Genetic analysis of resistance and PK/PD model-based study of Moxifloxacin and Levofloxacin in multi-drug resistant tuberculosis patients attending hospitals in Southern Ethiopia

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Dissertation paper submitted to the Department of Pharmacology and Clinical Pharmacy, School of Pharmacy, College of Health Sciences, Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy (PhD) in Pharmacology.

June 2023

Addis Ababa

Addis Ababa University

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This is to certify that the dissertation prepared by Temesgen Sidamo, entitled: ***Genetic analysis of resistance and PK/PD model-based study of Moxifloxacin and Levofloxacin in multidrug resistant tuberculosis patients attending hospitals in Southern Ethiopia****, and* submitted in fulfillment of the requirements for the Degree of Doctor of Philosophy (Pharmacology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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This dissertation is based on the following papers:

1. **Paper I: Temesgen Sidamo,** Workineh Shibeshi, Getnet Yimer, Eleni Aklillu, Ephrem Engidawork. Explorative Analysis of Treatment Outcomes of Levofloxacin- and Moxifloxacin-Based Regimens and Outcome Predictors in Ethiopian MDR-TB Patients: A Prospective Observational Cohort Study. *Infection and Drug Resistance 2021:14 5473–5489. https://doi.org/10.2147/IDR.S342964*.
2. **Paper II**: **Temesgen Sidamo,** Prakruti S Rao, Eleni Aklillu, Workineh Shibeshi, Yumi Park, Yong-soon Cho, Jae-Gook Shin, Scott K Heysell, Stellah G Mpagama, Ephrem Engidawork. Population Pharmacokinetics of Levofloxacin and Moxifloxacin, and the Probability of Target Attainment in Ethiopian Patients with Multidrug-Resistant Tuberculosis. *Infection and Drug Resistance 2022:15 6839–6852. https://doi.org/10.2147/IDR.S389442*
3. **Paper III: Temesgen Sidamo,** Yumi Park, Workineh Shibeshi, Eleni Aklillu, Jae Gook Shin, Melaku Tilhahun, Muluye Abebe, Sunyoung Lee, Ephrem Engidawork (submitted to PLOSE ONE): Phenotypic and genotypic characterization of resistance to fluoroquinolones and some second-line antitubercular drugs, and their correlation with clinical outcomes in Ethiopian patients with multidrug-resistant tuberculosis.

Abstract

Genetic analysis of resistance and PK/PD model-based study of Moxifloxacin and Levofloxacin in multidrug resistant tuberculosis patients attending hospitals in Southern Ethiopia

Temesgen Sidamo

Addis Ababa University, 2023

Background: *Multi-drug resistant tuberculosis (MDR-TB) has hindered the therapeutic success of TB. Patient, drug, and pathogen-related factors are critical to understanding, characterizing, and predicting drug resistance, which help to tailor drug dosing taking into account inter-individual differences and patient needs. The highly active World Health Organization (WHO) Group A fluoroquinolones, FQs (moxifloxacin, MXF, and levofloxacin, LFX), exhibit inter-individual pharmacokinetic variability (IIV) that may result in low drug exposure. The 24-hour area under the concentration-time curve over the minimum inhibitory concentration (AUC0-24/MIC) is a strong predictor of FQ exposure and positive treatment outcome. Ethiopia is one of the world nations with a high prevalence of MDR-TB. However, there is a lack of data on factors affecting MDR-TB treatment success and one-size-fits-all scenario is the single most common therapeutic strategy of TB in Ethiopia.*

Objective: *This dissertation aimed to study treatment outcome and its predictors, characterize and predict MXF and LFX resistance in MDR-TB patient population using phenotypic and genotypic methods as well as a PK/PD model-based study.*

Methodology: *A prospective observational study of 80 MDR-TB patients was carried out. Treatment outcomes in patients receiving MXF-and LFX-based regimens were compared at the end of treatment. We determined the plasma concentrations of MXF and LFX and their PK parameters. A PK/PD (AUC0-24/MIC) analysis to predict the recommended probability of target attainment (PTA) was carried out for*

both drugs. The potential patient covariates which impact the model estimated PK profiles were also screened. Drug resistance was characterized by phenotypic and genotypic methods, and the association between patient and pathogen related variables, FQ-resistance and treatment outcome were evaluated.

Result: *The total treatment success in this study was lower than the report in previous local and international studies. The LFX-based MDR-TB regimen outperformed the MXF-based regimen. Unsuccessful treatment outcome was predicted by a delayed culture conversion rate, history of alcohol intake, lesion of lung cavities, serum levels of creatinine (Cr.). A one-compartment model with adjustments was fit to the LFX and MXF concentrations. Cr. and body mass index (BMI) were covariates identified to have impact on clearance and apparent volume distribution (Vd) of LFX and MXF, respectively. Exposures to LFX and MXF were lower in study participants than in other settings. However, LFX-treated groups experienced higher drug levels and exposure with dose. Nine clinical isolates (11.3%) were resistant overall, all of which were at least resistant to FQs, while 3 were resistant to both FQs and injectable drugs. Mutation in gyrA 94 was identified in 5 isolates. The MIC values were associated with patient treatment outcomes. Cavitary lesion and serum creatinine predict FQ-resistant tuberculosis.*

Conclusion: *Exposure to either LFX or MXF may be inadequate, but LFX appears to provide better treatment outcomes. Patient clinical and behavioral variables can predict drug exposure, drug resistance and the overall treatment outcome. Even though the line probe assay (LPA) showed a moderate level of resistance in Ethiopian patients, genotypic test results do not always correlate well with phenotypic results. The actual drug resistance level could be higher than anticipated. Higher MIC values of clinical isolates correlate with a higher risk of treatment failure. In such scenarios, dosage optimization may improve outcome. GyrA mutation is associated with FQ-resistance in Ethiopian patients. Further controlled clinical studies are needed to establish optimal doses and exposure of FQs.*

Keywords: *Fluoroquinolones, multi-drug resistant tuberculosis, population pharmacokinetics, treatment outcome, gyrA sequencing*

Acknowledgments

First of all, I would like to thank the Almighty God for everything in my life. I owe him my studies and my whole life.

I would like to acknowledge institutions and individuals who have contributed significantly to this study. First and foremost, I am grateful to Addis Ababa University and Wolaita Sodo University for supporting this study financially. Next, I would like to express my deep gratitude to my principal research supervisor Prof. Ephrem Engidawork for his unconditional and invaluable support throughout my studies. His encouragement, patience, and a constant will to help throughout this study were essential to keep going in this long journey. The support of Prof. Eleni Aklillu was very important to bring this study to life and achieve its goals. Furthermore, her continued guidance during my trip to Korea was key to the success of this study. Please accept my sincere appreciation. I would like to thank the Armauer Hansen Research Institute (AHRI) for allowing me access to their laboratory and collaboration. I also thank the staff members of the four hospitals that collected data —Nigist Eleni Mohamed Memorial and Referral Hospital, Arbaminch, Butajira, and Yirgalem Hospitals—as well as the management of all four. I would also like to express my gratitude to the SNNP's regional laboratory staff memebrs who involved in the microbiological assay in this study.

I would like to extend my heartfelt appreciation to Prof. Jae-Gook Shin for inviting me to his laboratory at Inje University College of Medicine, Republic of Korea. Prof. Shin, thank you for all your invaluable support in my endeavor. I am also grateful to all the staff and researchers at the Inje University College of Medicine who rendered me their unreserved support in various ways. I want to express my deep gratitude to Dr. Workineh Shibeshi for securing a research grant from the

European and Developing Countries Clinical Trials Partnership (EDCTP) and for his continuous guidance in this study. Additionally, I appreciate EDCTP for paying me a monthly stipend for more than a year. I also would like to thank Dr. Getnet Yimer for his mentor-ship during my study.

Let me extend my sincere appreciation to Prof. Heysell Scott, Ms. Rao Prakruti, and Dr. Setellah Mpagam for their collaboration and immense support to achieve the objectives of the Population Pharmacokinetic study. Indeed, it has been a wonderful opportunity to work with you. Thank you!

I acknowledge my beloved wife, Yenework Desalegn. Her prayers, selflessness, and compassion were tenets of my academic and life success. Additionally, I am grateful to God for my precious two daughters, Handakina and Amen Temesgen. Tariku Markos and Adanech Deboch are a wonderful couple who have stood by me throughout the challenges of my life. May the Almighty God bless you dears for all your kind support and care. My sincere gratitude should go to Dr. Tesfaye Wolde, who helped me out financially when I was in South Korea. I also would like to thank Tesfahun Eromo and Mamo Abiyo for their generous financial support for this study. There are several people who have contributed in one way or another to my studies and life. Since I can't list everyone here, I just want to say thank you to everyone.

Dedication

I dedicate this Ph.D. Dissertation to my late son **Amanuel Temesgen** who suffered a tragic high voltage electrocution and lost his life suddenly and far too early. Dear my son (Bucu), my firstborn, I never thought that you would go so quickly. I also never doubted that we would celebrate your dad's achievement together. Did you know how painful that is for your dad and your desperately loving family? I have no idea how to face the tragedy of your loss for the rest of my life. But I would like to tell you that you are always with us and we are not willing to accept you as a dead. I miss and we miss you. May your beautiful soul rest in everlasting peace until I join you again.

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Statement of Author

With my signature below, I declare and confirm that this dissertation is my own work. I have followed all ethical principles of science in the preparation, data collection, data analysis, and completion of this dissertation. To the best of my capacity and knowledge, I have cited and referenced all sources used in this document. Efforts have been made to avoid plagiarism in the preparation of this dissertation. This dissertation is submitted in fulfillment of the requirements for the degree of Doctor of Philosophy (Pharmacology) from the School of Graduate Studies Directorate, Addis Ababa University. I hereby declare that this dissertation has not been submitted to any other institution for the award of a degree, diploma or certificate. Brief quotations from this dissertation may be used without special permission provided the source is cited accurately and completely. Requests for permission to extend citations from this dissertation or to reproduce this dissertation in whole or in part may be granted by the Director of Graduate Studies if, in his opinion, the proposed use of the material is in the interests of scholarship. In all other cases, however, the permission of the author of the dissertation must be obtained.

