0.31	<0.001*
Weight (kg) $^{\phi}$	136 (100.0)	0.15	0.090	0.11	0.218
Dietary diversity $^{\phi}$	135 (100.0)	-0.001	0.995	-0.1	0.257
Sex			0.358		0.398
Female	37 (27.2)	2.16 (1.36 - 2.57)		3.47 (2.40 - 4.38)	
Male	99 (62.2)	1.89 (1.13 - 2.53)		3.09 (1.95 - 4.15)	
Total	136 (100.0)	1.94 (1.22 - 2.55)		3.12 (2.13 - 4.20)	
Current smoker			0.071		0.104
Non-smoker	126 (95.5)	1.89 (1.23 - 2.50)		3.10 (2.19 - 4.19)	
Smoker	6 (4.5)	2.65 (2.21 - 3.30)		4.01 (3.89 - 5.56)	
Total	132 (100.0)	1.94 (1.25 - 2.55)		3.14 (2.22 - 4.20)	
Alcohol drinker			0.772		0.796
Non-drinker	111 (82.2)	1.89 (1.23 - 2.53)		3.11 (2.06 - 4.22)	
Drinker	24 (17.8)	2.07 (1.18 - 2.64)		3.42 (2.11 - 4.06)	
Total	135 (100.0)	1.92 (1.21 - 2.56)		3.11 (2.06 - 4.21)	
Diabetes Co-morbidity			0.098		0.031*
Non-diabetic	127 (93.4)	1.89 (1.15 - 2.53)		3.05 (1.98 - 4.19)	
Diabetic	9 (6.6)	2.17 (1.96 - 2.70)		3.97 (3.60 - 4.85)	
Total	136 (100.0)	1.94 (1.22 - 2.55)		3.12 (2.13 - 4.20)	
HIV co-infection			0.194		0.451
Negative	112 (82.4)	1.89 (1.14 - 2.53)		3.12 (1.99 - 4.17)	
Poisitive	24 (17.6)	2.24 (1.47 - 2.74)		3.29 (2.52 - 4.60)	
Total	136 (100.0)	1.94 (1.22 - 2.55)		3.12 (2.13 - 4.20)	
Concomitant drug intake			0.684		0.812
No	42 (30.9)	1.99 (1.36 - 2.58)		3.31 (1.95 - 4.04)	
Yes	94 (69.1)	1.89 (1.21 - 2.53)		3.05 (2.19 - 4.38)	
Total	136 (100.0)	1.94 (1.22 - 2.55)		3.12 (2.13 - 4.20)	
Adherence			0.363		0.203
Adherent	127 (93.4)	1.95 (1.27 - 2.57)		3.16 (2.25 - 4.23)	
Non-adherent	9 (6.6)	1.40 (0.96 - 2.49)		2.65 (1.61 - 3.64)	
Total	136 (100.0)	1.94 (1.22 - 2.55)		3.12 (2.13 - 4.20)	

*p < 0.05; $^{\phi}$spearman rank correlation constant reported; $AUC_{(0-4)}$: Area under concentration-time curve from 0 to 4 hours after drug administration; C_{max}: peak plasma concentration; IQR: interquartile range; r: spearman's rank correlation coefficient

Age was positively correlated with ethambutol C_{max}. This was observed from q0.25 (β = 0.03, 95% CI: 0.0003, 0.05) through q0.90 (β = 0.05, 95% CI: 0.03, 0.07) but not q0.10 (β = 0.004, 95% CI: -0.02, 0.03). Whereas the multivariable sequential quantile regression model did not find a significant association between ethambutol C_{max} and smoking at any quantile, the multivariable linear regression model determined that the mean ethambutol C_{max} increased by

1.05µg/ml (95% CI: 0.18, 1.92) among current smokers compared to non-smokers. Alcohol users had a lower q0.90 C_{max} by 0.64µg/ml (95% CI: -1.24, -0.05) compared to non-users. At low quantiles (q0.10: $\beta = 1.61$, 95% CI: 0.67, 2.54; q0.25: $\beta = 0.91$, 95% CI: 0.10, 1.72) those with diabetes comorbidity had a higher C_{max} than those without diabetes (Table 19).

Table 19: Results of multivariable ordinary least square regression and multivariable sequential quantile regression of ethambutol C_{max}

	OLS	q0.10	q0.25	q0.50	q0.75	q0.90
Characteristic	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**	**β (95% CI)**
Age (years)	0.03 (0.01, 0.04) *	0.004 (-0.02, 0.03)	0.03 (0.0003, 0.05) *	0.02 (0.004, 0.03) *	0.04 (0.01, 0.06) *	0.05 (0.03, 0.07) *
Sex						
Female	Ref	Ref	Ref	Ref	Ref	Ref
Male	-0.29 (-0.78, 0.19)	-0.27 (-0.99, 0.45)	-0.02 (-0.85, 0.82)	0.17 (-0.57, 0.92)	-0.14 (-0.63, 0.35)	-0.23 (-0.93, 0.46)
Current smoker						
Non-smoker	Ref	Ref	Ref	Ref	Ref	Ref
Smoker	1.05 (0.18, 1.92) *	0.89 (-0.54, 2.33)	0.95 (-0.54, 2.44)	0.91 (-0.22, 2.03)	1.02 (-0.18, 2.22)	0.71 (-0.33, 1.74)
Alcohol drinker						
Non-drinker	Ref	Ref	Ref	Ref	Ref	Ref
Drinker	-0.12 (-0.61, 0.37)	0.22 (-0.73, 1.17)	0.34 (-0.82, 1.51)	-0.28 (-0.88, 0.31)	-0.16 (-0.75, 0.42)	-0.64 (-1.24, -0.05) *
Diabetes Co-morbidity						
Non-diabetic	Ref	Ref	Ref	Ref	Ref	Ref
Diabetic	0.51 (-0.19, 1.22)	1.61 (0.67, 2.54) *	0.91 (0.10, 1.72) *	0.46 (-0.25, 1.17)	-0.02 (-1.29, 1.26)	0.57 (-1.53, 2.67)
HIV co-infection						
Negative	Ref	Ref	Ref	Ref	Ref	Ref
Positive	0.37 (-0.14, 0.88)	0.04 (-1.25, 1.33)	0.81 (-0.15, 1.77)	0.67 (-0.05, 1.40)	0.48 (-0.09, 1.06)	-0.05 (-0.74, 0.63)
Concomitant drug intake						
No	Ref	Ref	Ref	Ref	Ref	Ref
Yes	-0.25 (-0.69, 0.19)	-0.21 (-0.98, 0.56)	-0.42 (-1.29, 0.45)	-0.31 (-0.76, 0.14)	-0.56 (-1.10, -0.02) *	-0.25 (-0.84, 0.34)
Adherence						
Adherent	Ref	Ref	Ref	Ref	Ref	Ref
Non-adherent	-0.23 (-0.97, 0.51)	-0.06 (-1.33, 1.21)	-0.23 (-1.58, 1.12)	-0.15 (-0.89, 0.60)	0.10 (-0.67, 0.88)	-0.29 (-0.92, 0.33)
Pseudo R2	**0.19**	**0.10**	**0.07**	**0.11**	**0.11**	**0.23**

*p<0.05; ^R: squared estimate; β: parameter coefficient; R2: R-squared; CI, confidence interval; OLS, ordinary least square; Ref, reference category.

Notes: The quantiles used for the quantile regression are 0.10, 0.25, 0.50, 0.75, and 0.90, and are denoted by q0.10, q0.25, q0.50, q0.75, and q0.90, respectively.

The plot of the coefficients of the covariates from the multivariable sequential quantile regression against the quantiles of the distribution of the ethambutol C_{max} with an overlay of the coefficients from the ordinary least square regression are shown in Figure 10. The significant effect of age and alcohol use were observed at the extreme highs of the quantiles while that of diabetes comorbidity were observed at the extreme lows. Age had a preponderance for increasing the C_{max} going from low to high quantiles. Smoking had effects at certain low and high quantiles.

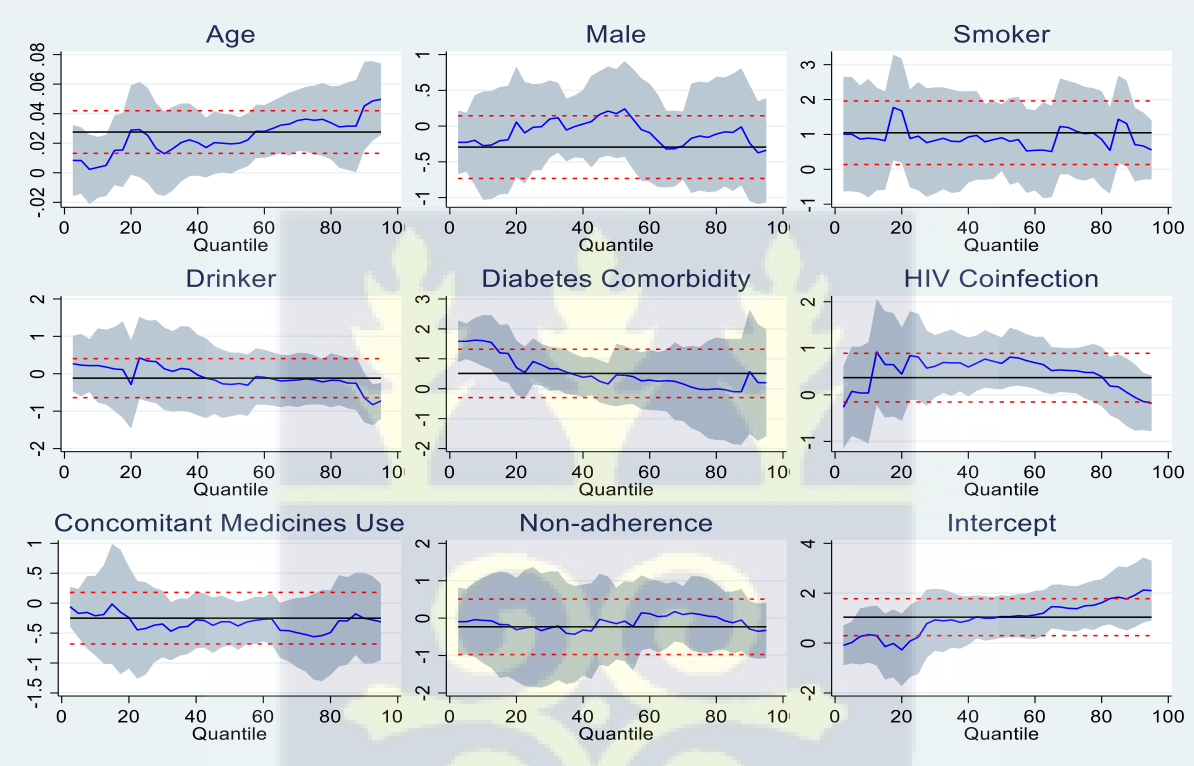


Figure 9: Plot of the sequential quantile regression coefficients and ordinary least squares coefficients of ethambutol C_{max} on covariates of interest

Notes: sequential quantile regression coefficients (solid blue lines) and their 95% CI (shaded gray regions) by their respective quantiles with an overlay of the plot of the coefficient of the ordinary least squares (black solid lines) and their 95% CI (red dotted lines).

4.3 Predictors of Low Peak Plasma Concentration of Anti-TB drugs

The association between the covariates of interest and low C_{max} for rifampicin and isoniazid are summarized in Table 20. The mean age of participants with normal rifampicin C_{max} was higher than those with low rifampicin C_{max} (54.9 (95% CI: 46.9, 62.9) vs 41.3 (95% CI: 39.0,

43.5), p = 0.004). On the other hand, the difference between the mean age of those with normal isoniazid C_{max} and those with low C_{max} was not significant statistically (45.8 (9% CI: 39.5, 52.1) vs 41.2 (38.9, 43.5), p = 0.161). Similarly, the mean weight of participants with normal rifampicin C_{max} was 66.8 kg (95% CI: 53.1, 80.5) and that for those with low rifampicin C_{max} was 54.9kg (95% CI: 53.1, 56.7) (p = 0.004). In terms of sex, the prevalence of low rifampicin C_{max} was higher among males (98.1%) than females (85.0%). No other associations were significant between low rifampicin or isoniazid C_{max} and the other covariates.

Table 20: Bivariate analysis of low rifampicin and isoniazid C_{max} and covariates of interest

Characteristic	Rifampicin C_{max} < 8µg/ml			Isoniazid C_{max} < 3µg/ml		
	Low	Normal	p-value	Low	Normal	p-value
Age (years) $^{\phi}$	41.3 (39.0, 43.5)	54.9 (46.9, 62.9)	0.004*	41.2 (38.9, 43.5)	45.8 (39.5, 52.1)	0.161
Weight (kg) $^{\phi}$	54.9 (53.1, 56.7)	66.8 (53.1, 80.5)	0.004*	55.2 (53.2, 57.2)	60.2 (54.6, 65.8)	0.079
Dietary diversity $^{\phi}$	6.8 (6.3, 7.2)	5.6 (2.9, 8.3)	0.224	6.8 (6.4, 7.3)	5.7 (4.5, 6.9)	0.094
Sex			0.006*			0.173
Female	34 (85.0)	6 (15.0)		33 (80.5)	8 (19.5)	
Male	102 (98.1)	2 (1.9)		94 (89.5)	11 (10.5)	
Current smoker			1.000			0.596
Non-smoker	126 (94.7)	7(5.3)		117 (86.7)	18 (13.3)	
Smoker	7 (100.0)	0 (0.0)		7 (100.0)	0 (0.0)	
Alcohol drinker			1.000			0.53
Non-drinker	109 (100.0)	7 (6.0)		100 (86.2)	16 (13.8)	
Drinker	26 (96.3)	1 (3.7)		27 (93.1)	2 (6.9)	
Diabetes Co-morbidity			0.446			0.124
Non-diabetic	127 (94.8)	7 (5.2)		120 (88.2)	16 (11.8)	
Diabetic	9 (90.0)	1 (10.0)		7 (70.0)	3 (30.0)	
HIV co-infection			0.644			0.335
Negative	111 (94.9)	6 (5.1)		106 (88.3)	14 (11.7)	
Positive	25 (92.6)	2 (7.4)		21 (80.8)	5 (19.2)	
Concomitant drug intake			0.440			0.277
No	40 (97.6)	1 (2.4)		39 (92.9)	3 (7.1)	
Yes	96 (93.2)	7 (6.8)		88 (84.6)	16 (15.4)	
Adherence			1.000			0.606
Adherent	127 (94.1)	8 (5.9)		118 (86.1)	19 (13.9)	
Non-adherent	9 (100.0)	0 (0.0)		9 (100.0)	0 (0.0)	

*p<0.05; $^{\phi}$ mean (95% CI) reported; C_{max}: peak plasma concentration

The crude and adjusted odds ratios from the logistic regression analyses involving low rifampicin C_{max} and selected covariates are presented in Table 21. Every year increase in age reduced the odds of low rifampicin C_{max} by 8.0% (COR = 0.92, 95% CI: 0.87, 0.97, p = 0.001) but by 12.0% (AOR = 0.88, 95% CI: 0.82, 0.94, p < 0.001) after adjusting for weight and sex. A 1kg increase in weight was linked with a 6.0% (AOR = 0.94, 95% CI: 0.89, 0.99, p = 0.022) reduction in the odds of low rifampicin C_{max} after accounting for age and sex. Males had a 22 times higher odds of low rifampicin C_{max} compared to females (AOR = 21.65, 95% CI: 3.43, 136.70, p = 0.001).

Table 21: Univariable and multivariable logistic regression between low rifampicin C_{max} and covariates of interest

Characteristics	COR (95% CI)	p-value	AOR (95% CI)	p-value
Age (years)	0.92 (0.87, 0.97)	0.001 *	0.88 (0.82, 0.94)	< 0.001 *
Weight (kilograms)	0.93 (0.88, 0.99)	0.017 *	0.94 (0.89, 0.99)	0.022 *
Sex				
Female	1		1	
Male	9.00 (1.72, 46.98)	0.009 *	21.65 (3.43, 136.70)	0.001 *

*p < 0.05, AOR: adjusted odds ratio; COR: crude odds ratio

Table 22 presents the output of the univariable and multivariable logistic regression of low isoniazid C_{max} on age, weight, dietary diversity scores, sex and diabetes comorbidity. Although increasing weight appeared to reduce the odds of low isoniazid C_{max}, neither that association nor any other that was assessed was significant by the crude and adjusted odds ratios.

Table 22: Univariable and multivariable logistic regression between low isoniazid C_{max} and covariates of interest

Characteristics	COR (95% CI)	p-value	AOR (95% CI)	p-value
Age (years)	0.97 (0.94, 1.01)	0.148	0.98 (0.94, 1.02)	0.368
Weight (kilograms)	0.97 (0.93, 1.00)	0.054	0.97 (0.94, 1.00)	0.054
Dietary diversity	1.17 (0.97, 1.42)	0.093	1.16 (0.94, 1.44)	0.154
Sex				
Female	1		1	
Male	2.07 (0.76, 5.61)	0.152	2.05 (0.74, 5.71)	0.169
Diabetes Comorbidity				
Non-diabetic	1		1	
Diabetic	0.31 (0.07, 1.33)	0.116	0.43 (0.09, 2.17)	0.310

*p < 0.05, AOR: adjusted odds ratio; COR: crude odds ratio

Table 23 presents the associations between the covariates of interest and low C_{max} for pyrazinamide and ethambutol. Statistically significant associations were found between low pyrazinamide C_{max} and body weight, and for low ethambutol C_{max}, with age and weight. As regards body weight, those with normal C_{max} had a bigger mean weight than those with low C_{max} (pyrazinamide: 57.3kg (95% CI: 55.0, 59.7) Vs 53.0kg (95% CI: 49.8, 56.2), p = 0.038; ethambutol: 58.4kg (95% CI: (55.5, 61.4) Vs 54.4kg (95% CI: 51.7, 57.0), p = 0.045). Those with normal ethambutol C_{max} had a higher mean age than those with low ethambutol C_{max} (44.1 (95% CI: 40.7, 47.6) vs 38.7 (95% CI: 36.0, 41.4), p = 0.045).

Table 23: Bivariate analysis of low pyrazinamide and ethambutol C_{max} and covariates of interest

Characteristic	Pyrazinamide C_{max} < 20µg/ml			Ethambutol C_{max} < 2µg/ml		
	Low	Normal	p-value	Low	Normal	p-value
Age (years) $^{\phi}$	40.0 (36.8, 43.2)	41.7 (39.0, 44.5)	0.457	38.7 (36.0, 41.4)	44.1 (40.7, 47.6)	0.014 *
Weight (kg) $^{\phi}$	53.0 (49.8, 56.2)	57.3 (55.0, 59.7)	0.038 *	54.4 (51.7, 57.0)	58.4 (55.5, 61.4)	0.045 *
Dietary diversity $^{\phi}$	6.62 (25.8, 7.4)	6.8 (6.2, 7.3)	0.747	6.6 (6.0, 7.2)	6.8 (6.1, 7.5)	0.759
Sex			0.229			0.442
Female	9 (23.1)	30 (76.9)		17 (46.0)	20 (54.0)	
Male	36 (34.3)	69 (65.7)		54 (54.5)	45 (45.5)	
Current smoker			0.438			0.103
Non-smoker	42 (31.6)	91 (68.4)		68 (54.0)	58 (46.0)	
Smoker	1 (14.3)	6 (85.7)		1 (16.7)	5 (83.3)	
Alcohol drinker			0.816			0.824
Non-drinker	36 (30.8)	81 (69.2)		59 (53.2)	52 (46.8)	
Drinker	9 (34.6)	17 (65.4)		12 (50.0)	12 (50.0)	
Diabetes Co-morbidity			0.725			0.310
Non-diabetic	43 (32.1)	91 (67.9)		68 (53.5)	59 (46.5)	
Diabetic	2 (20.0)	8 (80.0)		3 (33.3)	6 (66.7)	
HIV co-infection			0.646			0.122
Negative	38 (32.5)	79 (67.5)		62 (55.4)	50 (44.6)	
Positive	7 (25.9)	20 (74.1)		9 (37.5)	15 (62.5)	
Concomitant drug intake			0.436			0.853
No	11 (26.2)	31 (73.8)		21 (50.0)	21 (50.0)	
Yes	34 (33.3)	68 (66.7)		50 (53.2)	44 (46.8)	
Adherence			0.720			1.000
Adherent	43 (31.9)	92 (68.1)		66 (52.0)	61 (48.0)	
Non-adherent	2 (22.2)	7 (77.8)		5 (55.6)	4 (44.4)	

*p<0.05; $^{\phi}$ mean (95% CI) reported; C_{max}: peak plasma concentration

Only weight met the criteria for the low pyrazinamide C_{max} multivariable logistic regression model building. A 1kg increase in weight reduced the odds of low pyrazinamide C_{max} by 4% (OR = 0.96, 95% CI: 0.93, 0.99, p = 0.045).

Age and weight had comparable effects on low ethambutol C_{max} both before and after adjustment of the factors of interest (Table 24). The odds of low ethambutol C_{max} was reduced by 3% for each year increase in age (AOR = 0.97, 95% CI: 0.94, 0.99, p = 0.022). For each

kilogram increase in body weight the odds of low ethambutol reduced by 3% (OR = 0.97, 95% CI: 0.93, 0.99, p = 0.047). Smokers had 86% lower odds of low ethambutol C_{max} compared to non-smokers (AOR = 0.14, 95% CI: 0.02, 1.12, p = 0.064) after adjusting for the factors of importance but this effect seemed marginal.

Table 24: Univariable and multivariable logistic regression between low ethambutol C_{max} and covariates of interest

Characteristics	COR (95% CI)	p-value	AOR (95% CI)	p-value
Age (years)	0.97 (0.94, 0.99)	0.016 *	0.97 (0.94, 0.99)	0.022 *
Weight (kilograms)	0.97 (0.94, 0.99)	0.048 *	0.97 (0.93, 0.99)	0.047 *
Current smoker				
Non-smoker	1		1	
Smoker	0.17 (0.02, 1.51)	0.112	0.14 (0.02, 1.12)	0.064
HIV Co-infected				
Negative	1		1	
Positive	0.48 (0.19, 1.20)	0.118	0.42 (0.16, 1.13)	0.086

*p< 0.05

4.4 Association between Low concentration of Anti-TB drugs and treatment outcomes

4.4.1 Sputum Smear Conversion after Intensive Phase

A summary of the association and incidence rate ratio between sputum smear conversion and low C_{max} of the anti-TB drugs at the end of month 2 are presented in Table 25. At the end of the intensive phase, there was no difference in the prevalence of positive sputum smear status between participants with low C_{max} and those with normal C_{max} for all the drugs. The number of drugs with low concentration levels was determined for participants with all 4 drugs having known C_{max} alone. Number of drugs with low concentration was not associated with sputum smear status at the end of the intensive phase. After controlling for age, sex, body weight, dietary diversity, current smoking status, alcohol use status, household size, adherence,

concomitant use of other medicines, HIV coinfection, diabetes comorbidity and facility where treatment was provided, participants with low rifampicin C_{max} were 19.0% less likely to have sputum smear conversion than those with normal rifampicin C_{max} (IRR = 0.81, 95% CI: 0.73, 0.89, $p < 0.001$). For pyrazinamide, although low C_{max} appeared to increase the incidence of sputum smear conversion in comparison with normal C_{max}, this difference was not statistically significant (IRR = 1.18, 95% CI: 0.97, 1.44, p = 0.098) after adjusting for potential confounders. There was no evidence that having low plasma concentrations in exactly 1 (IRR = 1.01, 95% CI: 0.70, 1.45, p = 0.978), 2 (IRR = 1.02, 95% CI: 0.82, 1.26, p = 0.864), 3 (IRR = 0.87, 95% CI: 0.64, 1.18, p = 0.359), or all 4 (IRR = 1.02, 95% CI: 0.78, 1.35, p = 0.863) of the drugs had an association with unsuccessful outcomes.