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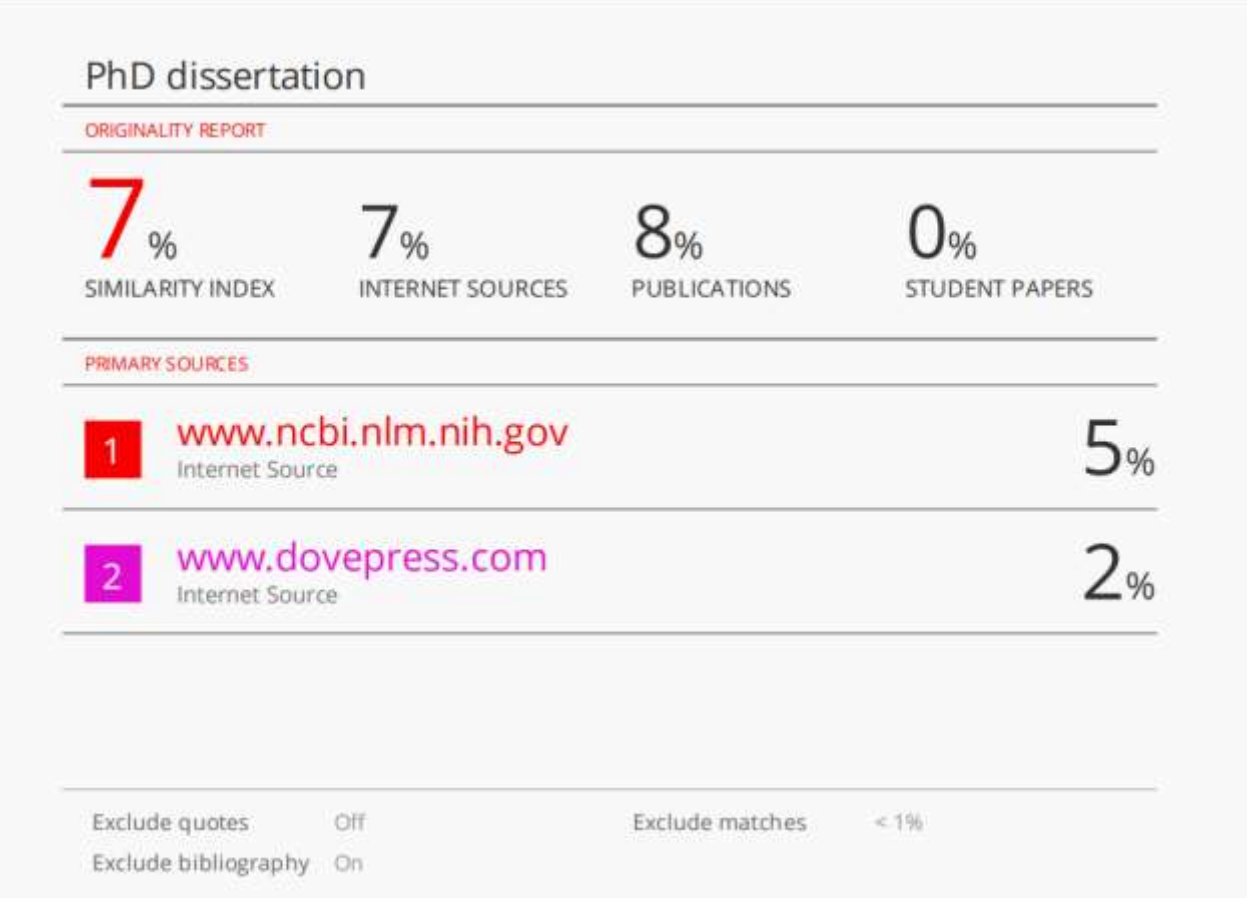

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Abbreviations

ADRs	Adverse Drug reactions
AHR	Adjusted Hazard Ratio
AHRI	Armauer Hansen Research Institute
AIC	Akaike Information Criterion
AIN	Acute Interstitial Nephritis (AIN)
AMR	Antimicrobial resistance
AUC	Area Under the Concentration Curve
AUC0-24	Area Under the Concentration over 24 hours
BDQ	Bedaquiline
BIC	Bayesian Information Criterion
BMI	Body Mass Index
CFU	Colony-forming units
CFZ	Clofazimine
CL	Clearance
C_{max}	Peak concentration
Cr.	Serum creatinine
CS	Cycloserine
%CV	Percent Coefficient of Variability
DILI	Drug-Induced Liver Injury
DLM	Delamanid
DOT	Directly Observed Treatment

DR-TB	Drug Resistant TB
DST	Drug Sensitivity Test
DW	Distilled Water
EDCTP	European and Developing Countries Clinical Trials Partnership
EMB	Ethambutol
*f*AUC0-24	Area under the unbound concentration Curve from 0 to 24 h
FQs	Fluoroquinolones
GM	Geometric Mean
gryA	Gyrase A gene
gryB	Gyrase B gene
HFS	The hollow fiber reactor system
IIV	Interindividual variability
INH	Isoniazid
K_a	Absorption rate constant
KNM	Kanamycin
LFX	Levofloxacin
LPA	Line probe assay
LS/MS/MS	Tandem Mass Spectrophotometry
LZD	Linezolid
MBDD	model-based drug development
MCSs	Monte Carlo Simulations
MDR-TB	Multidrug Resistant Tuberculosis
MGIT	Mycobacterium Growth Indicator Tube

MIPD	Model-Informed Precision Dosing
MTB	Mycobacterium Tuberculosis
MUT	Mutant DNA of TB
MXF	Moxifloxacin
PBPK	Physiologically based PK modeling
PK/PD	Pharmacokinetic – Pharmacodynamics
PopPK	Population Pharmacokinetics
PTA	Probability of Target Attainment
QRDR	Quinolone Resistance determining region
r/r	Resistance to both FQs and injectable second line drugs
r/s	Resistant to FQs but sensitive to Injectable second line drugs
Rif.	Rifampicin
RR-TB	Rifampin resistant Tuberculosis
RSE	Percent relative standard error
s/s	Sensitive to both FQs and injectable second line drugs
SLDs	Second-line TB drugs
SNNP	Southern Nations and Nationalities People
V_d	Volume of distribution
WGS	Whole Genome Sequencing
WHO	World Health Organization
WT	Wild-type DNA of TB
XDR-TB	Extensively Drug Resistant Tuberculosis

Chapter 1: Introduction

1.1.Background

Drug-resistant tuberculosis (DR-TB) has challenged decades of success in controlling TB (Kurz et al., 2016). There are 5 categories of DR-TB namely: Isoniazid (INH)-resistant TB, Rifampin resistant TB (RR-TB), MDR-TB (TB resistant to INH and rifampicin), pre-extensively drug-resistant TB (pre-XDR-TB, which is MDR-TB with resistance to a fluoroquinolones, FQ), and XDR-TB (MDR-TB plus resistance to any FQ and at least one of the injectables according to the previous definition (WHO, 2012), or plus at least one priority group A member (linezolid or bedaquiline) as per the recently revised WHO definition (WHO, 2021a). DR-TB continues to be a public health threat because it is more difficult to eliminate than the drug-susceptible one (Friedman et al., 2016, Oliveira et al., 2021, Oudghiri et al., 2018).

End TB strategy is one of the ambitious WHO effort which aims to end the global TB epidemic and has been considred as one of the United Nation's Sustainable Development Goals. It is a blueprint for countries to reduce TB incidence by 80%, TB deaths by 90%, and to eliminate catastrophic costs for TB-affected families by 2030. The strategy adapts diverse country settings instead of a one size fits all approach for its success (WHO, 2016).

However, MDR-TB is on the rise and has complicated all efforts to end TB (WHO, 2016, WHO, 2018b). Almost half a million MDR/(RR)-TB cases occur worldwide each year (WHO, 2019a, WHO, 2020). More than 100 countries have reported XDR-TB, and according to reliable sources, the prevalence of XDR-TB is approximately 8.5% (WHO, 2018b). Early detection and

characterization of drug resistance is critical to predicting clinical outcomes and the global effort to eradicate TB (Aziz and Wright, 2005, Gandhi et al., 2010).

MDR-TB has a poor outcome and is difficult to treat due to its large cost (Kang et al., 2006, White and Moore-Gillon, 2000) lengthy treatment, and higher risk of adverse drug reactions (ADRs) (Nathanson et al., 2004). According to a 2016 global report, treatment success in MDR-TB and XDR-TB were only 56% and 39%, respectively (WHO, 2019a). This implies that the available MDR-TB therapies are not optimal. Development of a treatment regimen that overcomes drug resistance and improves outcomes has become a high-priority area of research. However, only a few drugs were in the development pipeline up to recently (Dooley et al., 2013, Reynolds and Heysell, 2014, WHO, 2021a). The alarming rise in antimicrobial resistance (AMR) is rapidly limiting treatment options for infectious diseases and can have devastating consequences, potentially wiping out all gains made in global TB control (Reynolds and Heysell, 2014).

Due to their excellent *in vitro* and *in vivo* activity against *Mycobacterium tuberculosis* (MTB), the causative agent of all forms of TB, Fluoroquinolones (FQs) are the most important components of the MDR-TB regimens (WHO, 2018b). Moxifloxacin (MXF) and levofloxacin (LFX) are highly effective FQs (Aubry et al., 2004, Asif, 2017) that have a significant role in MDR-TB treatment success. Consequently, they are key components of WHO Group A drugs (WHO, 2018a). The antitubercular activity of FQs is by inhibiting DNA gyrase (type II topoisomerase) which results in blocked replication and transcription of the bacteria. Specifically, FQs target the *gyrA* and *gyrB* genes which encode the two subunits of the DNA gyrase present in MTB. When a FQ binds to the enzyme a stable drug-enzyme complex will be fomed and thereby blocks DNA replication and transcription (Aubry et al., 2006).

However, intra- and inter-individual pharmacokinetic (PK) variability among FQs is not uncommon and it may lead to inadequate amount of the drugs in the body and unsuccessful outcomes (Srivastava et al., 2011, Boveneind-Vrubleuskaya et al., 2017, Pranger et al., 2011). Interindividual variability (IIV) in this context is known as an intersubject variability and it refers the PK variability between different individuals. Intraindividual variability is usually known as interoccassion variability and is a PK varaibility within the same patient. A variety of host factors including co-illness, body size, co-medication, nutrition, critical illness, or genetics are the major sources of the IIV in FQs (Sachidanandam et al., 2001, Ahmed et al., 2016, Walter-Sack and Klotz, 1996). On the other hand, dosing patients in a fed-or fast state, for example, may result in interoccassion PK variability within the same patient (Requena-Mendez et al., 2019). These variability may result in inadequate or excess plasma levels of the drugs which may drive drug resistance or toxicities, respectively (Reynolds and Heysell, 2014). Genetic variation in MTB has also shown to result in a range of variable phenotypic resistance among FQs and it may negatively impact treatment outcome (Castro et al., 2020).

Understanding pharmacokinetic (PK) and Pharmacodynamic (PD) factors is important for dose optimization that can improve treatment outcomes (Pranger et al., 2019, Pranger et al., 2011). While PK refers to the drug concentration in serum, tissues and other body fluids, PD relates to the pharmacological effects of the drug for a particular exposure. An *in vitro* measure of the minimum concentration of drug needed to inhibit bacterial growth, called the minimum inhibitory concentration (MIC), is the PD parameter most frequently utilized clinically to describe antibacterial activity against a pathogen (Scaglione and Paraboni, 2008). In dosage optimization, population PK model (PopPK) is often developed first, and this is linked to PD parameter in what is called PK/PD modelling (Reynolds and Heysell, 2014). In recent years, PK/PD modelling and

simulation is the most widely used strategy for establishing rational dosing regimens of antimicrobial agents, drug sensitivity breakpoints, and prevention of AMR (Rodriguez-Gascon et al., 2021). The main tenet of PK/PD modelling is that drug dosage, exposure, and response all follow predictable patterns (Mager and Jusko, 2008).

Based on the *in vitro* PD drug impact, antibacterial drugs have three modes of bactericidal effects: time-dependent, concentration-dependent, or AUC/MIC ratio dependent (Craig, 1998). Time-dependent antibacterials efficiently kill the the pathogens when their concentrations are above the MIC for a larger portion of the dosing period. Some of the examples of time-dependent antibacterials include aztreonam, penicillins, cephalosporins, and carbapenems. Concentration-dependent antibacterials work by raising serum levels of the drug, which increases the bactericidal action. For an efficient bactericidal action, these drugs are dosed to reach high maximal concentrations at the infection site (for example, concentrations as large as 10 times the MIC for aminoglycosides). Antibacterial activity of drugs such as vancomycin and FQs depends on AUC0-24/MIC ratio during a 24-hour period (Ambrose et al., 2007).

The AUC0-24/MIC ratio is a strong predictor of FQs exposure and outcome (Craig, 2001, Firsov et al., 2001). A hollow fiber bioreactor system (HFS) experiment has established that an unbound AUC0-24/MIC ratio of 53 for MXF decreases the total population of MTB significantly and prevents emergence of drug resistance. Moreover, an AUC0-24/MIC ratio of ≥100 for MXF completely kills FQ-sensitive population of MTB without development of drug resistance (Gumbo et al., 2004). Similarly for LFX, AUC0-24/MIC associated with maximum MTB kill was found to be 146 whilst the ratio associated with resistance prevention was 360. It was suggested that up to 1500 mg LFX doses are needed for a high bactericidal effects if toxicities are not an issue (Deshpande et al., 2018).

Genotypic and phenotypic characterization of DR-TB are very essential to predicting appropriate treatment decisions and treatment outcome in patients. A clear understanding of the genetic basis of resistance in MTB can help to predict relevant mutations accounting for the drug resistance (Miotto et al., 2017). It promises to improve treatment outcomes for individuals with MDR-TB (Aubry et al., 2006). *In vitro* assays showed that the genetic changes in MTB influenced the MIC (a phenotypic measure of DST) and FQ-resistant mutation. Phenotypically detected low FQs drug susceptibility in MTB has been linked to a poor culture conversion rate in patients. Therefore, understanding how FQ-resistance is acquired in MTB is important since it help designing a strategy to mitigate further resistance and improve treatment outcomes (Castro et al., 2020).

Aquired resistance to FQs is primarily caused by mutation of *gyrA* and *gyrB* genes of DNA gyrase of MTB (Pang et al., 2016). It has been hypothesized that detection of mutation of these genes in FQs is equivalent to culture based DST for predicting MDR-TB treatment outcome (Farhat et al., 2017). However, all types of mutations in DNA gyrase may not result in poor drug susceptibility in MTB. Even some mutations have been shown to result in hyperscusceptibility to FQs. For example, *gyrA* mutations including A90V, D94G, and D94H and a mutation in *gyrB* (N510D) have been associated with resistance to FQs. In contrast, a novel combination of mutation of *gyrA* T80A or A90G has been associated with hypersusceptibility to FQs (Aubry et al., 2006).

1.2. Problem Statement

Ethiopia was recently removed from the list of high MDR/RR-TB burden world countries, but remains on the list of countries with high TB/HIV prevalence (WHO, 2021b). In 2018, it was reported that there were 151 occurrences of TB per 100,000 people and a mortality rate of 22 per 100,000 populations. In the identical year, roughly 0.71% of priorly untreated TB cases and 7.2% of TB cases that were treated in the past affected DR-TB, with an approximate 1,600 cases of

MDR/RR-TB emerged (FDRE, 2020). According to a recent systematic review, the prevalence of MDR-TB was 2.6% in newly diagnosed cases and 11.5% in retreated TB patients, while the overall pooled prevalence of MDR-TB was 10.8% (Reta et al., 2022).

As per the recommendations of WHO, individuals in Ethiopia diagnosed with MDR-TB are prescribed therapeutic regimens that are based on MXF or LFX (FDRE, 2017, WHO, 2019b). The drugs were chosen based on a number of factors, including the preference for oral over injectable agents, the DST, the validity of current DST procedures, drug resistance levels in the population, each patient's medical history, drug tolerability, and possible drug–drug interactions (WHO, 2018a).

There is also a dearth of evidence on the results of MDR-TB treatment outcome of FQs in Ethiopia. The available data indicates that the success rate of treatment ranges between 63% and 78.8%. A systematic review and meta-analysis conducted recently revealed that nearly 18% of patients with MDR/RR-TB who underwent treatment in Ethiopia experienced an unfavorable outcome (Alene et al., 2017, Girum et al., 2018, Woldeyohannes et al., 2019). Resistance to FQs due to low susceptibility of MTB and PK variability might have contributed to the variability in clinical outcomes. Based on the existing body of literature, PK variability of FQs is common and it could be associated with poor treatment outcomes and drug resistance (van den Elsen et al., 2020). Inappropriate dosing of the FQs (LFX and MXF) might have intensified the PK variability as well as the variable treatment outcomes in Ethiopian MDR-TB patients.

Despite the diverse ethnic backgrounds of Ethiopians, a one-size-fits-all scenario is a common practice in TB care (Adamu, 2013, FMOH/CDC, 2014). There is insufficient data on patient exposure to drugs, inter-individual variability (IIV), and how these affect MDR-TB therapy outcomes. To our knowledge, the PopPK and PK/PD model-based analysis that can guide the

dosing of the FQs (LFX and MXF) towards achieving an adequate *in vivo* drug exposures needed for a maximal mycobacterial kill and resistance prevention in the Ethiopian MDR-TB patients is not known. Genotypic and phenotypic profiles of MTB and their correlation with drug resistance and treatment outcomes are also not yet explored.

1.3. Research questions

- Is MDR-TB treatment by MXF-or LFX-based regimens effective in Ethiopian patients? What is the comparative efficacy of MXF- and LFX-based regimens in Ethiopian patients?
- What factors predict treatment outcome and drug exposure in patients treated with MXF- or LFX-based regimens?
- Are the standard doses of MXF and LFX optimal in order to achieve the required target drug exposure as defined by AUC0-24/MIC necessary to effectively kill (MXF≥53 and LFX≥146) and prevent drug resistance (MXF≥100 and LFX≥360) in Ethiopian MDR-TB patient population?
- Do both genotypic and phenotypic methods predict the actual drug susceptibility patterns for MXF and LFX in the clinical isolates of tuberculosis?

1.4. Hypotheses

- Treatment outcomes with MXF- or LFX-based regimens in MDR-TB patients may not be adequate and comparable between the regimens.
- Sociodemographic and clinical variables may influence resistance to FQs and treatment outcome as well as drug exposures of LFX and MXF in MDR-TB patients.

- Mutations in gyrase enzyme (*gyrA* gene) may contribute to MXF and LFX resistance in Ethiopian patients.
- There is adequate concordance between the phenotypic and genotypic methods of drug sensitivity test (DST) in clinical isolates
- Inadequate exposure of MXF and LFX predicts, a lower PTA and, therapeutic failure and drug resistance.

1.5. Objectives

General Objective

To predict drug resistance and compare treatment outcomes, and characterize PK and PD features of MXF and LFX in Ethiopian MDR-TB patients.

Specific Objectives

- To evaluate, compare treatment success and its predictors in MDR-TB patients who received MXF- and LFX-based MDR-TB therapy.
- To explore the PopPK and PK/PD relationships of LFX and MXF, and percent PTA defined by the $AUC_{0\text{-}24}$/MIC.
- To characterize drug resistances to FQs and some antitubercular drugs, and their correlation with clinical outcomes by phenotypic and genotypic methods.

1.6. Significance of the study

This study is essential as it generates local data on MDR-TB patients, pathogens (MTB), and drugs (MXF and LFX) that could be used as input to develop optimal drug regimens for MDR-TB to improve treatment outcomes. The data will also help to develop useful strategies for the clinical

implementation of therapeutic drug monitoring (TDM) in MDR-TB and to maximize its treatment outcome. The PopPK performed in this study is the first of its kind in Ethiopia and sheds light on the magnitude of IIV in the TB patient population. We believe that this study will pave the way for PK/PD studies for other antimicrobial drugs that aimed at optimizing their use and preventing AMR. In general, this study has immense relevance in terms of innovation and technology transfer trends from model-based PK/PD drug studies to Ethiopian institutions.

1.7. Limitations

This study has some limitations. These limitations are mainly due to our study design. Our study was observational and many factors that could influence the outcome of the study were not rigorously controlled in the study. In addition, although our study sample was small and not representative of all MDR-TB patients, it was sufficient for the PopPK study to provide some insight into Ethiopian MDR-TB patients. Maximal mycobacterial kill and resistance prevention primarily depends on the free fraction of drug concentrations. However, as we calculated PTA using total drug concentrations, the conclusions drawn may not be perfect. Although other mutations can be associated with drug resistance to FQs, logistical issues limited our sequencing experiment to only *gyrA*, the most common cause of resistance in FQs.

Chapter 2: Literature Review

2.1. Drug-resistant Tuberculosis

2.1.1. General Considerations

MDR-TB is a serious global health concern, and managing highly resistant strains of the pathogen is difficult (Redgrave et al., 2014). It may be a result of inadequate treatment regimens (Tiberi et al., 2022) and presents a significant hurdle for patients, healthcare providers, and healthcare facilities (WHO, 2022c). Due to its higher morbidity and mortality, sequelae, costs, and complexity; DR-TB is a global health crisis. Decades of progress in TB prevention and control are at risk of being reversed by DR-TB (Tiberi et al., 2022). In order to comprehend the circumstances in which resistance is selected and sustained, it is imperative to understand the mechanisms of resistance, the factors that drive resistance, and the destiny of resistant strains (Redgrave et al., 2014).

Generally, the causes of DR-TB are due to microbial aspects of gene mutation and clinical aspects of drug use (Muflihah et al., 2013). DR-TB arises due to the emergence of genomic variants that occur naturally (Zhang and Yew, 2015). People may acquire DR-TB in two ways. Firstly, acquired DR-TB is caused by inadequate medication, substandard drugs, inappropriate prescribing, patient non-adherence, failure of the health care system or delivery, or a cumulative effect of all of these. Secondly, DR-TB arises from the direct spread of DR-TB from infected individuals to other people (WHO, 2019a).