Table 25: Bivariate analysis of the low C_{max} of anti-TB drugs and sputum smear status and Incidence Rate Ratio of sputum smear conversion at the end of the intensive phase

Drug	Sputum Smear Status				Smear Conversion	
	Negative n(%)	Positive n(%)	Total n(%)	p-value	IRR (95% CI)	p-value
Rifampicin				1.000		< 0.001*
Normal C_{max}	5 (83.3)	1 (16.7)	6 (100.0)		1	
Low C_{max}	90 (77.6)	26 (22.4)	116 (100.0)		0.81 (0.73, 0.89)	
Isoniazid				1.000		0.621
Normal C_{max}	12 (80.0)	3 (20.0)	15 (100.0)		1	
Low C_{max}	86 (78.2)	24 (21.8)	110 (100.0)		0.93 (0.71, 1.23)	
Pyrazinamide				0.493		0.098
Normal C_{max}	64 (77.1)	19 (22.9)	83 (100.0)		1	
Low C_{max}	33 (82.5)	7 (17.5)	40 (100.0)		1.18 (0.97, 1.44)	
Ethambutol				0.768		0.847
Normal C_{max}	44 (78.6)	12 (21.4)	56 (100.0)		1	
Low C_{max}	45 (76.3)	14 (23.7)	59 (100.0)		0.98 (0.79, 1.21)	
Exactly one drug with low C_{max}				0.455		0.978
None or more than 1 with Low C_{max}	75 (77.3)	22 (22.7)	97 (100.0)		1	
Only one drug with low C_{max}	7 (63.4)	4 (36.6)	11 (100.0)		1.01 (0.70, 1.45)	
Exactly two drugs with Low C_{max}				0.754		0.864
More or less than 2 with low C_{max}	11 (73.3)	4 (26.7)	15 (100.0)		1	
Any two drugs with low C_{max}	71 (76.3)	22 (23.7)	93 (100.0)		1.02 (0.82, 1.26)	
Exactly three drugs with Low C_{max}				1.000		0.359
More or less than 3 with low C_{max}	45 (78.9)	12 (21.1)	57 (100.0)		1	
Any three drugs with low C_{max}	50 (78.1)	14 (21.9)	64 (100.0)		0.87 (0.64, 1.18)	
All four with Low C_{max}				0.602		0.863
At least 1 with normal C_{max}	64 (77.1)	19 (22.9)	83 (100.0)		1	
All four with low C_{max}	18 (72.0)	7 (28.0)	25 (100.0)		1.02 (0.78, 1.35)	

*p< 0.005; C_{max}: peak plasma concentration

4.4.2 Treatment Outcomes after Continuation Phase

Successful treatment outcomes were observed in 80.5% (132/164) of study participants with 62.2% (102/164) being cured and 18.3% (30/164) categorized as treatment completed. Those lost to follow up were 10.4% (17/164) of all participants. Four (2.4%) died, 11 (6.7%) failed treatment and no one was in the "not evaluated" category (0.00%).

Dietary diversity and adherence to the treatment regimen were significantly associated with treatment outcomes from the bivariate analysis between treatment outcomes and factors of interest (Table 26). Specifically, those with more diverse diets were more likely to have successful outcomes than those with less diverse diets. On adherence to the regimen, whereas 26.9% of those who missed at least 20% of their daily doses had successful treatment outcomes, 93.9% of those who adhered to more than 80% of their doses had successful outcomes. This difference was statistically significant ($p < 0.001$) (Table 26).

Table 26: Bivariate analysis of treatment outcomes by factors of interest

Characteristics	Successful outcome	Unsuccessful outcome	p-value
Age $^{\phi}$	41.0 (31.5 - 50.5)	42.0 (31.5 - 48.5)	0.713
Weight $^{\phi}$	58.0 (51.5 – 64.0)	56.0 (46.0 – 62.0)	0.236
Dietary Diversity $^{\phi}$	7.0 (5.6 - 9.5)	3.2 (1.7 - 5.6)	< 0.001 *
Household size $^{\phi}$	5.0 (3.0 – 7.0)	4.0 (3.0 - 7.5)	0.788
Sex (n = 164)			0.536
Female	38 (77.6)	11 (22.5)	
Male	94 (81.7)	21 (18.3)	
Current smoker (n = 160)			0.824
Non-smoker	122 (80.8)	29 (19.2)	
Smoker	7 (77.8)	2 (22.2)	
Alcohol drinker (n = 163)			0.336
Non-drinker	108 (81.8)	24 (18.2)	
Drinker	23 (74.2)	8 (25.8)	
Diabetes Co-morbidity (n = 164)			0.908
Non-diabetic	123 (80.4)	30 (19.6)	
Diabetic	9 (81.8)	2 (18.2)	
HIV co-infection (n = 164)			0.664
Negative	107 (79.9)	27 (20.2)	
Positive	25 (83.3)	5 (16.7)	
Concomitant drug intake (n = 164)			0.535
No	83 (79.1)	22 (20.9)	
Yes	49 (83.0)	10 (17.0)	
Adherence (n = 157)			< 0.001 *
Adherent	123 (93.9)	8 (6.1)	
Non-adherent	7 (26.9)	19 (73.1)	

*p<0.05; $^{\phi}$ median (IQR) reported.

Rifampicin (p = 0.351) and isoniazid (p = 0.503) did not differ in treatment outcomes by normal and low levels of C_{max} (Table 27). The proportion of participants with successful treatment outcomes among those with normal pyrazinamide C_{max} (92.9%) was higher compared to the proportion with successful outcomes among those with low pyrazinamide C_{max} (62.2%). Similarly, the prevalence of successful treatment outcomes among participants with normal ethambutol C_{max} was higher compared to the prevalence among those with low ethambutol C_{max} (93.8% vs. 74.7%, p = 0.002). The proportion of participants having exactly 2 (21.5% vs 6.1%, p = 0.024) or 4 drugs (44.8% vs 7.1%, p = <0.001) with low C_{max} who experienced unsuccessful outcomes was higher compared to the others.

The association between low rifampicin C_{max} and unsuccessful treatment outcomes was not assessed beyond the bivariate analysis since normal C_{max} perfectly predicted successful outcomes (Table 27). Low C_{max} of pyrazinamide and ethambutol were significantly associated with unsuccessful treatment outcomes with or without lost to follow up included as an outcome. The factors adjusted for in this relationship were facility where treatment was received, age, dietary diversity, sex, current smoking status, alcohol drinking status, household size, adherence to regimen, concomitant use of medication other than the anti-TB drugs, HIV coinfection, and diabetes comorbidity. With all outcomes included those with low pyrazinamide C_{max} had a 5.92-fold higher rate of unsuccessful outcomes relative to those with normal pyrazinamide C_{max} (IRR = 5.92; 95% CI: 12.72, 12.88; $p < 0.001$). Without loss to follow up in the outcomes, the adjusted rate of unsuccessful outcomes was 9.11 times higher among those with low C_{max} compared to those with normal C_{max} (IRR = 9.11; 95% CI: 1.66, 50.07; $p = 0.011$).

Low ethambutol C_{max} increased the rate of unsuccessful outcomes 5.74 times (95% CI: 1.88, 17.53) compared to normal C_{max} after adjustment of potential confounders. Having low C_{max} for 1 (IRR = 1.56, 95% CI: 0.27, 9.12, $p = 0.621$ (for all outcomes and when lost-to-follow up is excluded from outcomes)), 2 (IRR = 0.20, 95% CI: 0.02, 1.71, $p = 0.143$ (for all outcomes and when lost-to-follow up is excluded from outcomes)), or 3 drugs (IRR = 0.35, 95% CI: 0.08, 1.42, $p = 0.142$ (for all outcomes); IRR = 0.27, 95% CI: 0.04, 2.04, $p = 0.206$ (for outcomes which exclude lost-to-follow up) was not associated with unsuccessful outcomes. However, having low C_{max} for 4 drugs (IRR = 12.40, 95% CI: 2.20, 69.76, $p = 0.004$ (for all outcomes and when lost-to-follow up was excluded from outcomes) was significantly associated with poor outcomes.

Table 27: Bivariate analysis of treatment outcomes by C_{max} of anti-TB drugs and Incidence rate ratio of treatment outcomes at the end of the continuation phase

Characteristics	Treatment Outcomes				All outcomes included		Lost-to-follow up excluded	
	Successful n (%)	Unsuccessful n (%)	Total, N(%)	p-value	IRR (95% CI)	p-value	IRR (95% CI)	p-value
Rifampicin (n = 144)				0.351				
Normal	8 (100.0)	0 (0.0)	8 (100.0)		-Ψ		-Ψ	
Low	111 (81.6)	25 (18.4)	136 (100.0)		-Ψ		-Ψ	
Isoniazid (n = 146)				0.503		0.971		0.370
Normal	15 (78.9)	4 (21.1)	19 (100.0)		1		1	
Low	108 (85.0)	19 (15.0)	1127 (100.0)		0.98 (0.31, 3.12)		2.11 (0.41, 10.73)	
Pyrazinamide (n = 144)				<0.001 *		< 0.001 *		0.011 *
Normal	92 (92.9)	7 (7.1)	99 (100.0)		1		1	
Low	28 (62.2)	17 (37.8)	45 (100.0)		5.92 (2.72, 12.88)		9.11 (1.66, 50.07)	
Ethambutol (n = 136)				0.002 *		0.002 *		0.002 *
Normal	61 (93.8)	4 (6.2)	65 (100.0)		1		1	
Low	53 (74.7)	18 (25.3)	71 (100.0)		5.74 (1.88, 17.53)		5.73 (1.88, 17.44)	
Exactly one drug with low C_{max} (n = 128)				1.000		0.621		0.621
None or more than 1 with low C_{max}	95 (84.1)	18 (15.9)	113 (100.0)		1		1	
Only one drug with low C_{max}	13 (86.7)	2 (13.3)	15 (100.0)		1.56 (0.27, 9.12)		1.56 (0.27, 9.12)	
Exactly two drugs with Low C_{max} (n = 128)				0.736		0.143		00.143
More or less than 2 with low C_{max}	17 (89.5)	2 (10.5)	19 (100.0)		1		1	
Any two drugs with low C_{max}	91 (83.5)	18 (16.5)	109 (100.0)		0.20 (0.02, 1.71)		0.20 (0.02, 1.71)	
Exactly three drugs with Low C_{max} (n = 128)				0.007 *		0.142		0.206
More or less than 3 with low C_{max}	63 (92.7)	5 (7.3)	68 (100.0)		1		1	
Any three drugs with low C_{max}	45 (75.0)	15 (25.0)	60 (100.0)		0.35 (0.08, 1.42)		0.27 (0.04, 2.04)	
All four with Low C_{max} (n = 128)				<0.001 *		0.004 *		0.004*
At least 1 with normal C_{max}	92 (92.9)	7 (7.1)	99 (100.0)		1		1	
All four with low C_{max}	16 (55.2)	13 (44.8)	29 (100.0)		12.40 (2.20, 69.76)		12.40 (2.20, 69.76)	

*p < 0.05; Ψ Not estimated because normal concentration perfectly predicts successful outcomes; C_{max}, peak plasma concentration

4.5 Baseline and Longitudinal Risk Factors for Bacteriologic Failure

The proportion of participants with sputum smear positivity reduced consistently from the baseline (95.5%, 146/156) to the third month (1.9%, 2/106) of treatment after which it increased marginally to month 6 (9.8%, 11/112). The lowest number of positive cases was observed at the third month (2/104, 1.9%). The sputum smear negativity rate of 4.5% (7/156) at baseline increased sharply to 98.1% (104/106) at month 3 after which it remained fairly stable with a marginal reduction to 90.2% (101/112) at the end point (Figure 11).

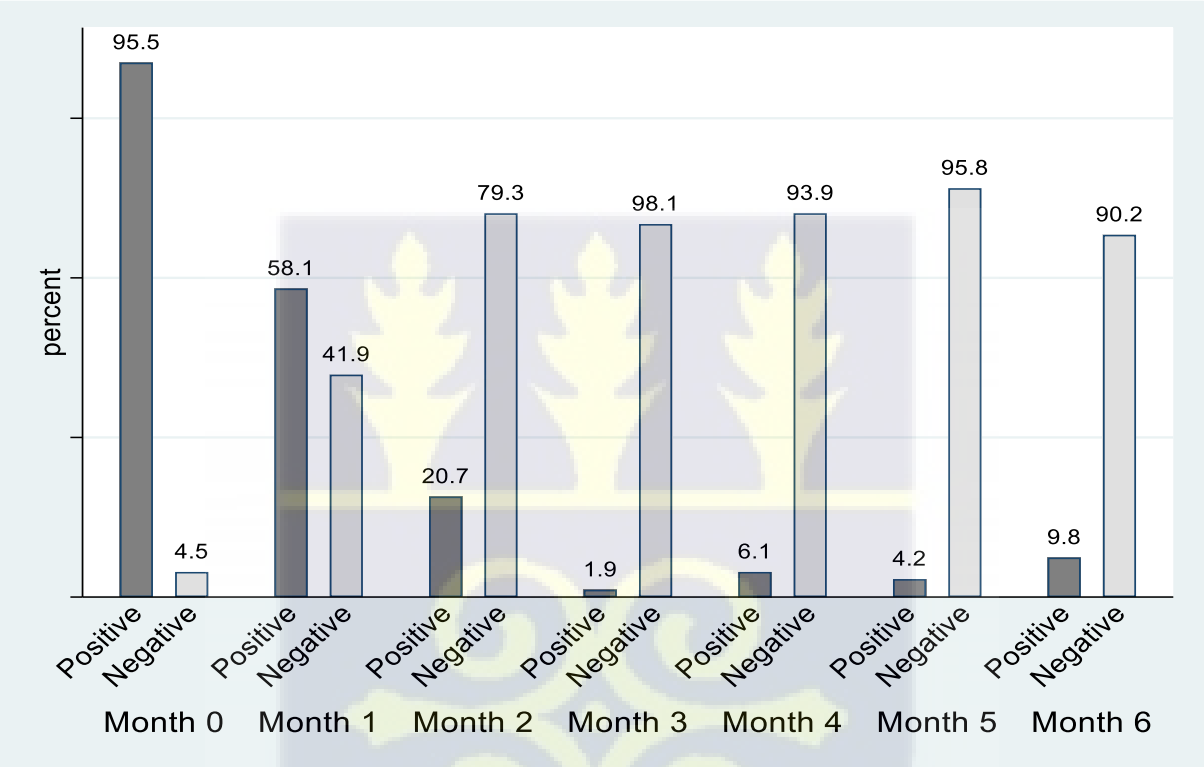


Figure 10: Bar chart of the number of sputum smear positive and negative cases at baseline and the end of each month during treatment

NB: The y-axis scale is not uniform across the bar charts for different months. Instead, it is adjusted individually for each month to appropriately display rate of positive relative to the rate of negativity for that specific month.

The association between smear positivity and factors of interest assessed at baseline, midline and endline are presented in Table 28 and Table 29. A significant association was found between smear positivity and treatment site ($p = 0.019$) at baseline and low pyrazinamide C_{max} ($p = 0.001$) at month 6 (Table 29). For the pyrazinamide C_{max}, the prevalence of smear

positivity was 26.7% among participants with low pyrazinamide C_{max} and 3.7% among those with normal pyrazinamide C_{max} (Table 29). No other factors were associated with smear positivity at the time points assessed although adherence was close to statistical significance at months 3 ($p = 0.057$) and 6 ($p = 0.064$), and low ethambutol C_{max} at month 6 ($p = 0.060$).

Table 28: Bivariate analysis of sputum smear status by factors of interest at Months 0, 3, and 6 during treatment with anti-TB medication

Characteristics	Month 0			Month 3			Month 6		
	Negative	Positive	p-value	Negative	Positive	p-value	Negative	Positive	p-value
Age ϕ	44.0 (41.0 - 46.0)	42.0 (31.0 - 50.0)	0.459	40.0 (31.5 - 47.0)	46.0 (31.0 - 61.0)	0.719	40.0 (32.0 - 47.0)	42.0 (33.0 - 62.0)	0.639
Weight ϕ	50.0 (49.0 - 56.2)	54.0 (47.0 - 60.0)	0.518	56.0 (50.3 - 64.4)	48.5 (37.0 - 60.0)	0.371	58.0 (52.0 - 64.0)	56.0 (46.0 - 62.0)	0.198
Dietary Diversity ϕ	8.0 (6.0 - 10.0)	6.0 (4.0 - 9.0)	0.123	8.0 (6.0 - 10.0)	7.0 (4.0 - 10.0)	0.722	8.0 (5.0 - 10.0)	10.0 (6.0 - 10.0)	0.563
Household size ϕ	5.0 (2.0 - 10.0)	5.0 (3.0 - 7.0)	0.887	4.0 (3.0 - 6.0)	4.5 (2.0 - 7.0)	0.824	5.0 (3.0 - 7.0)	4.0 (2.0 - 8.0)	0.487
Sex			0.675			0.063			0.503
Female	1 (2.2)	45 (97.8)		25 (92.6)	2 (7.4)		31 (93.9)	2 (6.1)	
Male	6 (5.4)	104 (94.6)		79 (100.0)	0 (0.0)		70 (88.6)	9 (11.4)	
Current smoker			0.250			1.000			0.450
Non-smoker	6 (4.1)	140 (95.9)		96 (98.0)	2 (2.0)		93 (91.2)	9 (8.8)	
Smoker	1 (16.7)	5 (83.3)		7 (100.0)	0 (0.0)		5 (83.3)	1 (16.7)	
Alcohol drinker			0.621			1.000			0.414
Non-drinker	5 (4.0)	120 (96.0)		85 (97.7)	2 (2.3)		83 (91.2)	8 (8.8)	
Drinker	2 (6.7)	28 (93.3)		18 (100.0)	0 (0.0)		17 (85.0)	3 (15.0)	
Diabetes Co-morbidity			0.436			1.000			1.000
Non-diabetic	6 (4.2)	138 (95.8)		98 (98.0)	2 (2.0)		93 (89.4)	11 (10.6)	
Diabetic	1 (8.3)	11 (91.7)		6 (100.0)	0 (0.0)		8 (100.0)	0 (0.0)	
HIV co-infection			0.353			1.000			0.455
Negative	7 (5.5)	121 (94.5)		84 (97.7)	2 (2.3)		79 (88.8)	10 (11.2)	
Positive	0 (0.0)	28 (100.0)		20 (100.0)	0 (0.0)		22 (95.6)	1 (4.4)	

ϕ median (IQR) reported

Table 29: Bivariate analysis of sputum smear status by factors of interest at Months 0, 3, and 6 during treatment with anti-TB medication

Characteristics	Month 0			Month 3			Month 6		
	Negative	Positive	p-value	Negative	Positive	p-value	Negative	Positive	p-value
Concomitant drug intake			0.678			0.220			0.532
No	2 (5.3)	36 (94.7)		48 (96.0)	2 (4.0)		62 (88.6)	8 (11.4)	
Yes	5 (4.2)	113 (95.8)		56 (100.0)	0 (0.0)		39 (92.9)	3 (7.1)	
Adherence			NA			0.057			0.064
Adherent	NA	NA		101 (99.0)	1 (1.0)		97 (92.4)	8 (7.6)	
Non-adherent	NA	NA		2 (66.7)	1 (33.3)		3 (60.0)	2 (40.0)	
Facility			0.019			0.062			0.448
KATH	3 (3.3)	88 (96.7)		51 (100.0)	0 (0.0)		57 (91.9)	5 (8.1)	
SGH	0 (0.0)	6 (100.0)		3 (75.0)	1 (25.0)		3 (75.0)	1 (25.0)	
KSH	2 (11.1)	16 (88.9)		12 (100.0)	0 (0.0)		9 (81.8)	2 (18.2)	
TGH	2 (33.3)	4 (66.7)		5 (100.0)	0 (0.0)		5 (100.0)	0 (0.0)	
HFH	0 (0.0)	35 (100.0)		33 (97.1)	1 (2.9)		27 (90.0)	3 (10.0)	
Rifampicin			0.984			1.000			0.604
Normal	1 (3.7)	26 (96.3)		13 (100.0)	0 (0.0)		12 (100.0)	0 (0.0)	
Low	6 (4.7)	123 (95.4)		91 (97.8)	2 (2.2)		89 (89.0)	11 (11.0)	
Isoniazid			0.647			0.328			1.000
Normal	2 (5.9)	32 (94.1)		18 (94.7)	1 (5.3)		17 (89.5)	2 (10.5)	
Low	5 (4.1)	117 (95.9)		86 (98.8)	1 (1.2)		84 (90.3)	9 (9.7)	
Pyrazinamide			1.000			1.000			0.001 *
Normal	5 (4.5)	106 (95.5)		74 (97.4)	2 (2.6)		79 (96.3)	3 (3.7)	
Low	2 (4.4)	43 (95.6)		30 (100.0)	0 (0.0)		22 (73.3)	8 (26.7)	
Ethambutol			0.138			0.500			0.060
Normal	6 (6.8)	82 (93.2)		56 (96.5)	2 (3.5)		59 (95.2)	3 (4.8)	
Low	1 (1.5)	67 (98.5)		48 (100.0)	0 (0.0)		42 (84.0)	8 (16.0)	

P < 0.05; $^{\phi}$ median (IQR) reported; HFH, Holy Family Hospital, Techiman; KATH, Komfo Anokye Teaching hospital; KSH, Kumasi South Hospital; SGH, Suntreso Government Hospital; TGH, Tafo Government Hospital; NA, not applicable as medication administration is yet to start

The results of the generalized estimating equation analysis showing baseline and longitudinal factors and their association with sputum smear positivity with separate adjustments for the C_{max} of each of the anti-TB drugs are presented in Table 30. The baseline factors which were associated with smear positivity were smoking status, drinking status, and facility from which treatment was provided. The longitudinal factors on the other hand were body weight, dietary diversity, concomitant use of medicines other than the anti-TB drugs, and rifampicin C_{max}. Regardless of the model, the odds of smear positivity reduced monthly by 2% for every 1kg increase in body weight (AOR = 0.98; 95% CI: 0.97, 0.98; $p < 0.001$). An interaction effect was identified between baseline smoking and alcohol use and another between concomitant medication use and diabetes comorbidity for both models. The interaction effect between concomitant medication use and HIV coinfection was significant in the model that adjusted for rifampicin C_{max}.

From the model which had adjustment for the rifampicin C_{max}, participants who drank alcohol but did not smoke had a 5-fold (AOR = 5.07; 95% CI: 1.43, 17.96; $p < 0.012$) higher odds of smear positivity whereas those who neither drank alcohol nor smoked were 3.5 times (AOR = 3.47; 95% CI: 1.04, 11.54; $p < 0.043$) more likely to be smear positive compared to those who drank alcohol and smoked. If they smoked but did not drink alcohol, the odds of smear positivity was not significantly different from those who drank alcohol and smoked.