Whilst resistance in MTB is caused by mutations in the genome that occur spontaneously, the level of resistance depends on the specific mutation (Davies Forsman et al., 2017). The DNA gyrase,

encoded by *gyrA* and *gyrB*, is the primary target of FQs in MTB (Ginsburg et al., 2003). Around 60-70% of MTB isolates that are resistant to FQ exhibit mutations in the *gyrA* in its quinolone resistance-determining region (QRDR), with the greatest frequency observed at codon 94, followed by codons 90, 91, and 88 (Laurenzo and Mousa, 2011). Therefore, alterations in the *gyrA* gene give rise to an increased degree of FQ resistance, whereas changes in *gyrB* are associated with a resistance of lesser intensity (Dookie et al., 2018). Mutations in DNA gyrase result in MTB resistance to FQs. The *gyrA* protein in DNA gyrase, to which FQ binds, may change in structure and function as a result of missense mutations. Therefore, the gyrase enzyme remains active or uninhibited. Thus, the drugs (FQs) cannot inhibit negative supercoiling of bacterial DNA. As a result, the MTB DNA is not damaged and remains alive (Muflihah et al., 2013).

Drug resistance mechanisms have been understood better due to advances in sequencing technology (Dookie et al., 2018). Resistance detection has become easier with molecular assays and the MICs that are determined using the traditional susceptibility plates. For example, the most common genomic alterations responsible for FQ resistance can be detected quickly using the molecular line probe assay (LPA). Unlike genotypic DST, which simply identifies resistance, MICs provides a level of resistance (Davies Forsman et al., 2017).

Clinical aspects of drug use such as inappropriate prescriptions, irrational drug selection, inadequate dosing, poor quality drugs, or poor adherence to therapy, among others have led to increased drug resistance (Allue-Guardia et al., 2021, FDRE, 2020). Treatment-naïve patients are highly unlikely to acquire single- or multidrug-resistant infections from naturally occurring wild-type resistant strains, suggesting that there are additional causes for the development of resistant forms of TB. In a programmatic setting, DR-TB arises from the selection of resistant mutant organisms as a result of the administration of ineffective drugs that kill susceptible bacilli while

allowing resistant bacteria to grow and spread. Inappropriate treatment, such as direct or indirect administration of a single drug, aggravates the selection of resistant variants (Brode et al., 2022, FDRE, 2020).

Non-adherence has been thought to be the root cause of MDR-TB, and Directly Observed Treatment (DOT) has been considered the most important health breakthrough of recent decades (Garner and Volmink, 2003). However, it is proven that MDR-TB can occur even with full patient compliance to medication regimens (Srivastava et al., 2011). In spite of an adherence of over 98%, the prevalence of MDR-TB remains high (Calver et al., 2010). Based on a hollow fiber bioreactor system (HFS) experiment, unsuccessful outcomes arose only at a higher degree of non-adherence, implying that PK variability is a more probable cause of MDR-TB occurrence (Srivastava et al., 2011). There is increasing evidence that IIV in PK profiles may result in sub-therapeutic drug concentrations in patients (Davies Forsman et al., 2017, Pasipanodya et al., 2012). Exposure to low levels of essential anti-TB drugs, such as FQ used in human and animal non-tuberculosis cases, may provide an opportunity for resistance selection in mutant strains. FQs, for example, are utilized extensively in agriculture, animal clinics, and medical settings, making previous FQ usage history or exposure difficult to determine (Sukul and Spiteller, 2007).

2.1.2. Global Epidemiology of MDR-TB

MDR-TB possesses an enormous epidemiological effect, with 480,000 identified cases and 190,000 fatalities in 2014, 10% of which cases qualify the definition for XDR-TB (Rendon et al., 2016). With increasing resistance to drugs, the epidemic persists and will necessitate considerable focus and commitment to eradicate it (Tiberi et al., 2018). Globally, almost 15% of MDR/RR-TB infected people die from the disease (WHO, 2019a). In 2016, roughly 490,000 individuals with MDR-TB were identified, which constitutes 4% of treatment-naive cases and 19% of cases with

prior treatment worldwide (WHO, 2017a). In 2017, 558,000 people were diagnosed with RR-TB, and 82% of these cases had MDR-TB. In 2018, around 484,000 MDR/RR-TB incidents were reported globally, accounting for 3.4% of new infections and 18% of already treated cases (WHO, 2019a). Approximately 500,000 RR-TB cases were reported worldwide in 2019, with a 60% of successful outcome (WHO, 2020). According to the most recent WHO estimates, nearly 470,000 people were infected with MDR-TB, with nearly 180,000 (38.3%) dying from XDR-TB (WHO, 2021c).

Even though there was a decreasing trend in MDR-TB in previous years, there was an increase (up to 3.1%) in the estimated MDR-TB incidence globally between 2020 and 2021 (WHO, 2022a). This is likely due primarily to a general increase in TB from 2020 to 2021, which is presumed to be due to the effect of the COVID-19 on TB diagnosis (Dean et al., 2022). The COVID-19 pandemic is still negatively affecting TB diagnosis and care, where it has caused a slowdown, interruption, or reversal of the progress made up to 2019 in combating TB (WHO, 2022a). In 2021, 1.6 million individuals died from TB (187, 000 of these were HIV patients). TB ranks as the 13th most common cause of fatalities and, following COVID-19, is the second leading infectious cause of death worldwide. It kills more people than HIV/AIDS (WHO, 2022d). Despite the significant burden, just a quarter of the predicted number of MDR/RR-TB patients had been identified and reported in 2017 (WHO, 2018b). The rest is either among those known as "missing millions" or were provided with inadequate first-line treatment in the dearth of appropriate DR-TB diagnosis (WHO, 2018b). Therefore, undiagnosed DR-TB, which can spread silently, may complicate TB control and prevention efforts and reverse all previous gains (Li et al., 2017).

Resistance rates to FQs, the most effective drugs used to treat MDR-TB, has been predicted to vary between 0.15% to 30% based on the country (Bernard et al., 2015). These proportions are

greater among individuals who were exposed to FQs prior to a diagnosis of TB or in patients who developed resistance through repeated exposures (Lee et al., 2016). However, in certain locations in which FQ consumption is very substantial, resistance could be due to the spread of FQ-resistant pathogen in the population (Bernard et al., 2015). In more than 100 nations with adequate information, 20% of individuals with MDR/RR-TB additionally have FQ resistance, of which 8.5% were cases of XDR-TB (WHO, 2018b).

2.1.3. Anti-TB Drug Regimens

With the introduction of fast molecular diagnostics, novel and reinvented drugs, and meta-analyses of individual patient data, the care and management of DR-TB have advanced considerably over the past few decades (Mase and Chorba, 2019). Recently, the new drugs bedaquiline and delamanid, as well as the repurposed medicines linezolid, clofazimine, and carbapenems, are more widely used for the treatment of DR-TB (Tiberi et al., 2018). Regularly updated evidence-based WHO guidelines and categorization of anti-TB drugs are key to establishing appropriate therapeutic modalities for the effective care of MDR-TB (Tiberi et al., 2022). The previous WHO classification of MDR-TB anti-TB drugs (2011) was based on effectiveness and safety and ranged from group 1 to group 5. First-line drugs are categorized as group 1 whereas second-line drugs grouped as group 2-5. Group 5 drugs were with potentially lower efficacy and clinical data (WHO, 2014a). This grouping suggests at least four drugs with acceptable sensitivity to MTB isolates are combined. Drugs are chosen in a stepwise manner from the groups based on their effectiveness, safety, and affordability (Caminero et al., 2010).

The subsequent WHO classification (2016) was entirely designed for conquering drug-resistant cases, and drugs were listed in order of their therapeutic importance from group A to group D (WHO, 2016). The intensive phase consisted primarily of MXF, injectable medications,

protionamide, pyrazinamide, clofazimine, and high-dose isoniazid, followed by MXF, clofazimine, pyrazinamide, and ethambutol in the continuation phase. WHO issued this regimen as an interim recommendation based on non-response to other previous regimens, safety, or drug resistance to pyrazinamide, ethambutol, kanamycin, ethionamide, or clofazimine (WHO, 2016).

WHO approved an entirely oral standardized 20-month MDR-TB protocol (known as a longer MDR-TB regimen) in 2018, consisting primarily of LFX, bedaquiline, linezolid, clofazimine, cycloserine, and others. The earlier regimen was replaced by this later guideline, which omitted injectable medications from the regimen. The medications were chosen based on the preference for oral agents over injectable agents, existing DST profile and its reliability, population drug resistance data, medication use history, safety, and possible interactions between drugs (WHO, 2018a).

In 2019, the WHO consolidated guideline included the standardized shorter (9-12 months regimen) and longer (18-20 months regimen) treatment protocols for MDR/RR-TB, as well as the use of novel drugs (Bedaquiline or delamanid) with improved efficacy and safety as well as improved treatment success (Mase and Chorba, 2019, WHO, 2019b). The most recent WHO consolidated guideline includes two new recommendations: one for a 6-month regimen of bedaquiline, pretomanid, linezolid, and MXF (BPaLM) in patients with MDR/RR-TB and additional FQ resistance (although the addition of MXF depends on DST results), and another for a 9-month all-oral regimen in individuals with MDR/RR-TB and no FQ resistance (Tiberi et al., 2022, WHO, 2022c).

The only new therapeutic agents for MDR-TB that have been developed over the last four decades are Bedaquiline and delamanid (Kurbatova et al., 2015, Gupta-Wright et al., 2018). Additionally, the alarming rise in AMR is rapidly limiting treatment options for infectious diseases and can have

devastating consequences, potentially erasing gains in global TB control (Reynolds and Heysell, 2014). As there are few drugs in development for DR-TB, it is critical to maintain the utility of currently available TB drugs (Asin-Prieto et al., 2015). One approach to preserving the utility of key drugs such as FQs is to optimize their doses based on PK and PD principles.

2.2. Population pharmacokinetic studies

2.2.1. General concept

Modeling and simulation are quantitative methods that have long been used in non-pharmaceutical industries to improve the efficiency of products. Modeling and simulation use mathematical algorithms and statistics to design simplified descriptions of complex systems under investigation (Gieschke and Steimer, 2000). Therefore, a model usually has a simplified representation of the systems. The type of simplification is related to the purpose for which it is used. Neither are "true" models, but each may be suitable for its intended purpose. Population modeling is a tool for identifying and describing relationships between individual physiological characteristics and observed drug exposures or responses (Marsot, 2018). PopPK was first introduced in 1972 (Sheiner et al., 1972), and it is a very useful concept for the clinical use of existing drugs and the discovery of new ones.

It is highly recommended to incorporate PK and PD elements into the production of a medicine to make it more rational and efficient (Reigner et al., 1997). PK/PD modeling is an integral part of drug discovery and is a mathematical approach for studying PK, PD, and their relationships. A key tenet of the PK/PD model is that the relationship between drug exposure and effect is easily predicted (Mager and Jusko, 2008, Negus and Banks, 2018). The PK/PD models describe the relationship between administered dose, serum concentration, and response, as well as surrogate

variables, outcome measures, and ADRs (Zou et al., 2020). This approach is widely used in new drug development and dosing regimen optimization (known as model-based drug development, MBDD, and model-informed precision dosing, MIPD, respectively) (Zhang et al., 2022).

MBDD or MIPD encompasses drug development and the application of various models of preclinical and clinical data sets to guide the development of drugs and therapeutic decisions (Zhang et al., 2022). A PK/PD-driven approach has been shown to help optimize the process of developing drugs (Gieschke and Steimer, 2000). MBDD or MIPD also improves therapeutic outcomes and decreases ADRs by tailoring treatments to individual patients. MIPD approaches include population PK/PD, physiologically based PK (PBPK), and computational pharmacology (Kim et al., 2020). Regulatory organizations strongly advise including antimicrobial PK/PD profiles in drug development programs (Nielsen et al., 2011, Palmer et al., 2022).

Inter- and intra-individual PK variability is common in pharmacotherapeutics and has an impact on drug efficacy and safety (Karlsson and Sheiner, 1993, Reynolds and Heysell, 2014). Surrogate variables such as age (in neonates), comorbidities (diabetes mellitus or HIV), host pharmacogenetics, renal or liver functions, and concomitant drug therapy can be used to link this variability to physiological processes. These variables influence drug absorption, distribution, metabolism, and excretion (Karlsson and Sheiner, 1993, Sachidanandam et al., 2001, Ahmed et al., 2016, Walter-Sack and Klotz, 1996). Alterations in any one of these PK parameters lead to alterations in the remainder. Variations in PK lead to variations in drug response (Smith and Rawlins, 1973). PK/PD modeling may describe the inter-and intra-individual variability quantitatively since it characterizes the PK and PD as well as describes relationships between key covariates including age, body weight, HIV infection, degree of immunodeficiency, presence of

liver disease, genetic polymorphisms of drug-metabolizing enzymes, and demographic factors and PK/PD parameters (Holford and Sheiner, 1981, Vozeh et al., 1996).

Individual kinetic profiles are not required for a population model-based analysis. Even with sparse sampling (for example, only one or two concentration measurements per patient), population models enable the assessment and quantification of possible causes of variations in exposure/response in a specific population (Gieschke and Steimer, 2000, S. Karger GmbH, 2003). Standard PK studies often use multiple sampling time points over 24 (or more) hours to determine the AUC0-24 and peak concentration (Cmax). However, such PK dose-ranging studies are time-consuming and not suitable for young children and critically ill patients, who are much more likely to have severe abnormal PK that may be related to differences in treatment outcomes (Kim et al., 2020, Vozeh et al., 1996).

In antimicrobials, PK/PD modeling is used to identify drug resistance, insufficient or toxic doses, and to optimize drug doses that may ensure a desirable antimicrobial effect (Kim et al., 2020). PK/PD index is frequently used in the estimation of optimal dose and target concentration needed in the body as it predicts a suboptimal dose that promotes resistance selection in mutant microbes (Editorial, 2018). The most common antimicrobial effect measures in antimicrobial PK/PD analysis are microbial killing, resistance to drugs, and halting growth of the microbes. Designing dose-effect studies, identifying doses that may suppress drug resistance, and selecting susceptibility breakpoints are all crucial phases in antimicrobial drug development (Nielsen et al., 2011). PK/PD studies integrate all prospective drug exposures for standard dosing regimens as well as all likely MIC profiles into the breakpoints definitions for a positive outcome (Gumbo et al., 2015, Nielsen et al., 2011). In this respect, the probability of target PK/PD can predict the therapeutic success of a particular antimicrobial agent.

The probability of target attainment (PTA) is the likelihood of achieving a specific PK/PD index correlated with the efficacy of an antimicrobial drug at a given MIC value. As a result, the PTA can be estimated for various MIC values of a wide range of infectious agents. Therefore, PK/PD indices may be utilized to choose dosing regimens with PTA greater than 90% in the population explored, maximizing the probability of favorable outcomes, establishing clinical breakpoints, and suppressing resistance (Asin-Prieto et al., 2015, de Velde et al., 2018).

Monte Carlo simulation (MCS) takes into account patient variability in both PK parameters and PD (relative to MIC), and for antimicrobials, PTA is determined from stochastic simulations of these models (Gumbo et al., 2015). MCS generates random AUC, Cmax, and MIC values using a probabilistic density function. Following the calculation of the probability density function for both PK and microbiological parameters, a computer program is used to simulate multiple MIC drug exposure scenarios. For each scenario, a random MIC value and a random PK value are calculated, each corresponding to their respective probabilities (Gumbo et al., 2015). A significant quantity of PK/PD results is calculated throughout the computer simulation process, which eventually indicates a range of probabilities. The resulting probability distribution of AUC/MIC or Cmax/MIC can be used to explore the appropriate dose range that may help predict favorable antimicrobial outcomes (Gumbo et al., 2015, Dalhoff et al., 2009).

2.2.2. The PK and PK/PD of Fluoroquinolones

FQs are antimicrobials with a wider spectrum of activity that were first used to treat TB in 1984 and have ever since become a key member of TB medications, especially for drug-resistant infections (Hawkey, 2003). They are the mainstay of MDR-TB therapy, with patients prescribed MXF, LFX, or gatifloxacin (Sarathy et al., 2019, Johnston et al., 2009). WHO treatment guidelines show the higher bactericidal activity of later-generation FQs such as LFX, MXF, and gatifloxacin

compared to ofloxacin and ciprofloxacin (old-generation FQs) (Caminero, 2008). A meta-analysis found that the use of later-generation FQ improved cure rates in patients with XDR-TB isolate that was resistant to ofloxacin. However, the highly active WHO group A FQs (such as MXF and LFX) show intra-individual and inter-individual PK variability which can cause low drug exposure (van den Elsen et al., 2020). Optimizing the use of FQs for treating MDR-TB may improve outcomes, enabling the shortening of treatment duration, and prevent further resistance (Jacobson et al., 2010). The PK and PK/PD of FQs in TB are discussed briefly in the following section.

The PK of Fluoroquinolones

The FQs have excellent absorption profiles upon oral administration, with bioavailability of ofloxacin in the range of 85-95% (Turnidge, 1999), LFX >99% (Chien et al., 1997, Turnidge, 1999), and MXF 86 - 91.2% in healthy individuals (Ballow et al., 1999). The simultaneous use of the multivalent cations Zn2+, Al3+, and Fe3+ significantly reduces FQ absorption (Ballow et al., 1999, Stass and Kubitza, 2001). AUC and Cmax of MXF were decreased by 40% and 45%, respectively, when co-administered with sucralfate. These parameters were decreased when co-administered with iron by 61% and 41%, respectively (Polk et al., 1989). Food has no effect on FQ AUC while slowing absorption and slightly reducing Cmax (Ballow et al., 1999, Stass, 1999). LFX and ofloxacin are FQs that exhibit approximately 25% protein binding at the doses tested, regardless of concentration (Bergogne-Berezin, 2002), while MXF protein binding estimated to be 25-50% (Bergogne-Berezin, 2002, Stass, 1999), with a significant IIV (Peloquin et al., 2008). Ofloxacin, LFX (Pranger et al., 2011), and MXF generally have good penetration into body fluids (Bellmann et al., 2004), including into the cerebrospinal fluid (Alffenaar; et al., 2008, Siefert et al., 1999).

Depending on the individual drug, FQs have a different elimination profile. LFX and ofloxacin are excreted mostly unchanged in the urine, but only 5% are biotransformed by liver enzymes (Yew et al., 1991). MXF is excreted by multiple pathways, with approximately 50% undergoing hepatic glucuronidation and sulfation, and 25% and 20–25% excreted unchanged in the feces and urine, respectively (Tachibana et al., 2005). MXF metabolism does not involve the cytochrome P450 system. However, p-glycoprotein and other enzymatic systems in MXF metabolism can lead to drug interactions (Senggunprai et al., 2009).

Normalized PK values documented for MXF in healthy individuals were t1/2 10.7-13.3 hours, Cmax 4.34 g/ml/70 Kg, and AUC0-24 39.3 g*h/ml/70 Kg (Stass et al., 1998). Additionally, the normalized PK values reported for LFX in healthy individuals were t1/2 6-8 hours, Cmax 6.21 µg/mL/70 kg, and AUC24 44.8 µg*h/mL/70 kg. Infection with HIV had no effect on LFX PK, and no interaction was found between MXF and the antiretroviral agents (Goodwin et al., 1994, Villani et al., 2001). The PK of MXF 400 mg and 800 mg was reported on the basis of an investigation of intensified therapy for TB meningitis, which found that high dosages did not result in toxicity but raised *in vivo* exposure (Ruslami et al., 2013).

The PK/PD of Fluoroquinolones

The ratio between the 24 h AUC and MIC (AUC0-24/MIC) strongly predicts FQs exposure and a positive treatment outcome (Craig, 1998, Craig, 2001, Firsov et al., 2001). The PTA that can predict a favorable outcome can be estimated using these PK and PD parameters (Stass and Kubitza, 2001). The hollow fiber bioreactor system (HFS) and murine studies revealed that the AUC over MIC ratio is a very strong predictor of the antimicrobial effects of FQs (Gumbo et al., 2004, Ruslami et al., 2013). An unbound AUC0-24/MIC ratio of at least 100 or greater was shown to be a reliable measure of the maximal antibacterial effect of FQ against Gram-positive

and Gram-negative bacteria (Hu et al., 2003, Shandil et al., 2007). In the HFS experiment, a free MXF AUC0-24/MIC ratio of 53 was found to significantly reduce more than 3 log10 CFU/mL of MTB, resulting in suppression of the emergence of drug resistance. Moreover, MXF's AUC0-24/MIC ratio of 100 effectively kills the FQ-susceptible MTB and prevents drug resistance (Gumbo et al., 2004, Shandil et al., 2007).

The unbound ratio of AUC to actual MIC and published MIC90 of 1000 mg LFX was 187.67 and 96.51, respectively in previous studies (Bergogne-Berezin, 2002, Peloquin et al., 2008). Johnson and colleagues reported that the most favorable bactericidal effect was observed for LFX 1000 mg (Johnson et al., 2006). Gumbo *et al*. (2004) reported an AUC0-24/MIC ratio of 129.1 for LFX using MICs of 1.0 µg/ml. However, in a recent HFS experiment, it was found that the AUC0-24/MIC predicting maximum MTB kill for LFX was 146, while the ratio predicting resistance prevention was 360. According to the results of this experiment, maximal LFX dosages of 1500 mg are required to kill as many mycobacteria as possible, provided that undesirable effects of the drug can be avoided (Deshpande et al., 2018).