There was no participant that was diabetic who was not on other medication. For those who were diabetic and on other medication, and those who were non-diabetic and on other medication, the odds of smear positivity was lower by 53% (AOR = 0.47; 95% CI: 0.26, 0.85; $p = 0.012$) and 69% (AOR = 0.31; 95% CI: 0.09, 0.99; $p = 0.047$), respectively, compared to those who were neither diabetic nor on other medicines.

All participants who were HIV positive had medication other than the anti-TB drugs but they were omitted from the model due to multicollinearity. Those who were not HIV positive but took other medicines were 1.8 (AOR = 1.83; 95% CI: 1.08, 3.09; p = 0.024) times more likely to be smear positive compared to those who did not have HIV and did not take any other medicines.

There were differences in the odds of smear positivity between the hospitals. Participants who received care at the Suntreso Government hospital (AOR = ; 5.34% CI: 1.11, 25.84; p = 0.037) and Kumasi South hospital (AOR = 2.46; 95% CI: 1.13, 5.33; p = 0.023) had higher odds of being smear positive compared to those at KATH. Those seen at Tafo Government hospital (AOR = 0.25; 95% CI: 0.07, 0.85; p = 0.026) had lower odds of smear positivity than those at KATH. Bacteriologic failure at the Holy Family hospital (AOR = 1.28; 95% CI: 0.63, 2.63; p = 0.493) was no different relative to KATH.

Low rifampicin C_{max} predicted a 2.4 (AOR = 2.44; 95% CI: 1.41, 4.21; p = 0.001) times higher odds of smear positivity compared to normal rifampicin C_{max} but Isoniazid C_{max} levels did not predict bacteriologic failure.

Table 30: Generalized estimating equation results showing baseline and longitudinal factors associated with sputum smear positivity with separate adjustments for the plasma concentration of rifampicin and isoniazid.

Characteristics	Model with adjustment for rifampicin C_{max}		Model with adjustment for isoniazid C_{max}	
	AOR (95% CI)	p-value	AOR (95% CI)	p-value
Month of treatment # Weight	0.98 (0.97, 0.98)	< 0.001*	0.98 (0.97, 0.98)	< 0.001*
Age (years)	1.01 (0.99, 1.03	0.202	1.01 (0.99, 1.03)	0.280
Dietary diversity	0.87 (0.79, 0.96)	0.007*	0.87 (0.79, 0.97)	0.008*
Current smoker # Alcohol drinker *(Ref: Smoker # Drinker)*				
Non-smoker # Non-drinker	3.47 (1.04, 11.54)	0.043*	2.85 (0.88, 9.23)	0.080
Non-smoker # Drinker	5.07 (1.43, 17.96)	0.012*	3.94 (1.16, 13.35)	0.028*
Smoker # Non-drinker	2.34 (0.25, 22.31)	0.460	2.16 (0.24, 19.36)	0.491
Other medicines intake # diabetes comorbidity *(Ref: No # Non-diabetic)*				
No # Diabetic	1		1	
Yes # Diabetic	0.47 (0.26, 0.85)	0.012*	0.50 (0.28, 0.91)	0.023*
Yes # Non-diabetic	0.31 (0.09, 0.99)	0.047*	0.33 (0.11, 0.97)	0.043*
Other medicines intake # HIV coinfection *(Ref: No # Negative)*				
No # Positive	1		1	
Yes # Negative	1.83 (1.08, 3.09)	0.024*	1.62 (0.97, 2.69)	0.065
Yes # Positive	1		1	
Adherence *(Ref: Adherent)*				
Non-adherent	1.29 (0.46, 3.60)	0.624	1.41 (0.51, 3.88)	0.504
Facility *(Ref: Komfo Anokye Teaching Hospital)*				
Suntreso Government Hospital	5.34 (1.11, 25.84)	0.037*	4.39 (0.95, 20.35)	0.059
Kumasi South Hospital	2.46 (1.13, 5.33)	0.023*	2.15 (1.03, 4.47)	0.041*
Tafo Government Hospital	0.25 (0.07, 0.85)	0.026*	0.22 (0.05, 0.95)	0.043*
Holy Family Hospital, Techiman	1.28 (0.63, 2.63)	0.493	1.31 (0.63, 2.73)	0.471
Drug C_{max} (*Ref: Normal)*				
Low	2.44 (1.41, 4.21) [r]	0.001*	0.97 (0.50, 1.87) [i]	0.927

*P < 0.05; # denotes interaction; [i] low isoniazid C_{max}; [r] low rifampicin C_{max}; C_{max}, peak plasma concentration; AOR, adjusted odds ratio;

4.6 Distinct Anti-TB drug Non-adherence Trajectories

The distribution of symptoms presented by study participants is summarized in Table 31. The Most prevalent symptom was cough. Although fever was the second most frequently reported symptom among those who did not miss any dose (16.6%), and also among those who missed 1-7 doses (16.4%) in a month, loss of appetite (14.3%) and loss of weight (14.3%) were more frequently reported among those who missed more than 7 doses in a month. Some of the least reported symptoms were vomiting (1.1%), diarrhoea (0.6%), finger clubbing (0.4%), and bleeding from the nose (0.4%).

Table 31: Distribution of presenting symptoms by number of doses missed per month.

Presenting symptoms	Number of doses missed			
	0 (n = 246)	1-7(n = 81)	>7 (n = 11)	Total (N = 338)
Cough	209 (34.6)	72 (34.8)	10(35.7)	291(34.7)
Fever	100 (16.6)	34(16.4)	3(10.7)	137(16.6)
Loss of appetite	56 (9.3)	14 (6.8)	4(14.3)	74(8.8)
Chest pains	55 (9.1)	23(11.1)	3(10.7)	81(9.7)
Loss of weight	54(8.9)	17(8.2)	4(14.3)	75(8.9)
Difficulty in breathing	37(6.1)	17(8.2)	2(7.1)	56(6.7)
Headache	36(6.0)	10(4.8)	1(3.6)	47(5.6)
Night sweats	27(4.5)	5(2.4)	1(3.5)	33(3.9)
Chills	8(1.3)	5(2.4)	0(0.0)	13(1.5)
Vomiting	5(0.8)	4(1.9)	1(3.6)	9(1.1)
Finger clubbing	3(0.5)	0(0.0)	0(0.0)	3(0.4)
Diarrhoea	3 (0.5)	2(1.0)	0(0.0)	5(0.6)
bleeding from nose	1(0.2)	2(1.0)	0(0.0)	3(0.4)
Others[1]	10(1.7)	2(1.0)	0(0.0)	12(1.4)
Total	604(100.0)	207(100.0)	28(100.0)	839(100.0)

[1] Includes general weakness, back pains, waist and joint pains, sore throat.

Note: n refers to observations and not participants; it includes repeated reports by some participants at different time points.

Adverse effects reported by participants are summarized in Table 32. Headache (31.5%) and fever (30.3%) were the most frequently reported side effects. Others worthy of note are myalgia (7.9%), gastrointestinal disturbances (8.3%) and nausea/vomiting (6.3%).

Table 32: Distribution of anti-TB drugs' side effects by number of doses missed.

Side Effects	Number of doses missed			
	0 (n = 104)	1-7(n = 31)	>7 (n = 10)	Total (N = 145)
Fever	59(32.5)	13(26)	5(22.7)	77(30.3)
Headache	58(32.0)	17(33.3)	5(22.7)	80(31.5)
Myalgia	13(7.2)	5(7.2)	2(9.1)	20(7.9)
Gastrointestinal disturbances	12(6.6)	6(11.8)	3(13.6)	21(8.3)
Nausea/vomiting	9(5.0)	3(5.9)	4(18.2)	16(6.3)
Insomnia	8(4.4)	2(3.9)	1(4.5)	11(4.3)
Dizziness	8(4.4)	1(2.0)	0(0.0)	9(3.5)
Exfoliative dermatitis	1(0.6)	0(0.0)	0(0.0)	1(0.4)
Epigastric pain	1(0.6)	1(2.0)	2(9.1)	4(1.6)
Pruritus	2(1.1)	1(2.0)	0(0.0)	3(1.2)
Arthralgia	1(0.6)	0(0.0)	0(0.0)	1(0.4)
Acne	2(1.1)	1(2.0)	0(0.0)	3(1.2)
Decreased visual acuity	2(1.1)	0(0.0)	0(0.0)	2(0.8)
Hypersensitivity	1(0.6)	0(0.0)	0(0.0)	1(0.4)
Arthritis	1(0.6)	0(0.0)	0(0.0)	1(0.4)
Peripheral neropathy	3(1.7)	1(2.0)	0(0.0)	4(1.7)
Total	181(100.0)	51(100.0)	22(100.0)	254(100.0)

Note: n refers to observations and not participants; it includes repeated reports by some participants at different time points.

The final observations for those lost to follow up were left out of the analysis since any estimate of the number of doses missed would be prone to severe bias and would potentially give rise to intractable groups in the model. Table 33 presents the Bayesian information criterion (BIC) and other classification parameters considered in the selection of the number of groups and trajectory shapes. Although several model specifications met the low BIC criteria and were within a

difference of <10 of the BIC of other potential models, only models (0 2 2), (0 2 3), and (0 1 3) did not have any group being less than 10% of participants.

The considerations for selecting a model among optimal candidate models selected by the BIC were: (a) preference for a parsimonious model that effectively captured the data patterns; (b) ensuring a close correspondence between the estimated probabilities of each group and the actual proportions of study members classified to those groups using the maximum posterior probability assignment rule; (c) requiring an average posterior probability value greater than 0.7 for each group, indicating a high level of confidence in the assigned group membership; (d) ensuring an adequate number of samples in each group to support reliable estimates; (e) expecting reasonably narrow confidence intervals, indicating precision in the estimated parameters and; (f) requiring odds of correct classification, based on the posterior probabilities of group membership, to be greater than 5 for each group, indicating a strong likelihood of accurate classification.

Based on (a), the (0 1 3) model was chosen. Specifically, it had all but 2 parameter estimates being statistically insignificant whereas orders (0 2 2) and (0 2 3) had 2 more and 3 more, respectively. The other criteria presented in Table 31 show that the selected model met all specified criteria.

Table 33: Model fitness for group-based trajectory model according to number of groups and trajectory shapes

Number of groups	Trajectory group shapes	BIC [u] (N=890)	BIC [d] (N=163)	AIC	ll	entropy
1	0	-2324.94	-2324.09	-2322.55	-2321.55	.
2	0 0	-1802.26	-1799.71	-1795.07	-1792.07	0.939
2	0 1	-1798.89	-1795.49	-1789.31	-1785.31	0.934
2	1 0	-1805.44	-1802.04	-1795.85	-1791.85	0.938
2	0 2	-1785.72	-1781.48	-1773.75	-1768.75	0.945
2	0 3	-1783.66	-1778.57	-1769.29	-1763.29	0.943
3	0 0 0 *	-1713.72	-1709.48	-1701.75	-1696.75	0.895
3	0 0 1 *	-1643.16	-1638.06	-1628.78	-1622.78	0.92
3	0 1 1 *	-1589.74	-1583.8	-1572.98	-1565.98	0.942
3	0 1 2 *	-1593.12	-1586.33	-1573.95	-1565.95	0.943
3	0 2 2	-1592.81	-1585.18	-1571.25	-1562.25	0.947
3	0 1 3	-1595.59	-1587.95	-1574.03	-1565.03	0.939
3	0 2 3	-1594.98	-1586.49	-1571.02	-1561.02	0.943
3	0 1 4 *	-1587.26	-1578.77	-1563.3	-1553.3	0.942
4	0 0 0 0 *	-1699.23	-1693.29	-1682.46	-1675.46	0.792

Trajectory shapes: 0: zero order, 1: linear, 2: quadratic, 3: cubic, 4: quartic; AIC: Akaike information criterion; BIC [u]: Bayesian information criterion for the total number of eligible observations; BIC [d]: Bayesian information criterion for the total number of eligible participants.
* One of the resulting groups has a group membership proportion smaller than 10%.

The average posterior probabilities for each group were above the minimum value of 0.70; 0.98 for group 1, 0.99 for group 2 and 0.97 for group 3 (Table 34). The proportion of study participants assigned to each group based on the maximum posterior probability corresponded closely with groups' estimated probability. Finally, the lowest odds of classification based on the posterior probabilities of group membership was 16.95. All the groups had higher values than the minimum threshold of 5.

Table 34: Model accuracy and classification criteria

Model accuracy criteria	Low	Declining	Increasing
Average posterior probability value	0.98	0.99	0.97
Odds of correct classification	16.95	342.72	310.81
Estimated group probability	71.24	18.23	10.54
Proportion assigned to group [a]	72.56	17.68	9.76
Sample numbers	119	29	16

[a] assignment done according to the maximum posterior probability assignment

The Parameter estimates for the group membership with confidence intervals and trajectories of the selected model is presented in Table 35. The 3 distinct non-adherence trajectory groups consisted of 71.2% of participants for group 1, 18.3% for group 2, and 10.5% for group 3. Group 1 trajectory was adequately characterized by an intercept only model while groups 2 and 3 were described by linear and cubic models, respectively. However, the quadratic and cubic terms for group 3 were not statistically significant. The linear term for group 2 was negative indicating a decrease in non-adherence and that for group 3 suggested an increase in non-adherence over time.

Table 35: Parameter estimates of group membership and likelihood of non-adherence over time.

	Group membership	Non-adherence over time (trajectory)			
Group	**%[a] (95% CI)**	**Intercept(β_0)**	**Linear(β_1)**	**Quadratic(β_2)**	**Cubic(β_3)**
Low Non-adherence	71.2 (64.5, 77.98)	-1.28*			
Declining Non-adherence	18.2 (12.4, 24.0)	2.01*	-0.38*		
Increasing Non-adherence	10.5 (6.1, 15.0)	-2.02*	2.00*	-0.45	0.04

*P < 0.05; [a] Percentage of participants in each risk group

Figure 12 shows the estimated trajectories of non-adherence to anti-TB regimen (solid lines), the observed average non-adherence rate at each month for the groups (dot symbols) during the 6

months period of treatment, and the estimated group percentages. The largest group of participants are categorized into group 1 (blue line). They began and ended treatment with a low level of non-adherence. They are as such labeled as the low non-adherence group. The second group represented by the green line (declining non-adherence), started at a moderate level of non-adherence. This declined steadily to a low level over time. Finally, the last group (red line), the increasing non-adherence group, consists of 10.5% of participants who ended the treatment having reached a high level of non-adherence although they started at a reasonably low level.

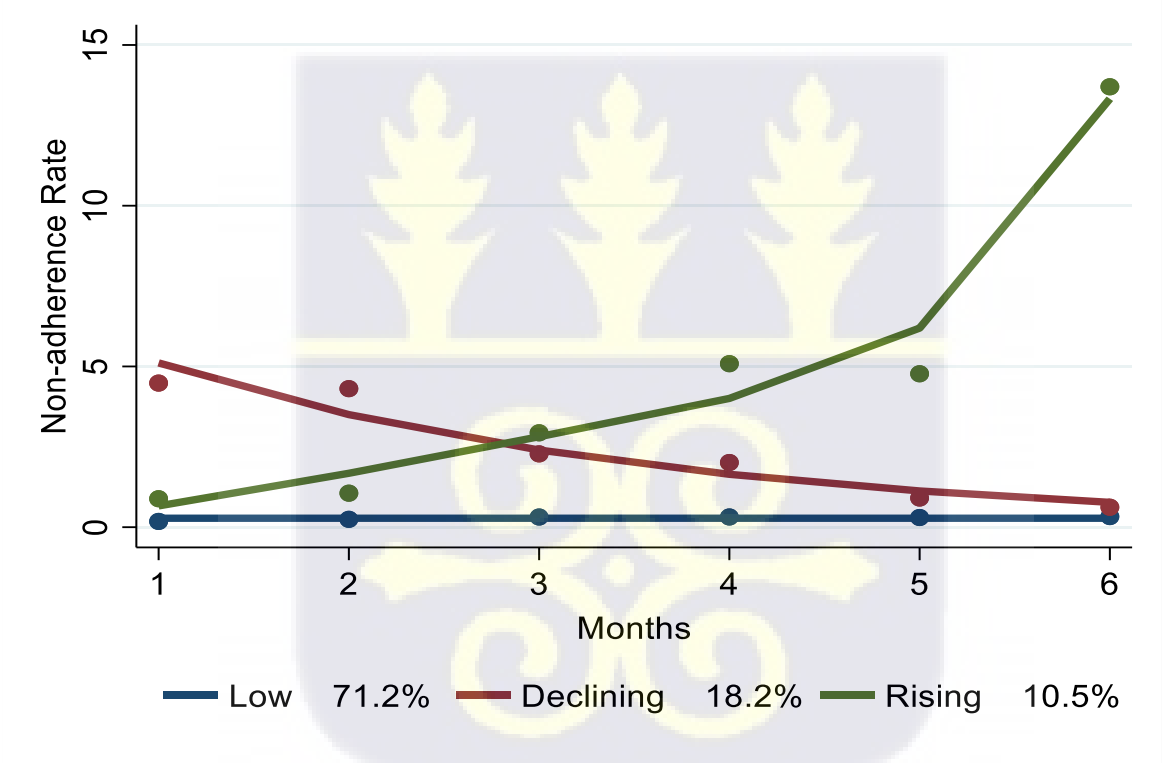


Figure 11: Estimated trajectories of non-adherence to anti-TB regimen (blue, green, and red solid lines), observed average non-adherence rate at each month for the groups (dot symbols) during the 6 months period of treatment, and estimated group percentages.

A "spaghetti" plot of each group members' trajectory is shown in Figure 13 to depict the individual patterns summarized into the groups presented in Figure 12.

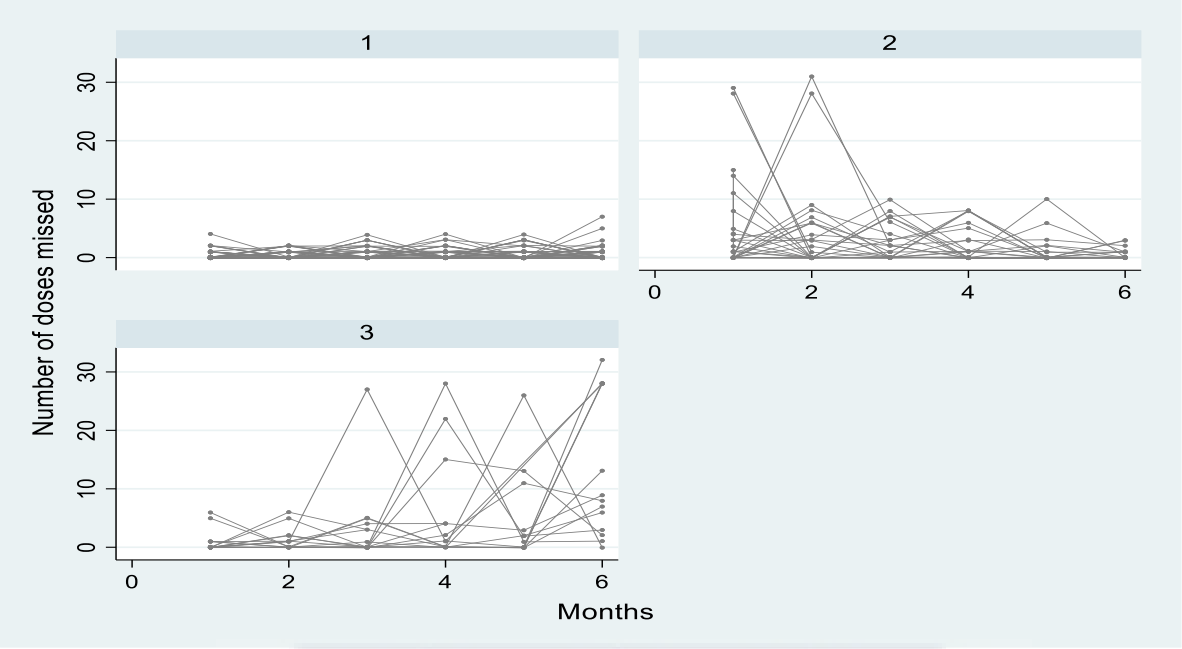


Figure 12: Spaghetti plot of individual trajectories of non-adherence to anti-TB medication over time

Factors that preexisted treatment initiation and how they influenced group membership as well as the effect of time-varying factors on non-adherence over time are summarized in Table 36. Group membership was modeled as a multinomial logistic regression using the low non-adherence group as the reference. It was modeled with the factors that predict trajectories simultaneously. Those who were employed were 86% less likely than the unemployed to be members of the increasing non-adherence group relative to the low non-adherence group (RR = 0.14; 95% CI = 0.03, 0.68; p = 0.016). Marital status had a marginal effect on group membership. Being married was associated with a lower risk of membership in the declining non-adherence group (RR = 0.44; 95% CI = 0.19, 1.04; p = 0.063) but a higher risk in the increasing non-adherence group (RR = 5.28; 95% CI = 0.93, 29.95; p = 0.061) compared to being unmarried.

On the trajectories of the groups, dietary diversity score had a statistically significant effect on all

three groups. It was associated with a 24% increased risk of non-adherence in the low group (RR = 1.24; 95% CI = 1.12, 1.38; $p < 0.001$), a 6% increased risk in the declining group (RR = 1.06; 95% CI = 1.02, 1.10, $p = 0.007$) and a 16% decreased risk in the increasing non-adherence group (RR = 0.84; 95% CI = 0.77, 0.91; $p < 0.001$). The increasing non-adherence group had a reduced likelihood of non-adherence when symptoms were present (RR = 0.0003, 95% CI = 0.00, 0.003; $p < 0.001$) but an increased likelihood when they had side effects (RR = 779.54; 95% CI = 63.49, 9570.51; $p < 0.001$). Being on medication other than the anti-TB drugs also reduced their risk for non-adherence by 69% (RR = 0.31; 95% CI = 0.23, 0.43; $p < 0.001$). In the declining group, however, taking other medicines increased the likelihood of non-adherence by 33% (RR = 1.33; 95% CI = 1.08, 1.64; $p = 0.008$).

Table 36: Factors that influence group membership and the trajectories of non-adherence to anti-TB medication.

Variable	Low		Declining		Increasing	
	RR (95% CI)	**p-value**	**RR (95% CI)**	**p-value**	**RR (95% CI)**	**p-value**
			Group membership			
Male ***(Ref: Female)***			1.32 (0.52 ,3.4)	0.560	1.76 (0.29 ,10.49)	0.536
Tertiary Education ***(Ref: Lower level)***			1.34 (0.29 ,6.19)	0.704	5.51 (0.68 ,44.59)	0.110
Marital status ***(Ref: Unmarried)***	Reference		0.44 (0.19 ,1.04)	0.063	5.28 (0.93 ,29.95)	0.061
Employed ***(Ref: Unemployed)***			1.64 (0.65 ,4.15)	0.294	0.14 (0.03 ,0.68)	0.016*
Alcohol drinker ***(Ref: Non-drinker)***			1.94 (0.75 ,5.01)	0.173	0.4 (0.03 ,4.76)	0.469
			Trajectory			
Symptom present ***(Ref: Absent)***	0.77 (0.47 ,1.28)	0.320	0.97 (0.78 ,1.22)	0.818	0.0003 (0.00, 0.003)	<0.001*
Dietary diversity score	1.24 (1.12 ,1.38)	< 001*	1.06 (1.02 ,1.1)	0.007*	0.84 (0.77 ,0.91)	<0.001*
Side effect present ***(Ref: Absent)***	0.98 (0.43 ,2.21)	0.95	1.2 (0.95 ,1.51)	0.12	779.54 (63.49 ,9570.51)	<0.001*
Taking other medicines ***(Ref: No)***	1.1 (0.73 ,1.65)	0.657	1.33 (1.08 ,1.64)	0.008*	0.31 (0.23 ,0.43)	<0.001*

CI: Confidence Interval; RR: Relative Risk; *p < 0.05

CHAPTER FIVE

5.0 DISCUSSION

This study sought to establish the link between the pharmacokinetics of the first-line anti-TB drugs and TB treatment outcomes in Ghana. The present study might be the first in Ghana. The study aimed to address several significant limitations observed in previous research on this topic. The first improvement involved using plasma concentrations at two specific time points instead of one for more precise peak plasma concentration estimation. Secondly, as opposed to the observational studies which assessed the association without adjustment for potential confounding variables, this study considered several key confounders in the evaluation. Thirdly, this study ensured a comparatively reasonable sample size capable of detecting small effect sizes. Other improvements worth mentioning are the use of data collected prospectively to fulfill the research objectives rather than retrospectively analyzing clinical data, assessing all four first-line anti-TB drugs, and maintaining continuous participant follow-up throughout the entire treatment duration.

5.1 Difference in Treatment Outcomes between Low and Normal Plasma Concentration of Drugs

This study hypothesized from the outset that "there is no difference in treatment outcomes between rifampicin-susceptible TB patients with low plasma concentration and those with normal plasma concentration of rifampicin, isoniazid, pyrazinamide, and ethambutol". This null hypothesis was rejected for rifampicin, pyrazinamide, and ethambutol but not isoniazid. There was evidence of an association between rifampicin plasma concentration and poor sputum smear conversion at the end of the intensive phase (initial two months of treatment), and at the end of the six months of treatment. It also provided evidence of rifampicin plasma levels' effect on bacteriologic failure throughout therapy. Although there was no evidence of an effect of low plasma concentration of

pyrazinamide and ethambutol on early treatment outcomes (end of initial two months of treatment), there was evidence of their association with unsuccessful end-of-treatment outcomes. There was no evidence whatsoever of an association between isoniazid plasma concentration and treatment outcomes assessed. Further findings showed that adherence to treatment was high (≥88.4%). Those whose adherence to treatment worsened over time were influenced by the increasing occurrence of symptoms and as well as side effects. Furthermore, their likelihood of non-adherence was lower if they had other non-TB medicines to take along with the TB regimen.

5.1.1 Rifampicin Plasma concentration and Treatment Outcomes

In the current investigation, low plasma concentrations of rifampicin correlated with poor sputum conversion at the end of the initial two months of treatment, influenced end-of-treatment results, as well as the varying patterns of predicting biological treatment failure throughout therapy. This is despite the fact that majority of participants exhibited successful treatment outcomes even though their plasma concentrations of rifampicin fell below the recommended levels. This implies that rifampicin concentration might play a role in the observed suboptimal treatment outcomes. Similar observations have been reported elsewhere (Aarnoutse et al., 2017; Pasipanodya et al., 2013a; Ramachandran et al., 2017, 2020; Sekaggya-Wiltshire, von Braun, et al., 2018; Svensson et al., 2018), while contrasting findings have also been documented by others (Park et al., 2015; Sekaggya-Wiltshire, Lamorde, et al., 2018a; Wilby et al., 2014). The studies that found no evidence of an association have attributed it to variations in the ethnic backgrounds of the populations studied and the relatively high prevalence of coexisting conditions, such as HIV and possibly other comorbidities like diabetes in these populations (Park et al., 2015).

The co-morbidities are believed to contribute to unfavorable outcomes by reducing the plasma concentrations of rifampicin. In the current study, HIV coinfection did not show evidence of impacting plasma concentration or influencing poor treatment outcomes. However, the relationship between diabetes and treatment outcomes yielded intriguing results. It was observed that TB patients with diabetes receiving anti-diabetic treatment had improved outcomes compared to non-diabetics. This improvement in treatment outcomes is likely attributed to the direct impact of diabetes on hepatic function, as opposed to the effect of anti-diabetic therapy inhibiting cytochrome P450 enzymes, which would have led to the opposite result. This effect on hepatic function, in turn, reduces the metabolism and elimination of rifampicin, resulting in elevated plasma levels (Dostalek et al., 2012).

The present study's examination of rifampicin plasma concentration highlighted several factors predicting these levels. These factors include age, body weight, gender, diabetes comorbidity, and the concurrent use of non-TB medications. Similar findings have been reported in other studies (Huerta-García et al., 2019). Among these factors, male sex and the concurrent use of non-TB medications were associated with decreased plasma levels. The reduced plasma concentration in males is attributed to a larger volume of distribution due to increased muscle mass and greater apparent drug clearance in men compared to women (Devaleenal Daniel et al., 2017). The positive correlation between increasing body weight and higher plasma levels of rifampicin can be attributed to its high affinity for fat tissue, primarily because of rifampicin's lipophilicity (Piccaro et al., 2015). An elevation in body weight could be linked to increased levels of adipose tissue (Arner, 2018). This change represents an important increase in the volume of distribution for a lipophilic drug like rifampicin, thus the higher plasma level (Gouju & Legeay, 2023).

5.1.2 Isoniazid Plasma concentration and Treatment Outcomes

In contrast to earlier studies, (Pasipanodya et al., 2013b; Prahl et al., 2014; Requena-Méndez et al., 2014; Sekaggya-Wiltshire, von Braun, et al., 2018), the present investigation did not find an association between isoniazid and unsuccessful treatment outcomes. This discrepancy may be attributed to various factors. It is possible that the study population had a higher proportion of fast acetylators, resulting in lower plasma concentrations compared to other studies. Another argument put forward by some is the use of C_{max} as an assessment measure, as opposed to AUC, C_{max}/minimum inhibitory concentration (MIC), or AUC/MIC (Burhan et al., 2013a). However, this argument loses its strength in light of the established correlation between C_{max} and AUC. This correlation was demonstrated in the present study and elsewhere (Pasipanodya et al., 2012; Peloquin, 2002).

Previous evidence has suggested that low isoniazid plasma concentrations may be linked to relapse and the development of drug resistance (Pasipanodya et al., 2013b; Sekaggya-Wiltshire, von Braun, et al., 2018). Low levels of isoniazid may diminish its synergistic effects with other antibiotics in the treatment regimen, underscoring the importance of optimizing its concentration (Deepak et al., 2009; Grosset et al., 1992). The current study identified body weight, alcohol use, and HIV coinfection as factors predicting plasma concentration. While it is widely recognized that HIV coinfection is associated with decreased plasma concentrations, the findings in this study contradicted this notion. It is believed that the decreased plasma level may be attributed to malabsorption due to HIV-related gastrointestinal issues and drug interactions with antiretrovirals (Bhatt et al., 2014). In conditions of liver impairment resulting from HIV-related liver disease or

coinfections like viral hepatitis, it is conceivable that isoniazid concentration would increase. Since liver function is not routinely assessed for TB, HIV or TB/HIV co-infected patients in Ghana, it may well be that the study sample had a high prevalence of participants with liver disease. Additionally, genetic factors that predispose individuals to slow acetylation may contribute to this observed phenomenon (Zabost et al., 2013). Data that lends credence to this explanation is that similar populations in Africa are known to have a high prevalence of slow acetylators (Ali et al., 2019; Gutiérrez-Virgen et al., 2023; T. Sileshi et al., 2023). This is further supported by a study among Ghanaian children (Dompreh et al., 2018).

5.1.3 Pyrazinamide Plasma concentration and Treatment Outcomes

Previous studies have documented the association between low pyrazinamide exposure and poor TB treatment outcomes (Burhan et al., 2013b; Pasipanodya et al., 2013b). Nevertheless, in the current investigation, this association was observed in terms of end-of-treatment outcomes but not with 2-month sputum smear conversion. Given its sterilizing activity, it was anticipated that there would be an association with 2-month sputum positivity, as this is the presently acknowledged biomarker for sterilizing activity (Perrin et al., 2007). One of the mechanisms contributing to low plasma concentrations of the first-line anti-TB medications is alterations in drug metabolism. This metabolic shift can be attributed to TB infection itself and other concomitant infections, particularly HIV, which, as part of disease progression, induce inflammation and elevate cytokine levels. Under such conditions, there is an upregulation of cytochrome P450 enzymes, resulting in accelerated drug metabolism and subsequently lower drug concentrations upon ingestion (Wang et al., 2022). Pyrazinamide plays a distinctive role in these circumstances due to its effectiveness against lingering bacilli in the low pH macrophage environment and in regions characterized by

acute inflammation (WHO, 2010b). In view of this, pyrazinamide may contribute to increased concentrations of the other drugs during the continuation phase by blocking the altered metabolism pathway to low concentration of drugs in the early stages of treatment when acute inflammation is prevalent. This may explain the delayed effect of pyrazinamide observed.

The findings in respect of the factors associated with pyrazinamide concentration demonstrate the dynamic nature of the relationships between them. While the linear regression model failed to identify any independent factors associated with pyrazinamide concentration, the results from the quantile regression model indicate that these relationships may not be linear. Instead, they appear to be complex, context-specific, and dynamic (Huang et al., 2017; Koenker & Bassett, 1978). This may also explain why some earlier studies aimed at identifying patient-related factors linked to the plasma levels of anti-TB medications found none (Fahimi et al., 2013; Hiruy et al., 2015). In the present study, the presence of diabetes comorbidity and the concurrent use of medications unrelated to TB were found to influence low pyrazinamide concentration, whereas age and adherence showed significant effects on high concentrations. These require further studies to explain the heterogeneity in the relationships across the different quantiles.

5.1.4 Ethambutol Plasma concentration and Treatment Outcomes

The positive correlation between age and ethambutol C_{max}, observed from q0.25 through q0.90, suggests that older individuals tend to have higher maximum plasma concentrations of ethambutol. This finding is in line with the known pharmacokinetic changes that occur with aging (Denti et al., 2015). As people age, physiological changes, such as alterations in body composition and organ function, can affect the absorption, distribution, metabolism, and excretion of drugs. In the case of

ethambutol, these age-related changes result in higher C_{max} levels. The negative impact of alcohol on ethambutol levels at high quantiles might be attributed to alcohol's influence on liver function and enzymes involved in drug metabolism. Individuals with diabetes comorbidity had a higher plasma concentration at low quantiles (q0.10 and q0.25). This could be due to diabetes-related changes in metabolism and drug clearance. It is important to note that these effects were observed at the lower quantiles, suggesting that diabetes may influence ethambutol plasma concentration primarily in patients with lower concentrations.

5.2 Adherence and Risk Factors

While low adherence undoubtedly contributes to suboptimal treatment outcomes, the present study did not yield sufficient evidence to support this notion due to the absence of widespread low adherence. Often when doses were missed, it was the missing of a dose or two irregularly throughout treatment as opposed to missing several doses consecutively (Fox et al., 2023). Nevertheless, it is crucial to underscore the importance of maintaining and enhancing adherence gains through DOTS. The present study found 3 distinct trajectories of non-adherence to TB treatment as did a study in the Philippines (Huddart et al., 2022). These groups were the low, reducing and increasing non-adherence groups. The trajectories in the present study were identical to those in the Philippine study, serving to confirm their findings. Of these groups, there is the need to target interventions to the increasing non-adherence group which has a high risk of consistently worsening non-adherence over time. Unemployment and, to a lower extent, being married are risk factors of membership in this high-risk non-adherence group.

Support by close family members tends to improve adherence (Appiah et al., 2023). Those who

were married were more likely to have increasing non-adherence to treatment over time suggesting that they may not be receiving their spouse's support. Considering the elevated levels of stigma linked to TB in Ghana, this is plausible (Amo-Adjei, 2016; Huq et al., 2022). On the other hand, unemployment predicted a high risk of decreasing adherence over time, possibly because of its negative impact on mental health, as employment can serve as a social support system (Knudsen et al., 2016). Also, negative effect of unemployment could manifest in difficulty in raising funds to travel to the health facility for refills (Pradipta et al., 2021).

The finding that the occurrence or persistence of symptoms increased the risk of non-adherence in the high-risk group may be because of the perception that the treatment was not effective. This contradicts the findings of Ágh et al. (2015), who acknowledge the potential for this relationship to change over time but suggest that an improved quality of life may lead to non-adherence. As earlier demonstrated, membership of the high-risk non-adherence group is characterized by unemployment and marriage. These are desperate circumstances that may drive individuals to search for alternative solutions if they suspect that the treatment was ineffective. This is corroborated by a study in Ghana which found that TB patients are likely to misperceive some TB symptoms (Dogah et al., 2021).

As expected, the incidence of side effects increased the likelihood of non-adherence in the high-risk non-adherence group (Pradipta et al., 2021). This risk might be mitigated by the clinical management of such side effects and through counseling.

5.3 Study Limitations

There were some notable limitations to this study. Firstly, AUC was not used to investigate the association with treatment outcomes although some studies have suggested that it is a better measure of exposure than C_{max}. Likewise, it has been argued that the ratio of C_{max} to MIC or AUC to MIC are even better metrics (Sileshi et al., 2021). Notwithstanding this, it is worth noting that evidence of the association of both C_{max} and AUC with treatment outcomes exist (Jayaram et al., 2003, 2004). In the present study, C_{max} was demonstrated to be correlated with the AUC as such the findings are likely similar to what would have been found if the AUC had been used. Secondly, although C_{max} was derived from 2 timepoints and as such is a better estimate than the one time point used by some studies in the literature, it may not necessarily be the specific maximum concentration reached. Additionally, the C_{max} was measured only once during treatment for each study participant despite the possibility of changes over time. Thirdly, bacteriologic failure was measured using florescence microscopy instead of solid culture which is the gold standard. This may introduce misclassification bias. For example, it was observed that at baseline some participants, about 5% tested negative to microscopy although they were shown by GeneXpert to be bacteriologically confirmed. This discrepancy may be due to the differences in the sensitivities of GeneXpert versus sputum microscopy. GeneXpert, being able to detect *M. tuberculosis* by analyzing its genetic information is highly sensitive even for low bacterial loads (<10 bacilli/ml) (Lawn & Nicol, 2011; WHO, 2014). In contrast, microscopy relies on the visual detection of acid-fast bacilli under a microscope requiring a high bacterial load for detection (>10,000 bacilli/ml) (Lawn & Nicol, 2011; WHO, 2014). As such, GeneXpert may detect dead bacilli while microscopy may yield false negatives in case of low bacillary concentration. This dynamic may have been at play from the third to the sixth month when a marginal increase in smear positivity is observed.

Another plausible explanation may be that those who could not produce sputum for microscopy as treatment progressed were more likely to be smear-negative than smear-positive, giving an appearance of the increasing rate of smear positivity. However, it must be considered that the evidence available suggests a good correlation between microscopy and culture (Olaru et al., 2014; Van Deun et al., 2008; World Health Organization, 2011). Further to that, the sensitivity, specificity, positive and negative predictive values of microscopy are high particularly when fluorescence is used by highly skilled medical laboratory scientists (Das et al., 2019; Lipsky et al., 1984). These were ensured to mitigate the effect of the potential misclassification bias.

5.4 Implications of Findings

5.4.1 Clinical implications

The association between low levels of all 4 drugs and end-of-treatment outcomes was estimated to be the highest. These suggest that adjusting doses of just one of the drugs in the regimen may not yield optimal treatment response although doing so may result in better outcomes than the status quo.

The low rifampicin levels observed may be prohibitive to killing or inhibiting the growth of the *M. tuberculosis*. This can allow the bacteria to continue multiplying and cause persistent infection which may result in chronic inflammation and contribute to the poor outcomes. Furthermore, rifampicin needs to reach therapeutic concentrations in the tissues and body compartments where the bacteria reside to be effective. Low rifampicin levels in the blood may translate into inadequate drug levels in these sites, allowing the bacteria to survive and proliferate. The other drugs in the regimen are added to make this unlikely to happen as each may exert their effect in controlling the

infection. Isoniazid does this through a direct rapid reduction in the bacterial population and pyrazinamide through its sterilizing effect. Unfortunately, these may not play out as intended since up to 22.7% of participants had low levels of all four drugs.

The need to achieve normal levels of rifampicin concentration is crucial for a couple of reasons other than those earlier discussed. Firstly, the present study has shown that low rifampicin plasma exposure predicts unsuccessful treatment outcomes at the end of both phases of treatment. Secondly, a dose-response relationship between rifampicin and treatment outcomes has been demonstrated elsewhere in support of the use of higher doses of rifampicin for treatment (Aarnoutse et al., 2017). Finally, the potential for a further shortening of the treatment duration also exists upon dose optimization of the first-line anti-TB drugs (Svensson et al., 2018; Velásquez et al., 2018). Consequently, dose adjustments for TB patients with low rifampicin, pyrazinamide and ethambutol concentration is imperative.

The observation that a monthly increase in body weight is linked to unsuccessful treatment outcomes may be attributed to weight-based dosing. As individuals move from a lower weight band to a higher one, the dose is adjusted accordingly. For instance, those weighing 30 to 39 kg were initially given 2 tablets, but this is increased to 3 tablets if their body weight reached 40 to 54 kg. Given the moderate weight gain during the treatment period, such dose increments across weight bands result in higher doses per kilogram body weight. This supports the notion that administering higher drug doses is associated with an increased likelihood of successful outcomes. Regardless of the rationale behind this finding, one reasonable interpretation is that a month-on-month increase in body weight serves as a significant indicator of successful TB treatment.

5.4.2 Public health impact

Low rifampicin concentrations can exert selective pressure on the bacteria, favouring the survival and multiplication of drug-resistant strains. This can lead to the development of drug resistance, making the infection more difficult to treat with rifampicin. Although this effect was not directly observed in this study, it is plausible, especially when it is considered that there was a high prevalence of low levels of ethambutol and isoniazid which are targeted for the control of the emergence of resistant strains in the intensive and continuation phases, respectively. As has been found by studies which investigated relapse or acquired drug resistance, high levels of low isoniazid concentration is a major risk factor. Even worse, a concerning observation was the relatively high occurrence of low plasma levels in precisely two drugs (38.3%) or three drugs (24.2%), highlighting the presence of what could be described as "functional mono or dual therapy." This situation poses a significant challenge by facilitating the development of drug-resistant strains. It may underlie the increasing prevalence of multidrug resistant TB in Ghana (Sylverken et al., 2021).

5.5 Directions for Further Research

Dose adjustment of rifampicin, pyrazinamide and ethambutol are required to optimize plasma concentration. Given the high cost of therapeutic drug monitoring for use in identifying patients requiring dose adjustment in clinical care, further studies exploring the use of higher doses of these three drugs, particularly rifampicin, for the treatment of rifampicin-susceptible TB would be a more workable approach. These studies may have to consider dose optimization for more than just one drug to increase the likelihood of improved outcomes. With the aim of making necessary dose

and policy changes, there is the need to scale up ongoing studies investigating this (Phillips et al., 2016; Seijger et al., 2019).

The present study has demonstrated the nuanced relationships between plasma concentrations of the first-line anti-TB drugs and their risk factors. Much remains to be understood regarding the mechanisms by which these effects vary across the quantile distribution of plasma concentrations.

Additional research focusing on the underlying mechanisms through which socioeconomic status impact adherence behavior would contribute valuable insights for shaping interventions intended to enhance TB treatment adherence.

5.6 Contribution to Knowledge

1. This study confirms that rifampicin-susceptible TB patients in Ghana with low plasma concentrations of rifampicin, pyrazinamide, or ethambutol are at risk of poor treatment outcomes even if they adhere well to the treatment regimen. Additionally, it has revealed that those with low plasma levels of all four of the first-line drugs are at a heightened risk of experiencing poor treatment outcomes.

2. The study has shown that the factors (such as age, weight, sex, diabetes co-morbidity, co-administration of other non-TB drugs) that influence plasma concentrations of the first-line anti-TB drugs may do so to different extents at different concentrations rather than equally across the mean concentration. This means that the relationships may not be linear as has been predominantly characterized in the literature.

3. This study has identified that there are three distinct trajectories of medication non-adherence that patients tend to follow throughout treatment. Specifically, these are the low, declining and increasing non-adherence developmental groups. It has further provided a more detailed characterization of the risk factors associated with the increasing non-adherence developmental group. Additionally, it has shed light on the dynamic factors that influence adherence behavior over time.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The current study aimed to investigate the association between the pharmacokinetics of the first-line anti-TB drugs and TB treatment outcomes in Ghana, addressing some limitations observed in previous research. The study concludes that low rifampicin plasma concentration is a key risk factor for poor treatment response. Low pyrazinamide and ethambutol plasma concentrations also predispose patients to unsuccessful treatment outcomes. In addition, having concurrently low plasma concentrations of all four first-line anti-TB drugs increases the susceptibility to poor treatment outcomes. Clinical characteristics that are associated with poor treatment outcomes are diabetes comorbidity, HIV co-infection, low body weight, and the co-administration of drugs not related to TB.

The plasma concentrations of the first-line anti-TB drugs are influenced by age, sex, body weight, smoking, alcohol use, diabetes comorbidity, HIV co-infection, adherence, and the co-administration of drugs not related to TB. These risk factors have a different influence on plasma concentrations in patients with high levels than those with low levels.

The study concludes that there are three distinct treatment non-adherence trajectories that patients tend to follow while on the first-line anti-TB medication. These are the low, declining and increasing non-adherence trajectories. The likelihood of increasing non-adherence over time is reduced by the incidence of TB disease symptoms and the co-administration of other medicines not related to TB. On the other hand, is increased by the occurrence of medication side effects.

6.2 Recommendations

To the Ministry of Health

The Ministry of Health should formulate and implement a policy requiring that all TB patients are routinely screened for diabetes and treated accordingly as is currently required of HIV for all TB patients.

To Health Practitioners

Clinicians should assess TB patients for diabetes. All those found to be diabetic must be linked to the diabetes clinics for care. In addition, clinicians should target the married and unemployed patients with interventions aimed at improving psycho-social support.

To the Government of Ghana and Partners

The government and health partners should provide resources to enable therapeutic drug monitoring in clinical practice as a means of assessing the need for dose adjustment in TB patients.

To the Research Community

The National Tuberculosis Programme (NTP) and its stakeholders should partner with research institutions to conduct further studies to determine doses of rifampicin, pyrazinamide and ethambutol which would optimize plasma concentrations and lead to improved treatment outcomes. Further studies should investigate the dynamic relationships between the pharmacokinetics of the first-line anti-TB drugs and their risk factors.

REFERENCES

Automatic citation updates are disabled. To see the bibliography, click Refresh in the Zotero tab.

APPENDICES

APPENDIX A: PARTICIPANT INFORMATION AND CONSENT FORM

Pharmacologic and Clinical Risk Factors of Poor TB Treatment Outcomes in Patients with Rifampicin-Susceptible TB in Selected Hospitals in the Ashanti, Bono and Bono East regions of Ghana

Introduction

Principal investigator: Michael Mireku Opoku, PhD student, School of Public Health, University of Ghana

Telephone: 0543380959

Email: mmirekuopoku@gmail.com

Background and study purpose

Tuberculosis is a disease that affects people from all walks of life in the world. Yet, TB occurs more frequently in Africa including Ghana than in most parts of the world. It is caused by bacteria called *Mycobacterium tuberculosis*. When an already infected person coughs or sneezes, the causative germs are expelled into the air. Uninfected persons could contact the germs when they breathe in the air with these germs. The disease as we know it, develops when the body's defence mechanism of the person who has been infected with the germs is compromised. TB is curable if treated with recommended drugs. However, a little more people than is expected do not end up with the consequences anticipated after treatment. It has been suggested that this could be because the amount of the drugs which eventually end up in the blood varies so much that for some people it is not enough to work as one would expect. This study is designed to find out if this could be the case in Ghana and also to find other factors which could explain why some people do not get the expected

response after treatment.