The MXF study identified AUC/MIC exposures that might potentially reduce acquired drug resistance (Angeby et al., 2010, Shandil et al., 2007). These exposures were then studied in MCSs, leading to the suggestion that amounts of 800 mg/d might be more effective for lung TB (Ruslami et al., 2013). The conventional 400 mg/day dose can achieve optimal target exposure in only 59% of simulated patients (Schentag et al., 2003). Other HFS model studies indicate that even a daily dose of 800 mg is insufficient to prevent a future acquisition of drug resistance (Drusano et al., 2010b).

Chapter 3: Methodology

3.1. Study setting, Design and Subjects

We carried out an observational cohort investigation from November 2017 to May 2020 at outpatient departments of Hospitals in Southern Ethiopia, which serve as MDR-TB treatment initiative centers (TICs). The Hospitals include Nigist Eleni Mohamed Memorial Referral Hospital (earlier known as Hosanna Zonal Hospital), Butajira hospital, Yirgalem Hospital, and Arbaminch general hospital. The Hospitals are located south of the capital, the distance ranging from 134 (Butajira Hospital) to 436 (Arbaminch Hospital) km. This study involved the collection of socio-demographic data, sputum cultures, and blood samples from adult MDR-TB patients from the hospitals. The clinical isolates of MTB were prepared from the baseline sputum cultures obtained from the regional reference laboratory of TB in Southern Ethiopia and these were used for LPA, determination of the MIC, and *gyrA* gene sequencing. Plasma separated from blood was used for the PopPK study.

3.2. Sample Size and Power

Sample size calculation was based on methods and assumptions made in another similar model-based study of Rifapentine and MXF (Drusano et al., 2010a). It was posited that rifapentine exposure (AUC0-tau per week) is significantly correlated with the anti-mycobacterial activity of therapy determined by the biomarker of "time to detection" of MTB (growth index) measured in liquid (MGIT) culture systems. The assumption was made that the correlation in patients with TB between rifapentine AUC0-tau/MIC per week and MGIT time to detection biomarker is only one fifth as powerful (r^2 =0.195) compared to the correlation (r^2=0.975) in the murine MTB-model

between rifampin AUC/MIC and MTB CFU from the lung. A sample size of 30 patients provides > 80% power for identifying a difference of 0.442 between the null hypothesis and alternative hypothesis using a one-sided test of the hypothesis with a significance level of 0.050. We increased the number by 33% to account for lost PK specimens, missing PD data, and protocol deviations. Thus, a cohort of 80 MDR-TB patients (n=43 on MXF-and n=37 LFX-based MDR-TB regimens) was included purposively for the exploration of treatment outcome and associated factors (**Paper I**), 39 (n=18 in MXF and n=21 in LFX groups) for the PK/PD model-based study of MXF and LFX (**Paper II**), and 70 clinical isolates (n=33 for LFX and n=37 for MXF) for the phenotypic and genotypic characterization of drug resistance (**Paper III**).

3.3. Inclusion and Exclusion Criteria

We included adult patients with confirmed MDR-TB who were 18 years or older and had begun treatment as of November 2017. Patients with or without HIV co-infection who received LFX (750 or 1000 mg/day) or MXF (600 mg/day) for ≥8 days and provided written informed consent were included. The study participants had been attending the TICs monthly for medication intake and treatment monitoring. Patients <18 years of age who were with yet not known treatment outcome (treatment stopped or still on treatment, or treatment results unknown from data source) and patients with hematocrit <25%, seriously ill and anemic were excluded. All patients in this study received standard TB treatment as per the national and WHO guidelines for TB and leprosy (FDRE, 2017, WHO, 2017b).

3.4. Data Collection and Laboratory Analysis

Demographic (age and gender), medical (radiographic, BMI, TB treatment history, co-illness, and therapeutic regimen), and laboratory (AFB and sputum culture) data were collected by trained

professionals in each hospital using a patient information sheet. Before acquiring data and patient follow-up, data collectors were trained and oriented about the purpose and design of the study.The majority of ADRs were documented in patient files and were taken exactly as they were. Hepatic and renal toxicities were deduced from at least three LFT and Cr. measurements taken during the course of the therapy period, respectively. Those patients with persistently higher than normal AST and ALT, and Cr. were considered hepatotoxic and nephrotoxic cases, respectively. Each patient also had five or more consecutive culture results recorded from their monthly culture tests from the start of medication. The accuracy and consistency of all the data collected was checked in every step.

3.4.1. Blood Sample Collection and Bioanalysis

After obtaining written informed consent, patients were asked to stay at the center for blood sampling. Patients had to stay in the hospital for 48 hours as they visit the hospitals from a remote areas and for the convenience of the blood sampling over the 24 hours as per the protocol of this study. At 0 hours (pre-dose sampling), 2, 4, 6, 9, 12, and 24 hours after dosing, 4 mL of blood was drawn with a vacutainer (Becton Dickinson Biosciences, San Jose, CA, USA) into EDTA tubes. The plasma was separated without a delay and each test tubes were labeled and coded after properly mixing the blood with the anticoagulant. The samples were shipped via the cold chain to referral laboratories for proper storage and bioassay. Plasma drug concentrations were quantified at Inje University College of Medicine (Korea) using a previously developed and validated liquid chromatography-electrospray-ionization-tandem mass spectrometry (LC-MS-MS) method (Kim et al., 2015).

3.4.2. Culture Identification of the Clinical Isolates

Sputum samples were decontaminated with an equal amount of 4% sodium hydroxide solution and centrifuged for 15 minutes at 3000 rpm. The supernatant was removed and a drop of phenol red solution was added as an indicator, and the precipitate was neutralized drop by drop with 2N HCL solution (Tilahun et al., 2018). LJ-pyruvate and LJ-glycerol media derived from eggs were prepared, aliquoted, and stored in the freezer at 2 - 8^0C for a period of two months (WHO, 2014b). To promote the growth of MTB lineages, the stored isolated strains were inoculated into conventional LJ-media slants supplemented with 0.3% glycerol and incubated at 37ºC for at least 2 months, with mycobacterial colonies being observed weekly (Tilahun et al., 2018). To identify AFB-positive isolates, the colonies were examined under a microscope with the Ziehl-Neelsen stain. Loop full colonies were harvested into 2 mL cryovials containing 500 µL sterile nuclease-free water for heat inactivation for the LPA test. The remaining colonies were collected and processed for determination of MIC and sequencing of the *gyrA* gene for those isolates identified to have mutation by the LPA and a few more suspected strains.

3.4.3. Line Probe Assay

FQ-resistant (LFX and MXF) MTB complex isolates were characterized with an LPA using Genotype® MTBDRsl VER 2.0 (Hain Life science, Germany). This test depends on DNA-STRIP technology and was carried out in accordance with the manufacturer's instructions (Ling et al., 2008, WHO, 2022b). Briefly, the whole procedure involves DNA extraction, multiplex polymerase chain reaction (PCR) amplification, and reverses hybridization. This assay detects the absence and/or presence of wild-type (WT) and/or mutant (MUT) DNA sequences within a specific region of the *gyrA* gene, which encodes the A subunit of DNA gyrase responsible for the negative super-coiling of double-stranded closed-circular DNA.

3.4.4. Determination of Minimum Inhibitory Concentration

MIC determinations were performed partly at AHRI in Ethiopia and partly at the Inje University College of Medicine (Korea) in 96 U bottom-shaped polystyrene microtiter using the EUCAST broth microdilution (Schon et al., 2020). Briefly, 7H9 broth-OADC and stock anti-tuberculous agents were prepared based on the protocol. Except for column 1, other wells were filled with 50 µL of 7H9-OADC media and column 1 was filled with 50 µL of the working solution. Column 2 is also filled with 50 µL of the working solution. Using a multichannel pipette 1:2 dilutions were then made by mixing and adding 50 µL of column 2 to the following column. Serially 2-fold dilution to column 11 was made and finally, 50 µL was discarded from the last row. A 0.5McFerland standard was prepared from fresh colonies (within 2 weeks from visible growth) and, subsequently, a 1:100 and a 1: 10,000 (Growth control, GC1%) bacterial suspensions were prepared in 7H9-OADC media. A 50 µL of the 1:100 inoculum of 0.5 McFerland were added to antibiotic-containing wells (**Paper III**). GC100% and GC1% were then inoculated and the plates were covered with a plastic lid and kept in O2-/CO2- permeable plastic bags followed by incubation at 37°C for a maximum of 21 days. The commercial available strains of the mycobacterium tuberculosis complex (MTBC) H37Rv was used as a reference in the MIC range of 0.0625-8.0 mg/L and 0.0625-16.0 mg/L for LFX and MXF, respectively. The targeted ranges for the rest of the drugs for which MIC was determined are provided in the Supplementary file (**Paper** III). The plate was read using an inverted mirror first after 7 days of incubation and then at 14 days of incubation. Finally, the MIC value was reported in mg/L.

3.4.5. *gyrA* Sequencing

The *gyrA* sequencing was conducted at the Inje University College of Medicine. Sequencing of *gyrA* (this gene was purposely selected based on previous information for the most prevalent

mutation for FQs) was performed for the strains identified as resistant in LPA and additionally suspected strains for resistance. One loop of selected clinical isolates was dispensed in Tris-EDTA buffer (pH 8.0) and incubated at 95°C for 30 minutes to obtain genomic DNA. DNA fragment of the *gyrA* gene was PCR amplified with primer sets (**Table 1, Paper III**) and SolgTM Pfu-X (Solgent, Republic of Korea).

3.5. Statistical Analyses

Details of the statistical analyses performed are presented in the attached papers. Data are expressed as percent, mean ± SD/SEM, and Median (Interquartile range). PK parameters estimated by PopPK were expressed as geometric mean (GM) and percent relative standard error (RSE), and IIV was expressed as percent coefficient of variation (%CV). The results were described as numbers and percentages and compared using the chi-square test or Fisher's exact test for categorical variables. On the other hand, the Mann-Whitney test was used to analyze continuous variables.

Treatment outcomes were evaluated, compared, and contrasted based on the WHO outcome criteria. Kaplan Meir survival analysis was carried out to compare outcome and assess the risks of unfavorable outcomes between LFX and MXF-based treatment groups. The chi-square test, Wilcoxon rank test, and Fisher exact test were used to compare the outcomes of the two treatment groups. Associations between outcome predicting factors and treatment outcome, MIC values and treatment outcome, and sociodemographic/clinical variables and FQ resistance were evaluated using a univariate and multivariate Cox Proportional hazard regression model, Chi-Square or Fisher Exact test, and binary logistic regression model, respectively. The findings were then presented as an adjusted hazard ratio (AHR), adjusted odds ratio (AOR), and 95% confidence intervals (CIs).