Duration/what is involved

As part of this study, we will be asking you (your ward) to provide us with information about yourself (your ward), your family and the treatment every time you (he or she) visit(s) the TB clinic. We will also take 2 separate samples of your (your ward's) blood, about 1 teaspoonful, on the same day, 2 hours and 6 hours after taking the medication on the 4th week after you (your ward) start(s) treatment. These samples will be refrigerated and transported to the USA to find the amount of the anti-TB drugs in them. For the blood sampling, you will be required to spend the night before at the hospital so that the first sample can be taken early the next morning before breakfast. Six hours after, the second blood sample will be taken. Kindly note that the process of drawing the blood may be painful but is not harmful. This study will last for 6 months which is the duration required for the treatment. During this period, you will be required to report to the clinic once every month in addition to the day that blood sampling will be done. On each of the clinic visit days the administration of the questionnaire is expected to last for about 1 hour. Also, we will be requiring you (your ward) to give us a sample of your sputum in containers every month during treatment to enable us assess your response to treatment. Whatever may be left of the blood and sputum samples collected will be discarded safely and completely. We assure you that every information you (your ward) provide(s) us will be kept in strict confidence. In sharing what we find from this study in reports, journals and meetings, you (your ward) will not be identified by name.

Benefits

There may be no personal benefit to you (or your ward) for participating in this study. Notably, you (your ward) would be helping us to find answers which could help solve the problem. It is possible that we may find something of clinical importance about you (your ward). In that case, you will be treated as the hospital's guidelines stipulate.

Potential Risks

You may be uncomfortable with some of the questions or procedures in the study. Kindly note that the process of drawing the blood may be painful but we do not anticipate any physical harm to you (or your ward) as those leading the process have been trained adequately for this exercise. We crave your indulgence to respond honestly to every question.

To reduce the likelihood of infection with the COVID-19 virus you will be provided with a face mask to be always worn. Alcohol hand rub will also be provided to ensure good hand hygiene. Kindly maintain a minimum distance of 6 feet from others, including the one interviewing you.

Costs

You will not be required to pay money for taking part in this study. The blood sampling may be done on a day which is not part of your routine visit for TB care. Due to the blood sampling which will be done 6 hours apart, reporting to the hospital will be at 9pm the night before and the process is expected to be done by 2pm that day.

Compensation

You (your ward) will be given a token of GHS50 for your time and GHS40 for transportation on the day of blood sampling since it will not be a day for routine TB care

services. Please note that NO compensation will be given for all the other visits to the clinic.

Confidentiality

The information that is collected from you will be used only for the purpose of this study. A code known only to the research team will be used instead of your name in the dataset and on the blood and sputum containers so that no information collected is traceable to you. We will not use your name or any information that will make it possible to identify you personally when we are talking or writing about this study.

Voluntary participation/withdrawal

You are at liberty to choose to participate or refuse participation in this study. If after choosing to participate in the study you later decide to withdraw, you could do so without having to pay any penalty whatsoever. Please be assured that if you refuse to take part in the study, it will not affect the care you receive at all. If you choose to participate you may choose not to answer any question you are uncomfortable with. We however encourage you to participate fully to enable us contribute to improving care for people with TB.

Outcome and feedback

Information gathered from this study will be presented at conferences and published in scientific journals. The data will be kept by the principal investigator on a password-protected computer. While we will not provide direct feedback to you on the information we gather from this study, you are at liberty to request same through the contacts provided below.

Funding information

This study is funded by the University of Ghana and the University of Florida Academic Partnership for TB/HIV Research Training in Ghana and funded by Fogarty International Center Grant (D43 TW010055).

Conflict of Interest

The data collected will be owned solely by the principal investigator.

Provision of information and consent for participants

If you agree to participate (allow your ward to participate) you will be given a copy of this information sheet and consent form to sign to show that you understand the information that has been given to you and you have willingly agreed to participate in the study.

Contacts

You may ask any questions you have pertaining to this study for clarification. Kindly reach the principal investigator, Michael Mireku Opoku on 0543380959 or mmirekuopoku@gmail.com with all your queries.

Also, if you think your rights have been harmed as a result of the study, you can contact the following agency:

Ghana Health Service, Ethics Review Administrator, Nana Abena Apatu, 0503539896.

Consent Form

I acknowledge that I have read or have had the purpose and contents of the Participants' Information Sheet read and all questions satisfactorily explained to me in a language I understand (English/Twi/Hausa). I fully understand the contents and any potential implications as well as my right to change my mind (i.e. withdraw from the research) even after I have signed this form.

I voluntarily agree to be part of this research.

Name of Participant……...

Participants' Signature ……...OR Thumb Print……

Date: …….

Interpreter's Statement

I interpreted the purpose and contents of the Participants' Information Sheet to the afore named participant to the best of my ability in the (English/Twi/Hausa) language to his proper understanding.

All questions, appropriate clarifications sort by the participant and answers were also duly interpreted to his/her satisfaction.

Name of Interpreter……

Signature of Interpreter…….. OR Thumb Print …….....

Date:……
Contact Details:

Statement of witness
I was present when the purpose and contents of the Participant Information Sheet was read and explained satisfactorily to the participant in the language he/she understood (English/Twi/Hausa).

I confirm that he/she was given the opportunity to ask questions/seek clarifications and same were duly answered to his/her satisfaction before voluntarily agreeing to be part of the research.

Name:……

Signature…….... OR Thumb Print …….....

Date:…….

Investigator Statement and signature

I certify that the participant has been given ample time to read and learn about the study. All questions and clarifications raised by the participant have been addressed.

Researcher's name…….

Signature …….

Date…….

Parental Consent Form

Introduction

Principal investigator: Michael Mireku Opoku, PhD student, School of Public Health, University of Ghana

Telephone: 0543380959

Email: mmirekuopoku@gmail.com

Background and study purpose

Tuberculosis is a disease that affects people from all walks of life in the world. Yet, TB occurs more frequently in Africa including Ghana than in most parts of the world. It is caused by bacteria called *Mycobacterium tuberculosis*. When an already infected person coughs or sneezes, the causative germs are expelled into the air. Uninfected persons could contact the germs when they breathe in the air with these germs. The disease as we know it, develops when the body's defence mechanism of the person who has been infected with the germs is compromised. TB is curable if treated with recommended drugs. However, a little more people than is expected do not end up with the consequences anticipated after treatment. It has been suggested that this could be because the amount of the drugs which eventually end up in the blood varies so much that for some people it is not enough to work as one would expect. This study is designed to find out if this could be the case in Ghana and also to find other factors which could explain why some people do not get the expected response after treatment.

Duration/what is involved

As part of this study, we will be asking your ward to provide us with information about them, their family and the treatment every time he or she visits the TB clinic. We will also

take 2 separate samples of your ward's blood, about 1 teaspoonful, on the same day, 2 hours and 4 hours after taking the medication on the 4th week after your ward starts treatment. These samples will be refrigerated and transported to the USA to find the amount of the anti-TB drugs in them. For the blood sampling, your ward may be required to spend the night before at the hospital so that the first sample can be taken early the next morning before breakfast. Four hours after, the second blood sample will be taken. Kindly note that the process of drawing the blood may be painful but is not harmful. This study will last for 6 months which is the duration required for the treatment. During this period, your ward will be required to report to the clinic once every month in addition to the day that blood sampling will be done. On each of the clinic visit days the administration of the questionnaire is expected to last for about 1 hour. Also, we will be requiring your ward to give us a sample of your sputum in containers every month during treatment to enable us assess your response to treatment. Whatever may be left of the blood and sputum samples collected will be discarded safely and completely. We assure you that every information your ward provides us will be kept in strict confidence. In sharing what we find from this study in reports, journals and meetings, your ward will not be identified by name.

Benefits

There may be no personal benefit to you or your ward for participating in this study. Notably, your ward would be helping us to find answers which could help solve the problem. It is possible that we may find something of clinical importance about your ward. In that case, you will be treated as the hospital's guidelines stipulate.

Potential Risks

You may be uncomfortable with some of the questions or procedures in the study. Kindly note that the process of drawing the blood may be painful but we do not anticipate any physical harm to your ward as those leading the process have been trained adequately for this exercise. We crave your indulgence to respond honestly to every question.

To reduce the likelihood of infection with the COVID-19 virus you and your ward will be provided with a face mask to be always worn. Alcohol hand rub will also be provided to ensure good hand hygiene. Kindly ensure you and your ward maintain a minimum distance of 6 feet from others, including the one interviewing you.

Costs

Neither you nor your ward will be required to pay money for taking part in this study. The blood sampling may be done on a day which is not part of your routine visit for TB care. Due to the blood sampling which will be done 4 hours apart, reporting to the hospital, if required, will be at 9pm the night before and the process is expected to be done by 2pm that day.

Compensation

Your ward will be given a token of GHS50 for your time and GHS40 for transportation on the day of blood sampling since it will not be a day for routine TB care services. Please note that NO compensation will be given for all the other visits to the clinic.

Confidentiality

The information that is collected from your ward will be used only for the purpose of this study. A code known only to the research team will be used instead of your name in the dataset and on the blood and sputum containers so that no information collected is traceable

to you. We will not use your ward's name or any information that will make it possible to identify him or her personally when we are talking or writing about this study.

Voluntary participation/withdrawal

You are at liberty to decide if your ward participates or to refuse participation in this study. If after choosing that your ward participates in the study you later decide to withdraw him or her, you could do so without having to pay any penalty whatsoever. Please be assured that if you refuse your ward participation in the study, it will not affect the care he or she receives at all. If you choose that your ward should participate, he or she may choose not to answer any question they are uncomfortable with. We however encourage that he or she participates fully to enable us to contribute to improving care for people with TB.

Outcome and feedback

Information gathered from this study will be presented at conferences and published in scientific journals. The data will be kept by the principal investigator on a password-protected computer. While we will not provide direct feedback to you on the information we gather from this study, you are at liberty to request same through the contacts provided below.

Funding information

This study is funded by the University of Ghana and the University of Florida Academic Partnership for TB/HIV Research Training in Ghana and funded by Fogarty International Center Grant (D43 TW010055).

Conflict of Interest

The data collected will be owned solely by the principal investigator.

Provision of information and consent for parent

If you agree to allow your ward to participate, you will be given a copy of this information sheet and consent form to sign to show that you understand the information that has been given to you and you have willingly agreed that your ward participates in the study.

Contacts

You may ask any questions you have pertaining to this study for clarification. Kindly reach the principal investigator, Michael Mireku Opoku on 0543380959 or mmirekuopoku@gmail.com with all your queries.

Also, if you think your rights or those of your ward have been harmed as a result of the study, you can contact the following agency:

Ghana Health Service, Ethics Review Administrator, Nana Abena Apatu, 0503539896.

Consent by Parent of child aged 15 – 17 years

I acknowledge that I have read or have had the purpose and contents of the Parental Information Sheet read and all questions satisfactorily explained to me in a language I understand (English/Twi/Hausa). I fully understand the contents and any potential implications as well as my right to change my mind (ie. withdraw my ward from the research) even after I have signed this form.

I voluntarily agree that my ward participates in this research.

Name of Participant……...

Participants' Signature ……...OR Thumb Print……

Date: …….

Interpreter's Statement

I interpreted the purpose and contents of the Participants' Information Sheet to the afore named parent to the best of my ability in the (English/Twi/Hausa) language to his/her proper understanding.

All questions, appropriate clarifications sort by the participant and answers were also duly interpreted to his/her satisfaction.

Name of Interpreter……

Signature of Interpreter…….. OR Thumb Print …….....

Date:……
Contact Details:

Statement of witness

I was present when the purpose and contents of the Parental Information Sheet was read and explained satisfactorily to the parent in the language he/she understood (English/Twi/Hausa).

I confirm that he/she was given the opportunity to ask questions/seek clarifications and same were duly answered to his/her satisfaction before voluntarily agreeing to be part of the research.

Name:……

Signature…….... OR Thumb Print …….....

Date:…….

Investigator Statement and signature

I certify that the parent has been given ample time to read and learn about the study. All questions and clarifications raised by the parent have been addressed.

Researcher's name…….

Signature …….

Date…….

Assent Form

Child Information sheet

Introduction

Principal investigator: Michael Mireku Opoku, PhD student, School of Public Health, University of Ghana

Telephone: 0543380959

Email: mmirekuopoku@gmail.com

Background and study purpose

Tuberculosis is a disease that affects people from all walks of life in the world. Yet, TB occurs more frequently in Africa including Ghana than in most parts of the world. It is caused by bacteria called *Mycobacterium tuberculosis*. When an already infected person coughs or sneezes, the causative germs are expelled into the air. Uninfected persons could contact the germs when they breathe in the air with these germs. The disease as we know it, develops when the body's defence mechanism of the person who has been infected with the germs is compromised. TB is curable if treated with recommended drugs. However, a little more people than is expected do not end up with the consequences anticipated after treatment. It has been suggested that this could be because the amount of the drugs which eventually end up in the blood varies so much that for some people it is not enough to work as one would expect. This study is designed to find out if this could be the case in Ghana and also to find other factors which could explain why some people do not get the expected response after treatment.

Duration/what is involved

As part of this study, we will be asking you to provide us with information about yourself, your family and the treatment every time you visit the TB clinic. We will also take 2 separate samples of your blood, about 1 teaspoonful, on the same day, 2 hours and 4 hours after taking the medication on the 4th week after you start treatment. These samples will be refrigerated and transported to the USA to find the amount of the anti-TB drugs in them. For the blood sampling, you may be required to spend the night before at the hospital so that the first sample can be taken early the next morning before breakfast. Four hours after, the second blood sample will be taken. Kindly note that the process of drawing the blood may be painful but is not harmful. This study will last for 6 months which is the duration required for the treatment. During this period, you will be required to report to the clinic once every month in addition to the day that blood sampling will be done. On each of the clinic visit days the administration of the questionnaire is expected to last for about 1 hour. Also, we will be requiring you to give us a sample of your sputum in containers every month during treatment to enable us assess your response to treatment. Whatever may be left of the blood and sputum samples collected will be discarded safely and completely. We assure you that every information you provide us will be kept in strict confidence. In sharing what we find from this study in reports, journals and meetings, you will not be identified by name.

Benefits

There may be no personal benefit to you for participating in this study. Notably, you would be helping us to find answers which could help improve TB treatment. It is possible that

we may find something of clinical importance about you. In that case, you will be treated as the hospital's guidelines stipulate.

Potential Risks

You may be uncomfortable with some of the questions or procedures in the study. Kindly note that the process of drawing the blood may be painful but we do not anticipate any physical harm to you as those leading the process have been trained adequately for this exercise. We crave your indulgence to respond honestly to every question.

To reduce the likelihood of infection with the COVID-19 virus you and your parent will be provided with a face mask to be always worn. Alcohol hand rub will also be provided to ensure good hand hygiene. Kindly maintain a minimum distance of 6 feet from others, including the one interviewing you.

Costs

You will not be required to pay money for taking part in this study. The blood sampling may be done on a day which is not part of your routine visit for TB care. Due to the blood sampling which will be done 6 hours apart, reporting to the hospital will be at 9pm the night before and the process is expected to be done by 2pm that day.

Compensation

You will be given a token of GHS50 for your time and GHS40 for transportation on the day of blood sampling since it will not be a day for routine TB care services. Please note that NO compensation will be given for all the other visits to the clinic.

Confidentiality

The information that is collected from you will be used only for the purpose of this study. A code known only to the research team will be used instead of your name in the dataset

and on the blood and sputum containers so that no information collected is traceable to you. We will not use your name or any information that will make it possible to identify you personally when we are talking or writing about this study.

Voluntary participation/withdrawal

You are at liberty to choose to participate or refuse participation in this study. If after choosing to participate in the study you or your parent later decide to withdraw, you could do so without having to pay any penalty whatsoever. Please be assured that if you or your parent refuse to take part in the study, it will not affect the care you receive at all. If you choose to participate you may choose not to answer any question you are uncomfortable with. We however encourage you to participate fully to enable us contribute to improving care for people with TB.

Outcome and feedback

Information gathered from this study will be presented at conferences and published in scientific journals. The data will be kept by the principal investigator on a password-protected computer. While we will not provide direct feedback to you or your parent on the information we gather from this study, you are at liberty to request same through the contacts provided below.

Funding information

This study is funded by the University of Ghana and the University of Florida Academic Partnership for TB/HIV Research Training in Ghana and funded by Fogarty International Center Grant (D43 TW010055).

Conflict of Interest

The data collected will be owned solely by the principal investigator.

Provision of information and consent for participants

The study and all that is required of you has been explained to your parent too. If you agree to participate following your parents' approval you will be given a copy of this information sheet and consent form to sign to show that you understand the information that has been given to you and you have willingly agreed to participate in the study.

Contacts

You may ask any questions you have pertaining to this study for clarification. Kindly reach the principal investigator, Michael Mireku Opoku on 0543380959 or mmirekuopoku@gmail.com with all your queries.

Also, if you think your rights have been harmed as a result of the study, you can contact the following agency:

Ghana Health Service, Ethics Review Administrator, Nana Abena Apatu, 0503539896.

Assent by participants between 15 and 17 years

I voluntarily agree to participate in this study.

....

Name of Participant Signature or thumb print

Date ……/……/……

Interpreter's Statement

I interpreted the purpose and contents of the Child Information Sheet to the afore named participant to the best of my ability in the (English/Twi/Hausa) language to his proper understanding.

All questions, appropriate clarifications sort by the participant and answers were also duly interpreted to his/her satisfaction.

Name of Interpreter……

Signature of Interpreter…….. OR Thumb Print …….....

Date:……
Contact Details:

Statement of witness
I was present when the purpose and contents of the child Information Sheet was read and explained satisfactorily to the participant in the language he/she understood (English/Twi/Hausa).

I confirm that he/she was given the opportunity to ask questions/seek clarifications and same were duly answered to his/her satisfaction before voluntarily agreeing to be part of the research.

Name:……

Signature…….... OR Thumb Print …….....

Date:…….

Investigator Statement and signature

I certify that the participant has been given ample time to read and learn about the study. All questions and clarifications raised by the participant have been addressed.

Researcher's name…….

Signature …….

Date…….

APPENDIX B: QUESTIONNAIRE/DATA ABSTRACTION TOOL

Pharmacologic and Clinical Risk Factors of Poor TB Treatment Outcomes in Patients with Rifampicin-Susceptible TB in selected Hospitals In the Ashanti, Bono and Bone East regions of Ghana	
Participant's ID (*studyID*): [ ][ ][ ][ ]	Date of visit: _____ (dd-mm-yyyy)
Research Assistant's Initials:_____ Instructions: Please Tick (√) the appropriate Metropolis and circle the corresponding code	
METROPOLIS/MUNICIPALITY *(adminUnit)* [1] Kumasi Metropolis	CODE A 1
FACILITY *(facility)* [1] Komfo Anokye Teaching Hospital (KATH) [2] Suntreso Government Hospital [3] Manhyia Government Hospital	CODE KAA1 SGA1 MGA1
METROPOLIS/MUNICIPALITY *(adminUnit)* [2] Asokwa Municipal	CODE B 2
FACILITY *(facility)* [4] Kumasi South Hospital	CODE KSB2
METROPOLIS/MUNICIPALITY *(adminUnit)* [3] Old Tafo Municipality	CODE C 3
FACILITY *(facility)* [5] Tafo Government Hospital	CODE TGC3

STUDY VISIT 0
WEEK 0

Hospital ID *(hospID)*:_____ Participant's ID *(studyID)*: _____ Location *(location)*:_____ Participant's Tel: _____ Supporter's Tel:_____	Date of TB diagnosis:_____ *(diagnosed)* (dd-mm-yyyy) Date of Recruitment: _____ *(recruit)* (dd-mm-yyyy) Date of Interview:_____ *(interview)* (dd-mm-yyyy)

Participant's Name: _____
Municipality_____ Municipality code [ ][ ]
Facility_____ Facility code [ ][ ][ ][ ]
Instruction: Please Tick (√) appropriate response and write its code in the empty box
PART A: BACKGROUND CHARACTERISTICS

No.	Question	Answer	code	
1	Participant's Date of Birth	_____ (dd-mm-yyyy)		a1_dob
2	Participant's sex	[1] Female [0] Male	[ ]	a2_sex
3	Highest Level of Education	[1] No Formal Education [2] Primary school [3] High School [6] Tertiary	[ ]	a3_educ
4	Religion	[1] Christian [2] Muslim [3] Other, (Please specify)……	[ ]	a4_religion
5	Which of the following best describes your employment status?	[1] Unemployed [2] Employed [3] Student [4] Other, (Please specify)……..	[ ]	a5_employ
6	Which of the following best describes your Marital status?	[1] Unmarried [2] Married	[ ]	a6_maritalstatus
7	Do you stay with a guardian?	[1] Yes [0] No	[ ]	a7_guardian
If **"Yes"** to Question 7 **skip** to Question 9				
8	Who is the guardian you stay with?	[1] Parent(s) [2] Other relative [3] Non-relative	[ ]	a8_residGuardian

9	What is your guardian's employment status?	[1] Unemployed [2] Employed [3] Student	[ ]	a9_guardEmploy
10	How much do you or your guardian earn in a month?	[1] less than GH₵ 1,000 [2] GH₵ 1,001 – 2,000 [3] GH₵ 2,001 – 3,000 [4] More than GH₵ 3,000	[ ]	a10_income
11	How many are you in the house where you stay?	_____		a11_hseholdSize
12	Which of these do you have at home?	TV set [1] Yes [0] No	[ ]	a12_tv
		Radio set [1] Yes [0] No	[ ]	a12_radio
		Car [1] Yes [0] No	[ ]	a12_car
		Motor bicycle [1] Yes [0] No	[ ]	12_motorBicycle
		Mobile phone [1] Yes [0] No	[ ]	a12_mobilePhone
		Internet access [1] Yes [0] No	[ ]	a12_internet
		Refrigerator [1] Yes [0] No	[ ]	a12_fridge
		Gas cooker [1] Yes [0] No	[ ]	a12_gasCooker
		Bicycle [1] Yes [0] No	[ ]	a12_bicycle
		Computer [1] Yes [0] No	[ ]	a12_computer
		Fan [1] Yes [0] No	[ ]	a12_fan
		Air conditioner [1] Yes [0] No	[ ]	a12_ac
13	Which type of toilet facility do you use at home?	[1] Pit latrine [2] Water closet [3] Public toilet [4] Open space/ Bush [5] Other, (please specify) _____	[ ]	a13_toiletType
14	Where do you stay?	_____		a14_residence
15	Have you had meals containing any of the following in the past 24 hours?			
	All starchy foods	Roots and tubers, such as; cassava, yam, plantain, cocoyam, cereals such as; wheat, sorghum, millet, rice, oats, pasta and pasta products, potatoes, maize flour, wheat flour, cassava flour, millet flour [1] Yes [0] No	[ ]	a15_starchy