The SPSS software version 25.0 (IBM, Armonk, NY, USA) was used for data analysis in Paper I and Paper III. However, Phoenix WinNonlin version 8.0 (Certara, Princeton, NJ, USA) was used for the calculation of Cmax and AUC0-24, and the estimation of other PK parameters. We developed PopPK using nonlinear mixed-effects modeling and first-order conditional estimation algorithms. Various covariates were also tested for their influence on the PK parameters. One-compartment and two-compartment models with first-order absorption and elimination rates (with and without absorption lag time) were tested. Additive, proportional, and compound residual models were also tested. Model selection was based on visual inspection of model fitting and diagnostic plots, precision of parameter estimates, minus two log-likelihood values (-2LL), and Akaike's Information Criterion (AIC) and Bayesian Information Criterion (BIC). Reliability of the final model was assessed using a 95% CI of 2.5. and 97.5 percentile of the simulated concentration-time profile. The exponential function was used for the estimation of a typical population PK parameters and their IIV.

We constructed the simulated PK profiles for the individual patients at different doses and calculated the PTA using the AUC0-24/MIC ratio. Based on the original doses patients received and plasma drug concentrations in this study, three doses were simulated for the drugs. The doses simulated for LFX were 750 mg, 1000 mg and 1500 mg. Similarly, the doses simulated for MXF were 600 mg, 800 mg and 1000 mg. Accordingly, Cmax and AUC0-24 were estimated for each plasma concentration vs time profile of the simulated doses. Simulated drug exposure parameters (Cmax and AUC0-24) are depicted by the boxplots for each doses of the drugs. PTA, defined by the AUC0-24/MIC meeting previously recommended target values for a favorable treatment outcome, was calculated and analyzed descriptively for the simulated doses of LFX and MXF. Based on the available literature on LFX, 146 and 360 targets were selected for the evaluation of

maximal mycobacterial killing and resistance suppression in mutant strains, respectively (Deshpande et al., 2018). In the same analogy, the two values set in the literature, 53 and 100, respectively were used for MXF (Gumbo et al., 2004, Shandil et al., 2007). A P-value <0.05 was considered statistically significant in all analyses.

3.6. Ethical Consideration

We carried out this study in accordance with the Declaration of Helsinki. The research protocol in this study was ethically approved by the College of Health sciences, Addis Ababa University (Protocol No. 078/17/SoP), and the National Research Ethics Committee (NREC). A written consent form was signed by the study participants after they were provided with details of the study protocol regarding the purpose, benefits, and risks of the study. Data access was restricted and identifiers were removed to ensure confidentiality and anonymity of patients involved in this study.

3.7. Operational Definitions

The number of days from the initiation of MDR-TB treatment to the date of collection of the first two successive negative sputum cultures is defined as first sputum culture conversion (FSCC) (Kurbatova et al., 2015). WHO 2013 recommendations were used to evaluate and compare outcomes between patients receiving MXF-based and LFX-based therapy (WHO, 2014a). A successful outcome refers “cure” and “completion of treatment”, whereas unfavorable outcome includes death, failure, and lost-to-follow-up (LTFU).

“Cure” is defined as completion of guideline-recommended treatment, without treatment failure, and 3-5 successive negative sputum cultures collected at least 30-day intervals during the last months of treatment. “Completed treatment” cases are those who completed treatment and had

clinical improvement, but culture tests were either absent or nor received. The term "death" refers to the death of a study participant from any cause over the treatment period. Individuals who had two or more positive culture test results from the five cultures collected around the final months of treatment, or who had treatment stopped untimely as a result of poor medical or radiological prognosis or drug toxicity, were classified as “treatment failures”. LTFU refers to those that discontinued taking their treatment for two or more months for reasons other than medical requirements. *In Vitro* MIC: the smallest drug concentration that prevents the growth of more than 99% of a microbe in a solid medium or broth dilution susceptibility in a test scenario (Benkova et al., 2020). Mycobacterial strain refers the descendants of a single isolation in pure sputum culture, usually derived from a single initial colony on a solid growth medium. A strain may be considered an isolate or group of isolates that can be distinguished from other isolates of the same genus and species by phenotypic and genotypic characteristics (Chae and Shin, 2018).

Chapter 4: Results

4.1. General overview

We examined treatment outcomes and associated factors between MXF- and LFX-based regimens in MDR-TB patients based on the WHO-defined outcome criteria (WHO, 2014a), culture conversion rate, and LPA. Overall treatment outcome was assessed in a cohort of 80 patients on either MXF (n=43) or LFX-based regimen (n=37) from four hospitals (**Paper I**). In the second part and follow-up study, we developed a population PK model for the drug concentrations of LFX and MXF. The developed PopPK model was used to assess IIV, PTA, and covariates related to the PK parameters that can predict treatment outcomes (**Paper II**). In the third part, 70 clinical TB isolates obtained from the first cohort were used to characterize drug resistance to FQs by phenotypic and genotypic methods (**Paper III**).

4.2. Patient characteristics

In total, 80 subjects with MDR-TB or RR-TB were enrolled in this study. Of these, 43 received an MXF-based regimen, while 37 received an LFX-based regimen. Male patients outnumbered female patients in the total sample. The sociodemographic and clinical variables were statistically similar between the LFX and MXF groups. All 80 patients were included in the assessment of outcome and outcome predictors between the two treatment groups **(Paper I)**. Of these 80 patients, 62 were available for potential blood draw for the subsequent population PK/PD study of LFX and MXF. Blood samples were drawn from 55 patients, as 7 seriously ill patients were excluded. Of the 55 patient samples, 16 were excluded due to difficulty in collecting adequate blood and unreliable concentration-time profiles. Therefore, samples from 39 patients (n=21 receiving LFX

doses of 750 or 1000 mg and n=18 receiving MXF doses of 600 mg) were included in the PopPK studies **(Paper II).** Approximately 70 clinical isolates (n=33 for LFX and n=37 for MXF) obtained from our first study cohort of patients (80) were phenotypically and genotypically characterized for drug resistance profile **(Paper III)**.

4.3. Treatment outcome and its predictors

The overall successful outcome in both groups was 65% (52/80). The total number of cases cured and completed treatment was 43 and 9, respectively, while deaths, failure, and LTFU cases were 8, 9, and 11, respectively. Treatment success was higher in LFX groups than in MXF groups ($\chi 2$=6.40; p=0.012) (**Paper I**). Additionally, as indicated in Figure 2 (**Paper I**), the median survival (days) in the LFX group was approximately two times that of the MXF group. The cumulative survival probability was also twice as high for the LFX group as for the MXF group (27.4% vs. 14.4%). Furthermore, in the log-rank test ($\chi 2$ =13.88, p= 0.001) and Cox proportional hazards analysis, we found that the LFX group had a reduced risk of unfavorable outcome than MXF group (AHR= 0.25, 95% CI (0.09-0.71), p<0.05). LPA of the clinical isolates showed that 71 (88.8%) were susceptible and 9 (11.2%) were resistant to FQs. Of these resistant cases, 3 (11.2%) were also resistant to injectables. Culture conversion within 90 days starting the treatment predicted treatment outcomes in both groups. However, there was no statistically noteworthy disparity between the treatment groups in terms of culture conversion rates.

We also showed that treatment regimen type (LFX vs MXF-based regimens), habit of drinking alcohol, cavitray lesion, serum creatinine (Cr.) ≥0.87 mg/dl, ALT≥32.5 Iu/L predicted (p<0.05) the risk of treatment failure as described by the adjusted hazard ratio and 95% CI (**Table 4-5**, **Paper I**).

The overall incidence of ADRs was 75.0% for both treatment groups. Ototoxicity, muscle pain, blood disorders, gastrointestinal disturbances, liver toxicity, psychotic problems, and peripheral neuropathy were commonly observed adverse events in both groups. Although the proportion of ototoxicity tended to be higher in the MXF group than in the LFX-group, there was no statistically significant difference in the incidence of remaining individual ADRs. However, a higher cumulative ratio of ADRs occurred in the MXF groups than in the LFX-group ($\chi 2=6.051$; $p<0.05$) (**Table 6, Paper I**).

The low treatment outcome observed in our MDR-TB patient cohort further motivated the subsequent PopPK and PK/PD model-based study to assess whether the previously set drug exposure targets associated with positive treatment outcomes were met in the study participants. Therefore, in the following section, we present our results of the PopPK study.

4.4. Population PK and PK/PD studies

One-compartment additive and multiplicative error models with lag time described best LFX plasma concentrations profiles, whereas one-compartment model with additive error models without lag time described MXF concentrations (**Paper II**). All PK parameters were computed taking residual variations into account. All population PK estimates were presented in terms of GM and RSE, and IIV as CV (**Paper II, Table 2**). The goodness-of-fit and VPCs of the final models for both LFX and MXF are shown in Figure 1 and Figure 2, respectively (**Paper II**). A stepwise covariate screening showed that Cr. was a covariate affecting CL of the LFX, evidenced by a significant decrease in -2LL. In a similar scenario, BMI was a covariate affecting Vd of the MXF. Therefore Cr. and BMI were selected as covariates in the final model of the LFX and MXF, respectively.

Drug exposures estimated in terms of Cmax and AUC0-24 for both LFX and MXF is depicted in Figure 3 (**Paper II**). Individual Cmax and AUC0-24 values for both original and simulated doses of LFX and MXF are presented in Table 3 (**Paper II**). The previously established PTAs needed for the bacterial kill and resistance suppression for both drugs were calculated for the simulated doses of the drugs. The PTAs for the maximum bacterial kill at the critical MIC of 0.5 mg/L for LFX simulated doses (750 mg, 1000 mg, and 1500 mg) were 29%, 62%, and 95% whereas the corresponding PTAs for the resistance suppression were 0, 0, and 4.8%, respectively. Interestingly, at the MIC value of 1 mg/L, only 24% of the target achieved with the dose of 1500 mg, whereas none of the targets achieved at MIC≥ 1 mg/L (**Figure 4, Paper II**). Similarly, considering the WHO selected critical 0.25 mg/L MIC value, the PTA for the simulated doses (600 mg, 800mg, and 1000mg) of MXF was 94.4%. But the PTAs for the resistance suppression were calculated as 50%, 77.8%, and 94.4%, respectively. Upon increasing the MIC value to 1 mg/L, the PTAs for the maximal mycobacterial kill were 11.1%, 27.8%, and 38.9%, respectively. But none of the simulated doses attained the targets for the resistance prevention at MIC≥1 mg/L (**Figure 5, Paper II**).