	Beans and peas	White beans, red beans, Bambara beans, cowpea, soya beans, black eye beans, kidney beans [1] Yes [0] No	[ ]	a15_beanspeas
	Nuts and seeds	Melon seeds (agushie), almond, sesame seeds, peanuts, groundnuts, shea nuts, tiger nuts, cashew nuts, locust bean seeds [1] Yes [0] No	[ ]	a15_nutsseeds
	All dairy	Milk and milk products which includes any type of milk, yoghurt, cheese, butter, ice-cream, wagashi, brukina. [1] Yes [0] No	[ ]	a15_diary
	Flesh foods and organ meat	Organ meat and miscellaneous small protein. Such as beef, goat, rabbit, pork, turkey, chicken, lamb, sausage, offal's, meat of wild animals and games [1] Yes [0] No	[ ]	a15_fleshmeat
	Eggs	Chicken eggs, guinea fowl eggs, turkey eggs, duck eggs [1] Yes [0] No	[ ]	a15_eggs
	Vitamin A rich dark green leafy vegetables	Kontomire, alefu, ayoyo, green onion (spring onion), local spinach, dandelion leaves. [1] Yes [0] No	[ ]	a15_vegetables
	Other vitamin A-rich vegetables and fruits	Carrots, orange, sweet potatoes, sweet red pepper, lettuce, tangerine, yellow maize, mango, pawpaw [1] Yes [0] No	[ ]	a15_vitA
	Other vegetables	Cabbage, tomatoes, onion, cucumber, garlic, mushrooms, eggplant, shallot, okra. [1] Yes [0] No	[ ]	a15_otherveget ables
	Other fruits	Coconut, banana, soursop, apple, pear, lemon, lime, noni fruit, sisibi fruit, pineapple, guava, atemoya, jackfruit, melon, African star fruit (agbalumo), black velvet tamarind (yooyi), watermelon.	[ ]	a15_otherfruits

		[1] Yes [0] No		
PART B: CLINICAL CHARACTERISTICS				
16	Which of the following symptoms do you have now?	Cough [1] Yes [0] No	[ ]	b16_cough
		Fever [1] Yes [0] No	[ ]	b16_fever
		Haemoptysis [1] Yes [0] No	[ ]	b16_haemoptysis
		Loss of appetite [1] Yes [0] No	[ ]	b16_appetite
		Dyspnoea [1] Yes [0] No	[ ]	b16_dyspnoea
		Shortness of breath [1] Yes [0] No	[ ]	b16_orthopnoea
		Night sweats [1] Yes [0] No	[ ]	b16_nSweats
		Finger clubbing [1] Yes [0] No	[ ]	b16_finClubbing
		chest pains [1] Yes [0] No	[ ]	b16_chestPains
		Loss of weight [1] Yes [0] No	[ ]	b16_weightLoss
		Headache [1] Yes [0] No	[ ]	b16_headache
		Vomiting [1] Yes [0] No	[ ]	b16_vomiting
		Diarrhoea [1] Yes [0] No	[ ]	b16_diarrhoea
		Chills [1] Yes [0] No	[ ]	b16_chills
		Others, Specify:		
17	Which of the following best describes your functional Status?	[1] Working (Moves and works normally) [2] Ambulatory (moves but cannot work) [3] Bed-ridden (confined to bed)	[ ]	b17_funStatus
18	Type of TB case	[1] New [0] Relapse	[ ]	b18_tbCase
19	Anthropometric characteristics	Height _____cm		b19_height
		Weight _____kg		b19_weight

PART C: Investigations				
20	Do you have diabetes	[1] Yes	[ ]	c20_dm

	mellitus?	[2] No [3] Don't know		
If **"No"** or **"Don't know"** please **skip** to Question 20				
21	How long have you known?	_____ months		c21_knownDM
22	Have you been tested for HIV?	[1] Yes [0] No	[ ]	c22_hivTesting

Laboratory Tests				
No.	Test	Results	Date test was done dd-mm-yyyy	
23	Fasting blood sugar	_____	_____	c23_fbs
24	HIV status	[1] Positive [2] Negative [3] Not Known	_____	c24_hivStatus
25	HIV clinical stage	[1] Stage I [2] Stage II [3] Stage III [4] Stage IV [5] Not known	_____	c25_hivStage
26	CD4 cell count	_____cells/ml	_____	c26_cd4
27	Chest X-Ray; Extent of lesions Tick (√) all that apply	[1] Old lesion [2] Active lesion [3] Calcification [4] Fibrosis [5] Infiltrates [6] Cavity	_____	c27_xray
28	Smear Microscopy	[1] Negative [2] scanty [3] + [4] ++ [5] +++	_____	c28_microscope
29	Culture	[1] Positive [2] Negative [3] Not known	_____	c29_culture

PART D: Medical and Drug History

30	Which other diseases were diagnosed within the past week? Tick (√) all that apply	Hypertension [1] Yes [0] No	[ ]	d30_htn
		Diabetes mellitus [1] Yes [0] No	[ ]	d30_dm
		HIV [1] Yes [0] No	[ ]	d30_hiv
		Other STI [1] Yes [0] No	[ ]	d30_sti
		Respiratory Tract Infection [1] Yes [0] No	[ ]	d30_rti
		Pneumonia [1] Yes [0] No	[ ]	d30_pneumo
		Urinary Tract Infection [1] Yes [0] No	[ ]	d30_uti
		Malaria [1] Yes [0] No	[ ]	d30_malaria
		Prurigo [1] Yes [0] No	[ ]	d30_prurigo
		Skin disease [1] Yes [0] No	[ ]	d30_skinDx
		Anaemia [1] Yes [0] No	[ ]	d30_anaemia
		Other, specify:		
31	Do you have any chronic disease?	[1]Yes [2] No [3] Don't know	[ ]	d31_chronicDx
32	Which diseases are those? Tick (√) all that apply	Cardiovascular disease [1] Yes [0] No	[ ]	d32_cardio
		Diabetes mellitus [1] Yes [0] No	[ ]	d32_dm
		Kidney disease [1] Yes [0] No	[ ]	d32_kidneyDx
		Liver disease [1] Yes [0] No	[ ]	d32_liverDx
		Cancer [1] Yes [0] No	[ ]	d32_cancer
		Other, specify		
	Skip to 35 if **NOT** an ART client			

33	Have you started taking ART drugs?	[1] Yes [0] No	[ ]	*d33_startART*
If 'No" **skip** to Question 35				

34 Which ART drug(s) are you currently taking?

Name of Drug (d32_arv)	Dose *(d34_dose)*	Frequency *(d34_freq)*	Start Date *(d34_start)*	Stop Date *(d34_stop)*
[1] Tenofovir				
[2] Zidovudine				
[3] Lamivudine				
[4] Emtricitabine				
[5] Efavirenz				
[6] Nevirapine				
[7] Dolutegravir				
[8] Abacavir				

35 Which medicines other than TB or HIV drugs are you currently taking?

Name of Drug *(d35_meds)*	Dose *(d35_medDose)*	Frequency *(d35_medFreq)*	Start Date *(d35_medStart)*	Stop Date *(d35_medStop)*
[1] Cotrimoxazole				
[2] Pyridoxine				
[3]				
[4]				
[5]				
[6]				
[7]				

36 TB Medication Information

	Name of Drug (FDC) *(d36_dot)*	Body weight *(d36_dot Weight)*	Number of Tablets *(d36_dot Tablets)*	Start Date *(d36_dot Start)*	Stop Date *(d36_dot Stop)*	Pills pick up
Initiation	[1] Rifampicin 150mg					Week 0 [ ] Week 2 [ ]

	[2] Isoniazid 75mg					Week 4 [ ] Week 6 [ ] Week 8 [ ]
	[3] Pyrazinamide 400mg					
	[4] Ethambutol 275mg					
Continuation Phase	[1] Rifampicin 150mg					Month 3 [ ] Month 4 [ ] Month 5 [ ] Month 6 [ ]
	[2] Isoniazid 75mg					

37	Which of these events have you experienced (or been experiencing) after starting the medication? Tick (√) all that apply	Gastrointestinal disturbances [1] Yes [0] No	[ ]	d37_gastro
		Fever [1] Yes [0] No	[ ]	d37_fever
		Headache [1] Yes [0] No	[ ]	d37_headache
		Myalgia [1] Yes [0] No		d37_myalgia
		Nausea/vomiting [1] Yes [0] No	[ ]	*d37*_nausea
		Exfoliative dermatitis/ Skin disease [1] Yes [0] No	[ ]	d37_skin
		Epigastric pain [1] Yes [0] No	[ ]	d37_epigastric
		Pruritus [1] Yes [0] No	[ ]	d37_pruritus
		Arthralgia [1] Yes [0] No	[ ]	d37_arthralgia
		Liver disease [1] Yes [0] No	[ ]	d37_liverDx
		Acne [1] Yes [0] No	[ ]	d37_acne
		Decreased visual acuity [1] Yes [0] No	[ ]	d37_visual
		Hypersensitivity reactions [1] Yes [0] No	[ ]	d37_reactions
		Arthritis [1] Yes [0] No		d37_arthritis

		Peripheral neuropathy [1] Yes [0] No		d37_neuropathy
		Convulsions [1] Yes [0] No		d37_convulsions
		Insomnia [1] Yes [0] No		d37_insomnia
		Dizziness [1] Yes [0] No		d37_dizziness
		Other, specify:		

PART E: Behavioural characteristics					
38	Anti-TB medication				
	DOT Doses Received at last refill (e38_received)	DOT Doses Administered (e38_admin)	DOT Doses Missed (e38_missed)	DOT doses left on blister pack (e38_blister)	Reasons Given for Missing Doses

Morisky Medication-adherence scale				
39	Do you ever forget to take you anti-TB medication?	[1] Yes [0] No	[ ]	e39_forgetMed
40	Do you ever have problems remembering to take your anti-TB medication?	[1] Yes [0] No	[ ]	e40_remembMed
41	When you feel better, do you sometimes stop taking your anti-TB medicine?	[1] Yes [0] No	[ ]	e41_stopMed
42	Sometimes if you feel worse when you take your anti-TB medicine, do you stop taking it?	[1] Yes [0] No	[ ]	e42_worseMed
Alcohol and Tobacco use				

43	Do you ever drink alcohol these days?	[1]Yes [0]No	[ ]	e43_drink
44	Which of these best describe the frequency with which you drink alcoholic beverages?	[1] Everyday [2] Nearly everyday [3] 3 to 4 times a week [4] 1 to 2 times a week [5] 2 to 3 times a month [6] Once a month [7] 7 to 11 times in a month [8] 4 to 6 times in the past year [9] 2 or 3 times in the past year [10] Once in the past year [11] Never drank any alcohol [12] Never in my life	[ ]	e44_drinkFreq
45	How many drinks did you usually have on days when you drank alcohol in the past year?	[1] _____ 330ml glass, bottle [2] _____ 200ml glass of wine [3] _____ 40ml of Hard liquor	[ ]	e45_numberDrink
46	In the past year, how often did you drink 5 or more drinks of any alcoholic drink or combination of beverages in a single day?	[1] Everyday [2] Nearly everyday [3] 3 to 4 times a week [4] 1 to 2 times a week [5] 2 to 3 times a month [6] Once a month [7] 7 to 11 times in a month [8] 4 to 6 times in the past year [9] 2 or 3 times in the past year [10] Once in the past year [11] Never drank any alcohol [12] Never in my life	[ ]	e46_intenseDrink
47	Do you currently smoke tobacco?	[1] Daily [2] Less than daily [3] Not at all [4] Don't know	[ ]	e47_currentTob
If "Daily" or "Less than daily" skip to 48				
48	Have you used smoked tobacco in the past?	[1] Yes [2] No [3] Don't know	[ ]	e48_pastTob
49	In the past, have you smoked tobacco?	[1] Daily [2] Less than daily [3] Not at all [4] Don't know	[ ]	e49_pastFreqTob

50	On average, how much cigarette do you smoke currently? If day or week **does not apply**, kindly select 99 after indicating quantity against the type	[1] ____Manufactured Cigarettes per day or week [2] ____ Pipes of tobacco (shisha) per day or week [3] ____ Cigar per day or week [4] ____ Other, specify _____ per day or week [99] Not per day or week	[ ]	e50_intenseTob
51	Have you repeatedly been using any other hard substances recently?	[1] Yes [2] No [3] Don't know	[ ]	e51_useHard
52	Which substance(s) have you been using?	[1] Tramadol [2] Heroine [3] Cocaine [4] Other, specify _____		e52_substance

PART F: TREATMENT OUTCOMES				
53	Treatment Outcomes	[1] Cured [2] Treatment completed [3] Lost to follow up [4] Treatment failed [5] Died	_____	f53_outcomes

STUDY VISIT 1
WEEK 4

Hospital ID *(hospID)*:_____ Participant's ID *(studyID)*: _____ Location *(location)*:_____ Participant's Tel: _____ Supporter's Tel:_____	Date of TB diagnosis:_____ *(diagnosed)* (dd-mm-yyyy) Date of Recruitment: _____ *(recruit)* (dd-mm-yyyy) Date of Interview:_____ *(interview)* (dd-mm-yyyy)

Participant's Name: _____
Municipality_____ Municipality code [ ][ ]
Facility_____ Facility code [ ][ ][ ][ ]
Instruction: Please Tick (√) appropriate response and write its code in the empty box

CLINICAL CHARACTERISTICS				
16	Which of the following symptoms do you have now?	Cough [1] Yes [0] No	[ ]	b16_cough
		Fever [1] Yes [0] No	[ ]	b16_fever
		Haemoptysis [1] Yes [0] No	[ ]	b16_haemoptysis
		Loss of appetite [1] Yes [0] No	[ ]	b16_appetite
		Dyspnoea [1] Yes [0] No	[ ]	b16_dyspnoea
		Shortness of breath [1] Yes [0] No	[ ]	b16_orthopnoea
		Night sweats [1] Yes [0] No	[ ]	b16_nSweats
		Finger clubbing [1] Yes [0] No	[ ]	b16_finClubbing
		chest pains [1] Yes [0] No	[ ]	b16_chestPains
		Loss of weight [1] Yes [0] No	[ ]	b16_weightLoss
		Headache [1] Yes [0] No	[ ]	b16_headache
		Vomiting [1] Yes [0] No	[ ]	b16_vomiting
		Diarrhoea [1] Yes [0] No	[ ]	b16_diarrhoea
		Chills [1] Yes [0] No	[ ]	b16_chills
		Others, Specify:		

Laboratory Tests				
No.	Test	Results	Date test was done dd-mm-yyyy	

23	Fasting blood sugar	_____	_____	c23_fbs
24	HIV status	[1] Positive [2] Negative [3] Not Known	_____	c24_hivStatus
25	HIV clinical stage	[1] Stage I [2] Stage II [3] Stage III [4] Stage IV [5] Not known	_____	c25_hivStage
26	CD4 cell count	_____cells/ml	_____	c26_cd4
27	Chest X-Ray; Extent of lesions Tick (√) all that apply	[1] Old lesion [2] Active lesion [3] Calcification [4] Fibrosis [5] Infiltrates [6] Cavity	_____	c27_xray
28	Smear Microscopy	[1] Negative [2] scanty [3] + [4] ++ [5] +++	_____	c28_microscope
29	Culture	[1] Positive [2] Negative [3] Not known	_____	c29_culture

PART D: Medical and Drug History				
30	Which other diseases were diagnosed within the past week?	Hypertension [1] Yes [0] No	[ ]	d30_htn
		Diabetes mellitus [1] Yes [0] No	[ ]	d30_dm
		HIV [1] Yes [0] No	[ ]	d30_hiv
		Other STI [1] Yes [0] No	[ ]	d30_sti
		Respiratory Tract Infection [1] Yes [0] No	[ ]	d30_rti
		Pneumonia	[ ]	d30_pneumo

	Tick (√) all that apply	[1] Yes [0] No		
		Urinary Tract Infection [1] Yes [0] No	[ ]	d30_uti
		Malaria [1] Yes [0] No	[ ]	d30_malaria
		Prurigo [1] Yes [0] No	[ ]	d30_prurigo
		Skin disease [1] Yes [0] No	[ ]	d30_skinDx
		Anaemia [1] Yes [0] No	[ ]	d30_anaemia
		Other, specify:		
31	Do you have any chronic disease?	[1]Yes [2] No [3] Don't know	[ ]	d31_chronic Dx
32	Which diseases are those? Tick (√) all that apply	Cardiovascular disease [1] Yes [0] No	[ ]	d32_cardio
		Diabetes mellitus [1] Yes [0] No	[ ]	d32_dm
		Kidney disease [1] Yes [0] No	[ ]	d32_kidneyDx
		Liver disease [1] Yes [0] No	[ ]	d32_liverDx
		Cancer [1] Yes [0] No	[ ]	d32_cancer
		Other, specify		

	Skip to 35 if **NOT** an ART client			
33	Have you started taking ART drugs?	[1] Yes [0] No	[ ]	*d33_start ART*
If 'No" **skip** to Question 35				
34	Which ART drug(s) are you currently taking?			

Name of Drug (d32_arv)	Dose *(d34_dose)*	Frequency *(d34_freq)*	Start Date *(d34_start)*	Stop Date *(d34_stop)*
[1] Tenofovir				

	[2] Zidovudine				
	[3] Lamivudine				
	[4] Emtricitabine				
	[5] Efavirenz				
	[6] Nevirapine				
	[7] Dolutegravir				
	[8] Abacavir				

35	Which medicines other than TB or HIV drugs are you currently taking?				
	Name of Drug *(d35_meds)*	Dose *(d35_medDose)*	Frequency *(d35_medFreq)*	Start Date *(d35_medStart)*	Stop Date *(d35_medStop)*
	[1] Cotrimoxazole				
	[2] Pyridoxine				
	[3]				
	[4]				
	[5]				
	[6]				
	[7]				

36	TB Medication Information					
	Name of Drug (FDC) *(d36_dot)*	Body weight *(d36_dotWeight)*	Number of Tablets *(d36_dotTablets)*	Start Date *(d36_dotStart)*	Stop Date *(d36_dotStop)*	Pills pick up
Initiation Phase	[1] Rifampicin 150mg					Week 0 [ ] Week 2 [ ]
	[2] Isoniazid 75mg					Week 4 [ ] Week 6 [ ]

	[3] Pyrazinamide 400mg					Week 8 [ ]
	[4] Ethambutol 275mg					
Continuation Phase	[1] Rifampicin 150mg					Month 3 [ ] Month 4 [ ] Month 5 [ ] Month 6 [ ]
	[2] Isoniazid 75mg					

37	Which of these events have you experienced (or been experiencing) after starting the medication? Tick (√) all that apply	Gastrointestinal disturbances [1] Yes [0] No	[ ]	d37_gastro
		Fever [1] Yes [0] No	[ ]	d37_fever
		Headache [1] Yes [0] No	[ ]	d37_headache
		Myalgia [1] Yes [0] No		d37_myalgia
		Nausea/vomiting [1] Yes [0] No	[ ]	*d37*_nausea
		Exfoliative dermatitis/ Skin disease [1] Yes [0] No	[ ]	d37_skin
		Epigastric pain [1] Yes [0] No	[ ]	d37_epigastric
		Pruritus [1] Yes [0] No	[ ]	d37_pruritus
		Arthralgia [1] Yes [0] No	[ ]	d37_arthralgia
		Liver disease [1] Yes [0] No	[ ]	d37_liverDx
		Acne [1] Yes [0] No	[ ]	d37_acne
		Decreased visual acuity [1] Yes [0] No	[ ]	d37_visual
		Hypersensitivity reactions [1] Yes [0] No	[ ]	d37_reactions
		Arthritis [1] Yes [0] No		d37_arthritis
		Peripheral neuropathy		d37_neuropa

		[1] Yes [0] No		thy
		Convulsions [1] Yes [0] No		d37_convulsions
		Insomnia [1] Yes [0] No		d37_insomnia
		Dizziness [1] Yes [0] No		d37_dizziness
		Other, specify:		

PART E: Behavioural characteristics					
38	Anti-TB medication				
	DOT Doses Received at last refill (e38_received)	DOT Doses Administered (e38_admin)	DOT Doses Missed (e38_missed)	DOT doses left on blister pack (e38_blister)	Reasons Given for Missing Doses

PART F: TREATMENT OUTCOMES				
53	Treatment Outcomes	[1] Cured [2] Treatment completed [3] Lost to follow up [4] Treatment failed [5] Died	_____	f53_outcomes

BLOOD SAMPLE TAKEN? [ ] YES [ ] NO

DATE SAMPLE WAS TAKEN …… (DD/MM/YYYY)

STUDY VISIT 2
WEEK 8

Hospital ID *(hospID)*:_____ Participant's ID *(studyID)*: _____ Location *(location)*:_____ Participant's Tel: _____ Supporter's Tel:_____	Date of TB diagnosis:_____ *(diagnosed)* (dd-mm-yyyy) Date of Recruitment: _____ *(recruit)* (dd-mm-yyyy) Date of Interview:_____ *(interview)* (dd-mm-yyyy)

Participant's Name: _____
Municipality_____ Municipality code [ ][ ]
Facility_____ Facility code [ ][ ][ ][ ]
Instruction: Please Tick (√) appropriate response and write its code in the empty box

CLINICAL CHARACTERISTICS				
16	Which of the following symptoms do you have now?	Cough [1] Yes [0] No	[ ]	b16_cough
		Fever [1] Yes [0] No	[ ]	b16_fever
		Haemoptysis [1] Yes [0] No	[ ]	b16_haemoptysis
		Loss of appetite [1] Yes [0] No	[ ]	b16_appetite
		Dyspnoea [1] Yes [0] No	[ ]	b16_dyspnoea
		Shortness of breath [1] Yes [0] No	[ ]	b16_orthopnoea
		Night sweats [1] Yes [0] No	[ ]	b16_nSweats
		Finger clubbing [1] Yes [0] No	[ ]	b16_finClubbing
		chest pains [1] Yes [0] No	[ ]	b16_chestPains
		Loss of weight [1] Yes [0] No	[ ]	b16_weightLoss
		Headache [1] Yes [0] No	[ ]	b16_headache
		Vomiting [1] Yes [0] No	[ ]	b16_vomiting
		Diarrhoea [1] Yes [0] No	[ ]	b16_diarrhoea
		Chills [1] Yes [0] No	[ ]	b16_chills

		Others, Specify:		

Laboratory Tests				
No.	Test	Results	Date test was done dd-mm-yyyy	
23	Fasting blood sugar	_____	_____	c23_fbs
24	HIV status	[1] Positive [2] Negative [3] Not Known	_____	c24_hivStatus
25	HIV clinical stage	[1] Stage I [2] Stage II [3] Stage III [4] Stage IV [5] Not known	_____	c25_hivStage
26	CD4 cell count	_____cells/ml	_____	c26_cd4
27	Chest X-Ray; Extent of lesions Tick (√) all that apply	[1] Old lesion [2] Active lesion [3] Calcification [4] Fibrosis [5] Infiltrates [6] Cavity	_____	c27_xray
28	Smear Microscopy	[1] Negative [2] scanty [3] + [4] ++ [5] +++	_____	c28_microscope
29	Culture	[1] Positive [2] Negative [3] Not known	_____	c29_culture

PART D: Medical and Drug History				
30	Which other diseases were diagnosed within the past week? Tick (√) all that apply	Hypertension [1] Yes [0] No	[ ]	d30_htn
		Diabetes mellitus [1] Yes [0] No	[ ]	d30_dm
		HIV [1] Yes [0] No	[ ]	d30_hiv
		Other STI [1] Yes [0] No	[ ]	d30_sti
		Respiratory Tract Infection [1] Yes [0] No	[ ]	d30_rti
		Pneumonia [1] Yes [0] No	[ ]	d30_pneumo
		Urinary Tract Infection [1] Yes [0] No	[ ]	d30_uti
		Malaria [1] Yes [0] No	[ ]	d30_malaria
		Prurigo [1] Yes [0] No	[ ]	d30_prurigo
		Skin disease [1] Yes [0] No	[ ]	d30_skinDx
		Anaemia [1] Yes [0] No	[ ]	d30_anaemia
		Other, specify:		
31	Do you have any chronic disease?	[1]Yes [2] No [3] Don't know	[ ]	d31_chronic Dx
32	Which diseases are those? Tick (√) all that apply	Cardiovascular disease [1] Yes [0] No	[ ]	d32_cardio
		Diabetes mellitus [1] Yes [0] No	[ ]	d32_dm
		Kidney disease [1] Yes [0] No	[ ]	d32_kidneyDx
		Liver disease [1] Yes [0] No	[ ]	d32_liverDx
		Cancer [1] Yes [0] No	[ ]	d32_cancer
		Other, specify		

	Skip to 35 if **NOT** an ART client

33	Have you started taking ART drugs?	[1] Yes [0] No	[ ]	*d33_start ART*

If 'No" **skip** to Question 35

34 Which ART drug(s) are you currently taking?

Name of Drug (d32_arv)	Dose *(d34_dose)*	Frequency *(d34_freq)*	Start Date *(d34_start)*	Stop Date *(d34_stop)*
[1] Tenofovir				
[2] Zidovudine				
[3] Lamivudine				
[4] Emtricitabine				
[5] Efavirenz				
[6] Nevirapine				
[7] Dolutegravir				
[8] Abacavir				

35 Which medicines other than TB or HIV drugs are you currently taking?

Name of Drug *(d35_meds)*	Dose *(d35_medDose)*	Frequency *(d35_medFreq)*	Start Date *(d35_medStart)*	Stop Date *(d35_medStop)*
[1] Cotrimoxazole				
[2] Pyridoxine				
[3]				
[4]				
[5]				
[6]				
[7]				

36 TB Medication Information

Name of Drug (FDC)	Body	Number	Start	Stop	Pills pick up

	(d36_dot)	weight *(d36_dot Weight)*	of Tablets *(d36_dot Tablets)*	Date *(d36_dot Start)*	Date *(d36_dot Stop)*	
Initiation Phase	[1] Rifampicin 150mg					Week 0 [ ] Week 2 [ ] Week 4 [ ] Week 6 [ ] Week 8 [ ]
	[2] Isoniazid 75mg					
	[3] Pyrazinamide 400mg					
	[4] Ethambutol 275mg					
Continuation Phase	[1] Rifampicin 150mg					Month 3 [ ] Month 4 [ ] Month 5 [ ] Month 6 [ ]
	[2] Isoniazid 75mg					

37	Which of these events have you experienced (or been experiencing) after starting the medication?	Gastrointestinal disturbances [1] Yes [0] No	[ ]	d37_gastro
		Fever [1] Yes [0] No	[ ]	d37_fever
		Headache [1] Yes [0] No	[ ]	d37_headache
		Myalgia [1] Yes [0] No		d37_myalgia
		Nausea/vomiting [1] Yes [0] No	[ ]	*d37*_nausea
		Exfoliative dermatitis/ Skin disease [1] Yes [0] No	[ ]	d37_skin
		Epigastric pain [1] Yes [0] No	[ ]	d37_epigastric
		Pruritus [1] Yes [0] No	[ ]	d37_pruritus
		Arthralgia [1] Yes [0] No	[ ]	d37_arthralgia
		Liver disease [1] Yes [0] No	[ ]	d37_liverDx
		Acne	[ ]	d37_acne

	Tick (√) all that apply	[1] Yes [0] No		
		Decreased visual acuity [1] Yes [0] No	[ ]	d37_visual
		Hypersensitivity reactions [1] Yes [0] No	[ ]	d37_reactions
		Arthritis [1] Yes [0] No		d37_arthritis
		Peripheral neuropathy [1] Yes [0] No		d37_neuropathy
		Convulsions [1] Yes [0] No		d37_convulsions
		Insomnia [1] Yes [0] No		d37_insomnia
		Dizziness [1] Yes [0] No		d37_dizziness
		Other, specify:		

PART E: Behavioural characteristics					
38	Anti-TB medication				
	DOT Doses Received at last refill (e38_received)	DOT Doses Administered (e38_admin)	DOT Doses Missed (e38_missed)	DOT doses left on blister pack (e38_blister)	Reasons Given for Missing Doses

PART F: TREATMENT OUTCOMES

53	Treatment Outcomes	[1] Cured [2] Treatment completed [3] Lost to follow up [4] Treatment failed [5] Died	_____	f53_outcomes

BLOOD SAMPLE TAKEN? [ ] YES [ ] NO

DATE SAMPLE WAS TAKEN ……. (DD/MM/YYYY)

STUDY VISIT 3
MONTH 3

Hospital ID *(hospID)*:_____	Date of TB diagnosis:_____

Participant's ID *(studyID)*: _____ Location *(location)*:_____ Participant's Tel: _____ Supporter's Tel:_____	*(diagnosed)* (dd-mm-yyyy) Date of Recruitment: _____ *(recruit)* (dd-mm-yyyy) Date of Interview:_____ *(interview)* (dd-mm-yyyy)

Participant's Name: _____
Municipality_____ Municipality code [ ][ ]
Facility_____ Facility code [ ][ ][ ][ ]
Instruction: Please Tick (√) appropriate response and write its code in the empty box

CLINICAL CHARACTERISTICS				
16	Which of the following symptoms do you have now?	Cough [1] Yes [0] No	[ ]	b16_cough
		Fever [1] Yes [0] No	[ ]	b16_fever
		Haemoptysis [1] Yes [0] No	[ ]	b16_haemoptysis
		Loss of appetite [1] Yes [0] No	[ ]	b16_appetite
		Dyspnoea [1] Yes [0] No	[ ]	b16_dyspnoea
		Shortness of breath [1] Yes [0] No	[ ]	b16_orthopnoea
		Night sweats [1] Yes [0] No	[ ]	b16_nSweats
		Finger clubbing [1] Yes [0] No	[ ]	b16_finClubbing
		chest pains [1] Yes [0] No	[ ]	b16_chestPains
		Loss of weight [1] Yes [0] No	[ ]	b16_weightLoss
		Headache [1] Yes [0] No	[ ]	b16_headache
		Vomiting [1] Yes [0] No	[ ]	b16_vomiting
		Diarrhoea [1] Yes [0] No	[ ]	b16_diarrhoea
		Chills [1] Yes [0] No	[ ]	b16_chills
		Others, Specify:		

Laboratory Tests				
No.	Test	Results	Date test was done dd-mm-yyyy	
23	Fasting blood sugar	_____	_____	c23_fbs

24	HIV status	[1] Positive [2] Negative [3] Not Known	_____	c24_hivStatus
25	HIV clinical stage	[1] Stage I [2] Stage II [3] Stage III [4] Stage IV [5] Not known	_____	c25_hivStage
26	CD4 cell count	_____cells/ml	_____	c26_cd4
27	Chest X-Ray; Extent of lesions Tick (√) all that apply	[1] Old lesion [2] Active lesion [3] Calcification [4] Fibrosis [5] Infiltrates [6] Cavity	_____	c27_xray
28	Smear Microscopy	[1] Negative [2] scanty [3] + [4] ++ [5] +++	_____	c28_microscope
29	Culture	[1] Positive [2] Negative [3] Not known	_____	c29_culture

PART D: Medical and Drug History				
30	Which other diseases were diagnosed within the past week?	Hypertension [1] Yes [0] No	[ ]	d30_htn
		Diabetes mellitus [1] Yes [0] No	[ ]	d30_dm
		HIV [1] Yes [0] No	[ ]	d30_hiv
		Other STI [1] Yes [0] No	[ ]	d30_sti
		Respiratory Tract Infection [1] Yes [0] No	[ ]	d30_rti
		Pneumonia [1] Yes [0] No	[ ]	d30_pneumo

	Tick (√) all that apply	Urinary Tract Infection [1] Yes [0] No	[ ]	d30_uti
		Malaria [1] Yes [0] No	[ ]	d30_malaria
		Prurigo [1] Yes [0] No	[ ]	d30_prurigo
		Skin disease [1] Yes [0] No	[ ]	d30_skinDx
		Anaemia [1] Yes [0] No	[ ]	d30_anaemia
		Other, specify:		
31	Do you have any chronic disease?	[1]Yes [2] No [3] Don't know	[ ]	d31_chronic Dx
32	Which diseases are those? Tick (√) all that apply	Cardiovascular disease [1] Yes [0] No	[ ]	d32_cardio
		Diabetes mellitus [1] Yes [0] No	[ ]	d32_dm
		Kidney disease [1] Yes [0] No	[ ]	d32_kidneyDx
		Liver disease [1] Yes [0] No	[ ]	d32_liverDx
		Cancer [1] Yes [0] No	[ ]	d32_cancer
		Other, specify		

	Skip to 35 if **NOT** an ART client			
33	Have you started taking ART drugs?	[1] Yes [0] No	[ ]	*d33_start ART*
If 'No" **skip** to Question 35				
34	Which ART drug(s) are you currently taking?			

Name of Drug (d32_arv)	Dose *(d34_dose)*	Frequency *(d34_freq)*	Start Date *(d34_start)*	Stop Date *(d34_stop)*
[1] Tenofovir				
[2] Zidovudine				

	[3] Lamivudine				
	[4] Emtricitabine				
	[5] Efavirenz				
	[6] Nevirapine				
	[7] Dolutegravir				
	[8] Abacavir				

35	Which medicines other than TB or HIV drugs are you currently taking?				
	Name of Drug *(d35_meds)*	Dose *(d35_medDose)*	Frequency *(d35_medFreq)*	Start Date *(d35_medStart)*	Stop Date *(d35_medStop)*
	[1] Cotrimoxazole				
	[2] Pyridoxine				
	[3]				
	[4]				
	[5]				
	[6]				
	[7]				

36	TB Medication Information					
	Name of Drug (FDC) *(d36_dot)*	Body weight *(d36_dotWeight)*	Number of Tablets *(d36_dotTablets)*	Start Date *(d36_dotStart)*	Stop Date *(d36_dotStop)*	Pills pick up
Initiation Phase	[1] Rifampicin 150mg					Week 0 [ ] Week 2 [ ] Week 4 [ ] Week 6 [ ] Week 8 [ ]
	[2] Isoniazid 75mg					
	[3] Pyrazinamide 400mg					

	[4] Ethambutol 275mg					
Continuation Phase	[1] Rifampicin 150mg					Month 3 [ ] Month 4 [ ] Month 5 [ ] Month 6 [ ]
	[2] Isoniazid 75mg					

37	Which of these events have you experienced (or been experiencing) after starting the medication? Tick (√) all that apply	Gastrointestinal disturbances [1] Yes [0] No	[ ]	d37_gastro
		Fever [1] Yes [0] No	[ ]	d37_fever
		Headache [1] Yes [0] No	[ ]	d37_headache
		Myalgia [1] Yes [0] No		d37_myalgia
		Nausea/vomiting [1] Yes [0] No	[ ]	*d37*_nausea
		Exfoliative dermatitis/ Skin disease [1] Yes [0] No	[ ]	d37_skin
		Epigastric pain [1] Yes [0] No	[ ]	d37_epigastric
		Pruritus [1] Yes [0] No	[ ]	d37_pruritus
		Arthralgia [1] Yes [0] No	[ ]	d37_arthralgia
		Liver disease [1] Yes [0] No	[ ]	d37_liverDx
		Acne [1] Yes [0] No	[ ]	d37_acne
		Decreased visual acuity [1] Yes [0] No	[ ]	d37_visual
		Hypersensitivity reactions [1] Yes [0] No	[ ]	d37_reactions
		Arthritis [1] Yes [0] No		d37_arthritis
		Peripheral neuropathy [1] Yes [0] No		d37_neuropathy
		Convulsions		d37_convulsi

		[1] Yes [0] No		ons
		Insomnia [1] Yes [0] No		d37_insomnia
		Dizziness [1] Yes [0] No		d37_dizziness
		Other, specify:		

PART E: Behavioural characteristics						
38	Anti-TB medication					
	DOT Doses Received at last refill (e38_received)	DOT Doses Administered (e38_admin)	DOT Doses Missed (e38_missed)	DOT doses left on blister pack (e38_blister)	Reasons Given for Missing Doses	

PART F: TREATMENT OUTCOMES				
53	Treatment Outcomes	[1] Cured [2] Treatment completed [3] Lost to follow up [4] Treatment failed [5] Died	_____	f53_outcomes

BLOOD SAMPLE TAKEN? [ ] YES [ ] NO

DATE SAMPLE WAS TAKEN ……. (DD/MM/YYYY)

STUDY VISIT 4
MONTH 4

Hospital ID *(hospID)*:_____ Participant's ID *(studyID)*: _____ Location *(location)*:_____ Participant's Tel: _____ Supporter's Tel:_____	Date of TB diagnosis:_____ *(diagnosed)* (dd-mm-yyyy) Date of Recruitment: _____ *(recruit)* (dd-mm-yyyy) Date of Interview:_____ *(interview)* (dd-mm-yyyy)

Participant's Name: _____

Municipality_____ Municipality code [ ][ ]
Facility_____ Facility code [ ][ ][ ][ ]
Instruction: Please Tick (√) appropriate response and write its code in the empty box

CLINICAL CHARACTERISTICS				
16	Which of the following symptoms do you have now?	Cough [1] Yes [0] No	[ ]	b16_cough
		Fever [1] Yes [0] No	[ ]	b16_fever
		Haemoptysis [1] Yes [0] No	[ ]	b16_haemoptysis
		Loss of appetite [1] Yes [0] No	[ ]	b16_appetite
		Dyspnoea [1] Yes [0] No	[ ]	b16_dyspnoea
		Shortness of breath [1] Yes [0] No	[ ]	b16_orthopnoea
		Night sweats [1] Yes [0] No	[ ]	b16_nSweats
		Finger clubbing [1] Yes [0] No	[ ]	b16_finClubbing
		chest pains [1] Yes [0] No	[ ]	b16_chestPains
		Loss of weight [1] Yes [0] No	[ ]	b16_weightLoss
		Headache [1] Yes [0] No	[ ]	b16_headache
		Vomiting [1] Yes [0] No	[ ]	b16_vomiting
		Diarrhoea [1] Yes [0] No	[ ]	b16_diarrhoea
		Chills [1] Yes [0] No	[ ]	b16_chills
		Others, Specify:		

Laboratory Tests				
No.	Test	Results	Date test was done dd-mm-yyyy	
23	Fasting blood sugar	_____	_____	c23_fbs
24	HIV status	[1] Positive [2] Negative [3] Not Known	_____	c24_hivStatus
25	HIV clinical stage	[1] Stage I [2] Stage II [3] Stage III [4] Stage IV [5] Not known	_____	c25_hivStage
26	CD4 cell count	_____cells/ml	_____	c26_cd4

27	Chest X-Ray; Extent of lesions Tick (√) all that apply	[1] Old lesion [2] Active lesion [3] Calcification [4] Fibrosis [5] Infiltrates [6] Cavity	_____	c27_xray
28	Smear Microscopy	[1] Negative [2] scanty [3] + [4] ++ [5] +++	_____	c28_microscope
29	Culture	[1] Positive [2] Negative [3] Not known	_____	c29_culture

PART D: Medical and Drug History				
30	Which other diseases were diagnosed within the past week? Tick (√) all that apply	Hypertension [1] Yes [0] No	[ ]	d30_htn
		Diabetes mellitus [1] Yes [0] No	[ ]	d30_dm
		HIV [1] Yes [0] No	[ ]	d30_hiv
		Other STI [1] Yes [0] No	[ ]	d30_sti
		Respiratory Tract Infection [1] Yes [0] No	[ ]	d30_rti
		Pneumonia [1] Yes [0] No	[ ]	d30_pneumo
		Urinary Tract Infection [1] Yes [0] No	[ ]	d30_uti
		Malaria [1] Yes [0] No	[ ]	d30_malaria
		Prurigo [1] Yes [0] No	[ ]	d30_prurigo
		Skin disease [1] Yes [0] No	[ ]	d30_skinDx
		Anaemia	[ ]	d30_anaemia

		[1] Yes [0] No		
		Other, specify:		
31	Do you have any chronic disease?	[1]Yes [2] No [3] Don't know	[ ]	d31_chronic Dx
32	Which diseases are those? Tick (√) all that apply	Cardiovascular disease [1] Yes [0] No	[ ]	d32_cardio
		Diabetes mellitus [1] Yes [0] No	[ ]	d32_dm
		Kidney disease [1] Yes [0] No	[ ]	d32_kidneyDx
		Liver disease [1] Yes [0] No	[ ]	d32_liverDx
		Cancer [1] Yes [0] No	[ ]	d32_cancer
		Other, specify		

	Skip to 35 if **NOT** an ART client			
33	Have you started taking ART drugs?	[1] Yes [0] No	[ ]	*d33_start ART*
If 'No" **skip** to Question 35				
34	Which ART drug(s) are you currently taking?			

Name of Drug (d32_arv)	Dose *(d34_dose)*	Frequency *(d34_freq)*	Start Date *(d34_start)*	Stop Date *(d34_stop)*
[1] Tenofovir				
[2] Zidovudine				
[3] Lamivudine				
[4] Emtricitabine				
[5] Efavirenz				
[6] Nevirapine				
[7] Dolutegravir				
[8] Abacavir				

35	Which medicines other than TB or HIV drugs are you currently taking?				
	Name of Drug *(d35_meds)*	Dose *(d35_medDose)*	Frequency *(d35_medFreq)*	Start Date *(d35_medStart)*	Stop Date *(d35_medStop)*
	[1] Cotrimoxazole				
	[2] Pyridoxine				
	[3]				
	[4]				
	[5]				
	[6]				
	[7]				

36	TB Medication Information					
	Name of Drug (FDC) *(d36_dot)*	Body weight *(d36_dotWeight)*	Number of Tablets *(d36_dotTablets)*	Start Date *(d36_dotStart)*	Stop Date *(d36_dotStop)*	Pills pick up
Initiation Phase	[1] Rifampicin 150mg					Week 0 [ ] Week 2 [ ] Week 4 [ ] Week 6 [ ] Week 8 [ ]
	[2] Isoniazid 75mg					
	[3] Pyrazinamide 400mg					
	[4] Ethambutol 275mg					
Continuation Phase	[1] Rifampicin 150mg					Month 3 [ ] Month 4 [ ] Month 5 [ ] Month 6 [ ]
	[2] Isoniazid 75mg					

37	Which of these events have you experienced (or been experiencing) after starting the medication?			
		Gastrointestinal disturbances [1] Yes [0] No	[ ]	d37_gastro
		Fever [1] Yes [0] No	[ ]	d37_fever
		Headache [1] Yes [0] No	[ ]	d37_headache
		Myalgia [1] Yes [0] No		d37_myalgia

	Tick (√) all that apply	Nausea/vomiting [1] Yes [0] No	[ ]	*d37*_nausea
		Exfoliative dermatitis/ Skin disease [1] Yes [0] No	[ ]	d37_skin
		Epigastric pain [1] Yes [0] No	[ ]	d37_epigastric
		Pruritus [1] Yes [0] No	[ ]	d37_pruritus
		Arthralgia [1] Yes [0] No	[ ]	d37_arthralgia
		Liver disease [1] Yes [0] No	[ ]	d37_liverDx
		Acne [1] Yes [0] No	[ ]	d37_acne
		Decreased visual acuity [1] Yes [0] No	[ ]	d37_visual
		Hypersensitivity reactions [1] Yes [0] No	[ ]	d37_reactions
		Arthritis [1] Yes [0] No		d37_arthritis
		Peripheral neuropathy [1] Yes [0] No		d37_neuropathy
		Convulsions [1] Yes [0] No		d37_convulsions
		Insomnia [1] Yes [0] No		d37_insomnia
		Dizziness [1] Yes [0] No		d37_dizziness
		Other, specify:		

PART E: Behavioural characteristics

38	Anti-TB medication				
	DOT Doses Received at last refill (e38_received)	DOT Doses Administered (e38_admin)	DOT Doses Missed (e38_missed)	DOT doses left on blister pack (e38_blister)	Reasons Given for Missing Doses

PART F: TREATMENT OUTCOMES

53	Treatment Outcomes	[1] Cured [2] Treatment completed [3] Lost to follow up [4] Treatment failed [5] Died	_____	f53_outcomes

BLOOD SAMPLE TAKEN? [ ] YES [ ] NO

DATE SAMPLE WAS TAKEN ……. (DD/MM/YYYY)

STUDY VISIT 5

MONTH 5

Hospital ID *(hospID)*:_____ Participant's ID *(studyID)*: _____ Location *(location)*:_____ Participant's Tel: _____ Supporter's Tel:_____	Date of TB diagnosis:_____ *(diagnosed)* (dd-mm-yyyy) Date of Recruitment: _____ *(recruit)* (dd-mm-yyyy) Date of Interview:_____ *(interview)* (dd-mm-yyyy)

Participant's Name: _____
Municipality_____ Municipality code [ ][ ]
Facility_____ Facility code [ ][ ][ ][ ]
Instruction: Please Tick (√) appropriate response and write its code in the empty box

CLINICAL CHARACTERISTICS				
16	Which of the	Cough [1] Yes [0] No	[ ]	b16_cough

	following symptoms do you have now?	Fever [1] Yes [0] No	[ ]	b16_fever
		Haemoptysis [1] Yes [0] No	[ ]	b16_haemoptysis
		Loss of appetite [1] Yes [0] No	[ ]	b16_appetite
		Dyspnoea [1] Yes [0] No	[ ]	b16_dyspnoea
		Shortness of breath [1] Yes [0] No	[ ]	b16_orthopnoea
		Night sweats [1] Yes [0] No	[ ]	b16_nSweats
		Finger clubbing [1] Yes [0] No	[ ]	b16_finClubbing
		chest pains [1] Yes [0] No	[ ]	b16_chestPains
		Loss of weight [1] Yes [0] No	[ ]	b16_weightLoss
		Headache [1] Yes [0] No	[ ]	b16_headache
		Vomiting [1] Yes [0] No	[ ]	b16_vomiting
		Diarrhoea [1] Yes [0] No	[ ]	b16_diarrhoea
		Chills [1] Yes [0] No	[ ]	b16_chills
		Others, Specify:		

Laboratory Tests				
No.	Test	Results	Date test was done dd-mm-yyyy	
23	Fasting blood sugar	_____	_____	c23_fbs
24	HIV status	[1] Positive [2] Negative [3] Not Known	_____	c24_hivStatus
25	HIV clinical stage	[1] Stage I [2] Stage II [3] Stage III [4] Stage IV [5] Not known	_____	c25_hivStage
26	CD4 cell count	_____cells/ml	_____	c26_cd4
27	Chest X-Ray; Extent of lesions Tick (√) all that apply	[1] Old lesion [2] Active lesion [3] Calcification [4] Fibrosis [5] Infiltrates [6] Cavity	_____	c27_xray
28	Smear Microscopy	[1] Negative [2] scanty [3] + [4] ++ [5] +++	_____	c28_microscope
29	Culture	[1] Positive [2] Negative [3] Not known	_____	c29_culture

PART D: Medical and Drug History				
30	Which other diseases were diagnosed within the past week?	Hypertension [1] Yes [0] No	[ ]	d30_htn
		Diabetes mellitus [1] Yes [0] No	[ ]	d30_dm
		HIV [1] Yes [0] No	[ ]	d30_hiv
		Other STI [1] Yes [0] No	[ ]	d30_sti
		Respiratory Tract Infection [1] Yes [0] No	[ ]	d30_rti
		Pneumonia [1] Yes [0] No	[ ]	d30_pneumo
		Urinary Tract Infection [1] Yes [0] No	[ ]	d30_uti
		Malaria [1] Yes [0] No	[ ]	d30_malaria

	Tick (√) all that apply	Prurigo [1] Yes [0] No	[ ]	d30_prurigo
		Skin disease [1] Yes [0] No	[ ]	d30_skinDx
		Anaemia [1] Yes [0] No	[ ]	d30_anaemia
		Other, specify:		
31	Do you have any chronic disease?	[1]Yes [2] No [3] Don't know	[ ]	d31_chronic Dx
32	Which diseases are those?	Cardiovascular disease [1] Yes [0] No	[ ]	d32_cardio
		Diabetes mellitus [1] Yes [0] No	[ ]	d32_dm
		Kidney disease [1] Yes [0] No	[ ]	d32_kidneyDx
	Tick (√) all that apply	Liver disease [1] Yes [0] No	[ ]	d32_liverDx
		Cancer [1] Yes [0] No	[ ]	d32_cancer
		Other, specify		

	Skip to 35 if **NOT** an ART client			
33	Have you started taking ART drugs?	[1] Yes [0] No	[ ]	*d33_start ART*
If 'No" **skip** to Question 35				

34	Which ART drug(s) are you currently taking?				
	Name of Drug (d32_arv)	Dose *(d34_dose)*	Frequency *(d34_freq)*	Start Date *(d34_start)*	Stop Date *(d34_stop)*
	[1] Tenofovir				
	[2] Zidovudine				
	[3] Lamivudine				
	[4] Emtricitabine				
	[5] Efavirenz				

	[6] Nevirapine				
	[7] Dolutegravir				
	[8] Abacavir				

35	Which medicines other than TB or HIV drugs are you currently taking?				
	Name of Drug *(d35_meds)*	Dose *(d35_medDose)*	Frequency *(d35_medFreq)*	Start Date *(d35_medStart)*	Stop Date *(d35_medStop)*
	[1] Cotrimoxazole				
	[2] Pyridoxine				
	[3]				
	[4]				
	[5]				
	[6]				
	[7]				

36	TB Medication Information					
	Name of Drug (FDC) *(d36_dot)*	Body weight *(d36_dotWeight)*	Number of Tablets *(d36_dotTablets)*	Start Date *(d36_dotStart)*	Stop Date *(d36_dotStop)*	Pills pick up
Initiation Phase	[1] Rifampicin 150mg					Week 0 [ ] Week 2 [ ] Week 4 [ ] Week 6 [ ] Week 8 [ ]
	[2] Isoniazid 75mg					
	[3] Pyrazinamide 400mg					
	[4] Ethambutol 275mg					
Continuation Phase	[1] Rifampicin 150mg					Month 3 [ ] Month 4 [ ]

	[2] Isoniazid 75mg					Month 5 [ ] Month 6 [ ]

37	Which of these events have you experienced (or been experiencing) after starting the medication? Tick (√) all that apply	Gastrointestinal disturbances [1] Yes [0] No	[ ]	d37_gastro
		Fever [1] Yes [0] No	[ ]	d37_fever
		Headache [1] Yes [0] No	[ ]	d37_headache
		Myalgia [1] Yes [0] No		d37_myalgia
		Nausea/vomiting [1] Yes [0] No	[ ]	*d37*_nausea
		Exfoliative dermatitis/ Skin disease [1] Yes [0] No	[ ]	d37_skin
		Epigastric pain [1] Yes [0] No	[ ]	d37_epigastric
		Pruritus [1] Yes [0] No	[ ]	d37_pruritus
		Arthralgia [1] Yes [0] No	[ ]	d37_arthralgia
		Liver disease [1] Yes [0] No	[ ]	d37_liverDx
		Acne [1] Yes [0] No	[ ]	d37_acne
		Decreased visual acuity [1] Yes [0] No	[ ]	d37_visual
		Hypersensitivity reactions [1] Yes [0] No	[ ]	d37_reactions
		Arthritis [1] Yes [0] No		d37_arthritis
		Peripheral neuropathy [1] Yes [0] No		d37_neuropathy
		Convulsions [1] Yes [0] No		d37_convulsions
		Insomnia [1] Yes [0] No		d37_insomnia
		Dizziness [1] Yes [0] No		d37_dizziness
		Other, specify:		

PART E: Behavioural characteristics

38	Anti-TB medication				
	DOT Doses Received at last refill (e38_received)	DOT Doses Administered (e38_admin)	DOT Doses Missed (e38_missed)	DOT doses left on blister pack (e38_blister)	Reasons Given for Missing Doses

PART F: TREATMENT OUTCOMES				
53	Treatment Outcomes	[1] Cured [2] Treatment completed [3] Lost to follow up [4] Treatment failed [5] Died	_____	f53_outcomes

BLOOD SAMPLE TAKEN? [ ] YES [ ] NO

DATE SAMPLE WAS TAKEN ……. (DD/MM/YYYY)

STUDY VISIT 6
MONTH 6

Hospital ID *(hospID)*:_____ Participant's ID *(studyID)*: _____ Location *(location)*:_____ Participant's Tel: _____ Supporter's Tel:_____	Date of TB diagnosis:_____ *(diagnosed)* (dd-mm-yyyy) Date of Recruitment: _____ *(recruit)* (dd-mm-yyyy) Date of Interview:_____ *(interview)* (dd-mm-yyyy)

Participant's Name: _____
Municipality_____ Municipality code [ ][ ]
Facility_____ Facility code [ ][ ][ ][ ]
Instruction: Please Tick (√) appropriate response and write its code in the empty box

CLINICAL CHARACTERISTICS				
16	Which of the following symptoms do you have now?	Cough [1] Yes [0] No	[ ]	b16_cough
		Fever [1] Yes [0] No	[ ]	b16_fever
		Haemoptysis [1] Yes [0] No	[ ]	b16_haemoptysis
		Loss of appetite [1] Yes [0] No	[ ]	b16_appetite
		Dyspnoea [1] Yes [0] No	[ ]	b16_dyspnoea
		Shortness of breath [1] Yes [0] No	[ ]	b16_orthopnoea
		Night sweats [1] Yes [0] No	[ ]	b16_nSweats
		Finger clubbing [1] Yes [0] No	[ ]	b16_finClubbing
		chest pains [1] Yes [0] No	[ ]	b16_chestPains
		Loss of weight [1] Yes [0] No	[ ]	b16_weightLoss
		Headache [1] Yes [0] No	[ ]	b16_headache
		Vomiting [1] Yes [0] No	[ ]	b16_vomiting
		Diarrhoea [1] Yes [0] No	[ ]	b16_diarrhoea
		Chills [1] Yes [0] No	[ ]	b16_chills
		Others, Specify:		

Laboratory Tests				
No.	Test	Results	Date test was done dd-mm-yyyy	
23	Fasting blood sugar	_____	_____	c23_fbs
24	HIV status	[1] Positive [2] Negative [3] Not Known	_____	c24_hivStatus
25	HIV clinical stage	[1] Stage I [2] Stage II [3] Stage III [4] Stage IV [5] Not known	_____	c25_hivStage
26	CD4 cell count	_____cells/ml	_____	c26_cd4
27	Chest X-Ray; Extent of lesions Tick (√) all that apply	[1] Old lesion [2] Active lesion [3] Calcification [4] Fibrosis [5] Infiltrates [6] Cavity	_____	c27_xray
28	Smear Microscopy	[1] Negative [2] scanty [3] + [4] ++ [5] +++	_____	c28_microscope
29	Culture	[1] Positive [2] Negative [3] Not known	_____	c29_culture

PART D: Medical and Drug History				
30	Which other diseases were diagnosed within the past week?	Hypertension [1] Yes [0] No	[ ]	d30_htn
		Diabetes mellitus [1] Yes [0] No	[ ]	d30_dm
		HIV [1] Yes [0] No	[ ]	d30_hiv
		Other STI [1] Yes [0] No	[ ]	d30_sti

	Tick (√) all that apply	Respiratory Tract Infection [1] Yes [0] No	[ ]	d30_rti
		Pneumonia [1] Yes [0] No	[ ]	d30_pneumo
		Urinary Tract Infection [1] Yes [0] No	[ ]	d30_uti
		Malaria [1] Yes [0] No	[ ]	d30_malaria
		Prurigo [1] Yes [0] No	[ ]	d30_prurigo
		Skin disease [1] Yes [0] No	[ ]	d30_skinDx
		Anaemia [1] Yes [0] No	[ ]	d30_anaemia
		Other, specify:		
31	Do you have any chronic disease?	[1]Yes [2] No [3] Don't know	[ ]	d31_chronic Dx
32	Which diseases are those? Tick (√) all that apply	Cardiovascular disease [1] Yes [0] No	[ ]	d32_cardio
		Diabetes mellitus [1] Yes [0] No	[ ]	d32_dm
		Kidney disease [1] Yes [0] No	[ ]	d32_kidneyDx
		Liver disease [1] Yes [0] No	[ ]	d32_liverDx
		Cancer [1] Yes [0] No	[ ]	d32_cancer
		Other, specify		

	Skip to 35 if **NOT** an ART client			
33	Have you started taking ART drugs?	[1] Yes [0] No	[ ]	*d33_start ART*
If 'No" **skip** to Question 35				
34	Which ART drug(s) are you currently taking?			

	Name of Drug (d32_arv)	Dose *(d34_dose)*	Frequency *(d34_freq)*	Start Date *(d34_start)*	Stop Date *(d34_stop)*
	[1] Tenofovir				
	[2] Zidovudine				
	[3] Lamivudine				
	[4] Emtricitabine				
	[5] Efavirenz				
	[6] Nevirapine				
	[7] Dolutegravir				
	[8] Abacavir				

35	Which medicines other than TB or HIV drugs are you currently taking?				
	Name of Drug *(d35_meds)*	Dose *(d35_medDose)*	Frequency *(d35_medFreq)*	Start Date *(d35_medStart)*	Stop Date *(d35_medStop)*
	[1] Cotrimoxazole				
	[2] Pyridoxine				
	[3]				
	[4]				
	[5]				
	[6]				
	[7]				

36	TB Medication Information					
	Name of Drug (FDC) *(d36_dot)*	Body weight *(d36_dotWeight)*	Number of Tablets *(d36_dotTablets)*	Start Date *(d36_dotStart)*	Stop Date *(d36_dotStop)*	Pills pick up
Initiation Phase	[1] Rifampicin 150mg					Week 0 [ ] Week 2 [ ] Week 4 [ ] Week 6 [ ] Week 8 [ ]
	[2] Isoniazid 75mg					
	[3] Pyrazinamide 400mg					
	[4] Ethambutol 275mg					

Continuation Phase	[1] Rifampicin 150mg					Month 3 [ ] Month 4 [ ] Month 5 [ ] Month 6 [ ]
	[2] Isoniazid 75mg					

37	Which of these events have you experienced (or been experiencing) after starting the medication? Tick (√) all that apply	Gastrointestinal disturbances [1] Yes [0] No	[ ]	d37_gastro
		Fever [1] Yes [0] No	[ ]	d37_fever
		Headache [1] Yes [0] No	[ ]	d37_headache
		Myalgia [1] Yes [0] No		d37_myalgia
		Nausea/vomiting [1] Yes [0] No	[ ]	*d37*_nausea
		Exfoliative dermatitis/ Skin disease [1] Yes [0] No	[ ]	d37_skin
		Epigastric pain [1] Yes [0] No	[ ]	d37_epigastric
		Pruritus [1] Yes [0] No	[ ]	d37_pruritus
		Arthralgia [1] Yes [0] No	[ ]	d37_arthralgia
		Liver disease [1] Yes [0] No	[ ]	d37_liverDx
		Acne [1] Yes [0] No	[ ]	d37_acne
		Decreased visual acuity [1] Yes [0] No	[ ]	d37_visual
		Hypersensitivity reactions [1] Yes [0] No	[ ]	d37_reactions
		Arthritis [1] Yes [0] No		d37_arthritis
		Peripheral neuropathy [1] Yes [0] No		d37_neuropathy
		Convulsions [1] Yes [0] No		d37_convulsions
		Insomnia		d37_insomni

		[1] Yes [0] No		a
		Dizziness [1] Yes [0] No		d37_dizziness
		Other, specify:		

PART E: Behavioural characteristics						
38	Anti-TB medication					
	DOT Doses Received at last refill (e38_received)	DOT Doses Administered (e38_admin)	DOT Doses Missed (e38_missed)	DOT doses left on blister pack (e38_blister)	Reasons Given for Missing Doses	

PART F: TREATMENT OUTCOMES				
53	Treatment Outcomes	[1] Cured [2] Treatment completed [3] Lost to follow up [4] Treatment failed [5] Died	_____	f53_outcomes

BLOOD SAMPLE TAKEN? [ ] YES [ ] NO

DATE SAMPLE WAS TAKEN …… (DD/MM/YYYY)

APPENDIX C: ETHICAL CLEARANCE – GHS-ERC

GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

My Ref. GHS/RDD/ERC/Admin/App | 21 | 125
Your Ref. No.

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra
Digital Address: GA-050-3303
Mob: +233-50-3539896
Tel: +233-302-681109
Fax + 233-302-685424
Email: ethics.research@ghsmail.org
26th April, 2021

Michael Mireku Opoku
University of Ghana
School of Public Health
P. O. Box LG 13
Legon, Accra

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol.

GHS-ERC Number	**GHS-ERC 002/02/21**
Study Title	Pharmacologic and Clinical Risk Factors of Poor TB Treatment Outcomes in Patients with Rifampicin –Susceptible TB in Five Selected Hospitals in the Ashanti Region of Ghana
Approval Date	26th April, 2021
Expiry Date	25th April, 2022
GHS-ERC Decision	**Approved**

This approval requires the following from the Principal Investigator

- Submission of a yearly progress report of the study to the Ethics Review Committee (ERC)
- Renewal of ethical approval if the study lasts for more than 12 months,
- Reporting of all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.
- Submission of a final report after completion of the study
- Informing ERC if study cannot be implemented or is discontinued and reasons why
- Informing the ERC and your sponsor (where applicable) before any publication of the research findings.

You are kindly advised to adhere to the national guidelines or protocols on the prevention of COVID -19

Please note that any modification of the study without ERC approval of the amendment is invalid.

The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Kindly quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED.....
Dr. Cynthia Bannerman
(GHS ERC Chairperson)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra

APPENDIX D: ETHICAL CLEARANCE RENEWAL – GHS-ERC

GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

My Ref:ghs/rdd/erc-admin/ren/app/22
Your Ref. No.

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra.
Digital Address: GA-050-3303

Tel: +233-0302-960628
Mob + 233-050-3539896
Email: ethics.research@ghsmail.org

18th April 2022

Michael Mireku Opoku
University of Ghana
School of Public Health
P. O. Box LG 13 Legon, Accra

RE: REQUEST FOR RENEWAL OF ETHICAL APPROVAL

Reference is made to your letter dated 1st April 2022 on the above subject matter.

Please be informed that the Ghana Health Service Ethics Review Committee has reviewed the request and has given approval for renewal of the ERC letter.

GHS-ERC Number	**GHS-ERC: 002/02/21**
Study Title	Pharmacologic and Clinical Risk Factors of Poor TB Treatment Outcomes in Patients with Rifampicin-Susceptible TB in Selected Hospitals in the Ashanti, Bono and Bono East Regions of Ghana
Effective Date of Renewal	25th April, 2022
Expiry Date	24th April, 2023
GHS-ERC Decision	**Renewal Approved**

The following applies:

- Submission of yearly progress report of the study to the Ethics Review Committee (ERC).
- Renewal of ethical approval if the study lasts for more than 12 months.
- Reporting of all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.
- Submission of a final report **after completion** of the study.
- Informing the ERC if study is discontinued and reasons why.

1

- Informing the ERC and your sponsor (where applicable) before any publication of the research findings.

You are kindly advised to adhere to the national guidelines or protocols on the prevention of COVID -19

Please note that any modification of the study without ERC approval of the amendment is invalid.

The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Kindly quote the protocol identification number in all future correspondence in relation to this approval protocol.

SIGNED.....
Dr Cynthia Bannerman
(GHS-ERC Chairperson)

Cc: The Director, Research and Development Division, Ghana Health Service, Accra

APPENDIX E: ETHICAL CLEARANCE – KATH-IRB

KOMFO ANOKYE
TEACHING HOSPITAL

P. O. Box 1934
Kumasi - Ghana
Tel: +233 - 3200-22301 - 4
Fax: +233 - 3220-24654 / 24621
Website: www.kathhsp.org

Our Ref. No.: KATH IRB/AP/023/21

Your Ref... No:.....

Komfo Anokye Teaching Hospital Institutional Review Board

25th May 2021

Mr. Michael Mireku Opoku
University of Ghana, School of Public Health,
Department of Epidemiology and Disease Control;
Accra

Dear Mr. Opoku,

Ethics Approval

Protocol title:	Pharmacologic and clinical risk factors of poor TB treatment outcomes in patients with Rifampicin-Susceptible TB in Ghana
Study site:	Komfo Anokye Teaching Hospital, Kumasi.
Sponsor:	NIH Fogarty PhD Scholarship Award UG-Florida Partnership for TB/HIV Research Training in Ghana

We write in response to the clarifications and revised documents following review by the Komfo Anokye Teaching Hospital Institutional Review Board (KATH IRB) in respect of the research study referenced above.

We are pleased to inform you that KATH IRB, per your correspondence of 9th April 2021, has given approval for the following study documents:

- *Protocol version 2 last updated 9th April 2021*
- *Informed consent form version 2 last updated 9th April 2021*
- *Assent form (15 to 17 years) version 2 last updated 9th April 2021*
- *Case report form version 2 last updated 9th April 2021*
- *Material Transfer Agreement dated 4th May, 2021*

Approval for the study is in effect until **24th May 2022** and it is the responsibility of the Principal Investigator to maintain the study in good standing at the Komfo Anokye Teaching Hospital. The Board anticipates to be notified of the actual start date of your project.

Prior to the expiration of the study approval, you must submit to the KATH IRB an "Application for Continuing Review" along with provision of "Annual Report" when the study is ongoing, or a "Termination Report" if the research has been completed.

You must hastily report to the KATH IRB should a modification to the research be proposed, and without delay if an unanticipated development occurs before the next required review. Regulations do not permit you to modify conduct of the study in its present form prior to ethics approval; except where urgent action is required to eliminate an apparent immediate hazard to a study subject or other person. It is of utmost importance data generated from this study must be used for the intended purposes only.

Thank you.

Sincerely,

Prof. Kwabena Antwi Danso, BSc, MB ChB, FWACS, FGCS, FACOG
Chairman, KATH IRB

APPENDIX F: ETHICAL CLEARANCE RENEWAL – KATH-IRB

KOMFO ANOKYE
TEACHING HOSPITAL

P. O. Box 1934
Kumasi - Ghana
Tel: +233 - 3200-22301 - 4
Fax: +233 - 3220-24654 / 24621
Website: www.kathhsp.org

Our Ref. No.: KATH IRB/CR01/023/22

Your Ref... No:.....

Komfo Anokye Teaching Hospital Institutional Review Board

4th April 2022

Mr. Michael Mireku Opoku
University of Ghana, School of Public Health,
Department of Epidemiology and Disease Control;
Accra

Dear Mr. Opoku,

Continuing Review Approval # 01

Protocol title:	Pharmacologic and clinical risk factors of poor TB treatment outcomes in patients with Rifampicin-Susceptible TB in Ghana
Study site:	Komfo Anokye Teaching Hospital, Kumasi.
Sponsor:	NIH Fogarty PhD Scholarship Award UG-Florida Partnership for TB/HIV Research Training in Ghana

I write in respect of your request for continuing review approval of the research study referenced above. The Komfo Anokye Teaching Hospital Institutional Review Board (KATH IRB) reviewed the report for your study covering the period from 1st August 2021 to 29th April 2022.

The Board has given approval for the continuation of the research study for the next one year from **25th May 2022 to 24th May 2023.** It is the responsibility of the principal investigator to maintain the study in good standing at KATH.

Should any revision to the study, or other unanticipated development occur prior to the next required review, you must inform the Board without delay. It is of utmost importance that data generated from this study must be used for the intended purposes only.

Sincerely,

Prof. Kwabena Antwi Danso, BSc, MB ChB, FWACS, FGCS, FACOG
Chairman, KATH IRB

APPENDIX G: AMENDED PROTOCOL – GHS-ERC

GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted.

My Ref:ghs/rdd/erc/Admin/amend/app/ 21
Your Ref. No.

Research & Development Divis
Ghana Health Service
P. O. Box MB 190
Accra.
Digital Address GA-050-3303
Mob: +233-50-3539896
Tel: +233-0302681109
Fax: + 233-0302685424
Email: ethics research a ghsma

4th December 2021

Michael Mireku Opoku
School of Public Health
University of Ghana
Legon, Accra

RE: Request for Ethical Approval to Amended Protocol

Reference is made to your letter dated 23rd November 2021 on the above subject matter.

The Ghana Health Service Ethics Review Committee (GHS-ERC) has reviewed the documents submitted, and the rationale for the request for amendment. The GHS-ERC has given approval for the amendment to be implemented.

GHS-ERC Number	**GHSERC: 002/02/21**
Study Title	Pharmacologic and Clinical Risk Factors of Poor TB Treatment Outcomes in Patients with Rifampicin-Susceptible TB in Selected Hospitals in the Ashanti, Bono and Bono East Region of Ghana
Effective Date for Approval of Amendment	4th December, 2021
Expiry Date	25th April, 2022
GHS-ERC Decision	**Amendment Version 2.0 dated 23rd November 2021 Approved**

The approval covers the following only:

- **Addition of study sites, i.e., Sunyani Municipal Hospital, Bono Regional Hospital, Sunyani and Holy Family Hospital, Techiman**
- **Change to study title from *"Pharmacologic and Clinical Risk Factors Of Poor TB Treatment Outcomes In Patients with Rifampicin-Susceptible TB in Selected Hospitals in the Ashanti Region of Ghana."* to *"Pharmacologic and Clinical Risk Factors of Poor TB Treatment Outcomes in Patients with Rifampicin-Susceptible TB in Selected Hospitals in the Ashanti, Bono and Bono East Region of Ghana."***

The following applies:

1

- Submission of yearly progress report of the study to the Ethics Review Committee (ERC).
- Renewal of ethical approval if the study lasts for more than 12 months.
- Reporting of all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.
- Submission of a final report **after completion** of the study.
- Informing ERC if study is discontinued and reasons why.
- Informing the ERC and your sponsor (where applicable) before any publication of the research findings.

You are kindly advised to adhere to the national guidelines or protocols on the prevention of COVID -19

Please note that any modification of the study without ERC approval of the amendment is invalid.

The ERC may observe or cause to be observed procedures and records of the study during and after implementation.

Kindly quote the protocol identification number in all future correspondence in relation to this approved protocol.

SIGNED.....

Dr. Cynthia Bannerman
(GHS-ERC Chairperson)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra

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