4.5. Drug resistance characteristics

Of the 80 clinical isolates, 70 (n=33 from LFX and n=37 from MXF groups) were included in the characterization of drug resistance. The rest 10 clinical isolates were excluded due to contamination and non-growth at the referral laboratories. Figure 1 (**Paper III**) depicts the overall activities in this part of our study. Resistance profiles from geneXpert (n=28) and LPA (n=70) were evaluated against the MIC values for the individual drugs determined by the broth microdilution method. The frequency (number count) of MIC distributions of clinical isolates for the FQs is depicted in Figure 2. Moreover, *gyrA* sequencing profile was obtained for 14 of the

clinical isolates (9 were those found to be resistant in LPA result and 5 additional strains suspected clinically for resistance).

Seven (7/28, 25%) of the clinical isolates from RR-TB patients as diagnosed by geneXpert were later found to be sensitive to both isoniazid and rifampicin based on their MIC results (**Table 2, Paper III**). The LPA showed that about 9 (12.8%) clinical isolates were resistant to FQs (**Paper I**). Of these, 7 were from patients on MXF-based regimen, whereas 2 were from LFX-based regimen. Upon MIC determination, MIC profiles were ≥1 mg/L for the 7 and 9 of the isolates in LFX and MXF groups, respectively. Therefore, the discordance between LPA and MIC results were 5/33 (15.2%) and 2/37 (5.4%) for LFX and MXF, respectively (**Table 3, Paper III**). Out of the 14 clinical isolates sequenced for *gyrA* gene, mutations were identified in 5 of the clinical isolates in the D94 (Asp94). These were D94N, D94H, D94A, D94A and D94A. The MIC profiles for all of these strains were ≥2.0 mg/L (**Table 4, Paper III**).

MIC profiles of the FQs (LFX at ≤0.5 and MXF at ≤0.25) in the clinical isolates were found to have a positive association with treatment outcome (62% vs 38%, $\chi 2$=18.7; P=0.001 and 78% vs 22%, $\chi 2$=23.6; P=0.001, respectively). These same MIC thresholds of both drugs also had a positive association with culture conversion rates before 90 days ($\chi 2$ =14.7, p=0.03 and $\chi 2$=12.5; p=0.04, respectively). Various demographic and clinical variables were screened for their association with FQ-resistance using univariate and multivariate logistic regression (**Table 5, Paper III**). Our multivariate analysis finally showed that baseline cavitary lesion (AOR=1.2; 95%CI=0.1-2.4, p=0.01) and Cr. (AOR=11.3; 95%CI=1.2-14.1, p=0.03) predicted FQ resistance.

Chapter 5: Discussion

The present study aimed to predict drug resistance and treatment outcome and associated factors, PK, and PD features of MXF and LFX in Ethiopian MDR-TB patients. Overall, MDR-TB patients in our study cohort had a lower treatment outcome (65%) than outcome reports in other settings (**Paper I**). For example, a higher treatment success was reported in various settings: previous national study (75.7%) (Tola et al., 2021), Taiwan (82.4%) (Yu et al., 2018), Pakistan (75.8%) (Javaid et al., 2018), Korea (72.7%) (Kang et al., 2021), and Tanzania (75.7%) (TH et al., 2019). The inadequate plasma drug exposure as described by the low PTA in our second study (**Paper II**) might explain the relatively lower outcome in Ethiopian MDR-TB patients. LPA revealed that 11.3% of the study subjects had a FQ resistant TB. Out of the 28 unsuccessful outcome cases, 9 (32.1%) were directly related to FQ resistance. This rate was higher than previously reported from Tigray region of Ethiopia (Welekidan et al., 2020), which may indicate that resistance to the most important and effective MDR-TB medications (FQs) is increasing. The higher MIC values of FQs were demonstrated to have association with the majority of unsuccessful outcomes (**Paper III**).

We also showed that, in terms of the WHO outcome criteria and other outcome indicators, the LFX-based regimen performed better than the MXF-based regimen (**Paper I**). However, the earlier studies did not show difference between the two treatment groups (Kang et al., 2016). The discrepancy could be caused by variations in the study subjects, doses administered, companion drugs, and research methods. Presence of injectable agents in MXF-based regimen and new antitubercular drugs (Bedaquiline and delamanid) in LFX-based regimen is a likely cause of differences in the overall treatment success. It has to be noted that bedaquiline and delamanid were recently added to the WHO group A regimen due to their high efficacy of MDR-TB treatment

(WHO, 2022c) although the subjects in this study received these new drugs as companion drugs of LFX-based regimen even before this grouping. Patients in the current study received dosages of 750 mg or 1000 mg LFX and 600 mg MXF. However, individuals in the prior studies that we contrasted with our study either received 400 mg MXF or 750 mg LFX in the corresponding regimens (Kang et al., 2016). This difference in dosage could be another source of discrepancy, since there is an evidence, at least for LFX, showing that higher early bactericidal effect is associated with 1000 mg/day than 750 mg/day (Peloquin et al., 2008). The smaller sample size we used in our studies might have impacted the outcomes observed. The patient population we included in the present study could also be a reason for the differences due to the fact that an inter-ethnic or IIV influences the PK and PD of drugs (Ahmed et al., 2016, Shah, 2015, Naidoo et al., 2018). Interestingly, even though our model-based study of both drugs (**Paper II**) showed that the PTA in both cases was inadequate for favorable treatment outcome in many patients, higher simulated doses of LFX resulted in a more proportionate increase in the overall plasma drug exposure. Consistent to this observation, Peloquin et al (2008) showed that more favorable PK results were seen with LFX than MXF. A study from India also reported that higher MXF doses did not change treatment outcome (Tornheim et al., 2022). More interestingly, a model-based MXF study revealed that administering MXF twice a day at 400 mg instead of once a day at 800 mg was a preferred regimen for MDR-TB patients, providing a better outcome (Yun et al., 2022). This indicates that a more powered sample sized and controlled design study is needed to prove if the observed differences in treatment outcome and drug exposure is related to the inherent differences in the efficacy of the drugs and frequency of a daily dosing.

Alcohol consumption, cavitary lesions, Cr, and ALT levels predicted success an unsuccessful treatment outcome (**Paper I**). Culture conversion before 3 months led to a successful treatment of

MDR-TB patients as evidenced elsewhere (EV et al., 2015). Alcohol consumption negatively impacted MDR-TB outcome directly or indirectly in previous studies (K et al., 2014) and this concurs with our finding. Cavitary lesion was identified as a risk factor in previous studies (Charoensakulchai et al., 2020), which is in line with our findings (**Paper I & III**). Cavities may serve as a sanctuary to bacteria and limit access of drugs, making them ineffective, as a result of which the bacteria becomes non-responsive (MA et al., 2004, SE et al., 2018). In our PopPK study, BMI was a covariate that influenced Vd of MXF. In this regard, weight-based dosing practice of MXF in Ethiopian patients appears justifiable. Similarly, Cr. was a covariate influenced the CL of LFX (**Paper II**). Moreover, Cr. was demonstrated to have association with FQ resistance (**Paper III**). Whether Cr. directly influences the treatment outcome, PK of drugs, and resistance in TB remains unclear. Cr. particularly affected CL of LFX (**Paper II),** which is consistent with another study report (Peloquin et al., 2008). However, Cr. may not be a reliable prognostic parameter in critically ill patients as the PK of drugs is difficult to predict in these patients (Camargo et al., 2019, Claus et al., 2013). In another scenario, FQs elevate Cr. and may induce acute interstitial nephritis (AIN). AIN may result in end stage renal failure and this may interfere with drug clearance (Camargo et al., 2019, Claus et al., 2013, Y et al., 2011, Udy et al., 2013). Therefore, Cr. appears to be an influential covariate to consider for TDM with the goal of optimizing LFX therapeutic outcomes. ALT was not selected as a covariate in the PopPK study although it was a predictor of treatment outcome in Paper I. Elevated ALT levels may be suggestive of drug-induced liver injury (DILI) (Jeong et al., 2015), which may also influence treatment outcome in TB patients.

Our PopPK and PK/PD model-based analysis predicted that the PTA for both LFX and MXF is inadequate for a successful treatment outcome and there was a higher relative IIV (%CV) of population estimated Ka for MXF and Vd and CL for LFX (**Paper II**). However, Vd and CL for

both drugs are higher in this study than in previous studies (Pranger et al., 2011, van den Elsen et al., 2019), which may explain the lower drug exposure (Cmax and AUC0-24) in our study population. Genetic factors might have influenced drug exposures in this regard. According to a report, MXF in TB patients exhibits considerable PK variability as well as genetic variants in drug transporters and metabolic enzymes (Pranger et al., 2011). In fact, a study in black Africans also revealed that genetic variation in UGT1A rs8175347 depressed MXF clearance, whereas rs3755319 boosted it. The same study found that the ABCB1 rs2032582 SNP reduced the bioavailability of MXF (Mugusi et al., 2020, Naidoo et al., 2018). Therefore, a more in-depth study to determine the impact of genetic factors on drug PK in Ethiopian TB patients may be beneficial.

Similarly, neither a maximal bacterial kill nor a resistance prevention target was achieved with the lower standard doses (750 mg and 1000 mg) of LFX. Moreover, only 4.8% of the patients achieved a resistance prevention target even with the highest simulated (1500 mg) LFX dose. However, the target for the maximal kill of bacteria increased proportionately and more predictably for LFX than MXF with increased doses (29%, 62%, and 95% for 750 mg, 1000 mg, and 1500 mg, respectively). A lower risk of QT interval prolongation by LFX (Malik et al., 2009), its greater treatment success compared to MXF (**Paper I**), and a more predictable PK with increased doses (**Paper II**) suggest that LFX-based regimens are preferable for dosage optimization and TDM in Ethiopian MDR-TB patients. A recent study report revealed that 1500 mg LFX confers the same effect as MXF 800 mg/day (Deshpande et al., 2018). Another study suggested that at the current epidemiological cut-off points, up to 1750 mg of LFX may be required for a maximum kill (Al-Shaer et al., 2019).

We carried out phenotypic and genotypic studies to characterize drug resistance in TB isolates from patients in our study (**Paper III**). The MIC of the drugs was determined by a broth microdilution for the phenotyping study, and the genotype of the drugs was evaluated using the results of the geneXpert, LPA, and *gyrA* sequencing. In comparison to findings from other studies, the FQ-resistance detected by LPA (12.9%) in the current investigation is lower. For example, it is reported that about 36% of MDR-TB cases in India have resistance to FQ (WHO, 2020, Chatterjee et al., 2018) and 30.2% have in South Korea (Lee et al., 2019). However, according to our phenotypic analysis (based on MIC profiles of the clinical isolates, the gold standard method for resistance profiling) higher proportion of (16/70, 22.9%) the clinical isolates were observed with risk of resistance to FQs (MIC≥1.0 mg/L). Therefore, the FQ resistance and unfavorable clinical outcome could be higher than we estimated earlier (**Paper I vs Paper III**). The present PK/PD model-based found that none of the doses of the studied drugs attained the target for resistance prevention at MIC greater than 1 mg/L (**Paper II**). Cavitatary lesions and Cr. were identified as predictors of FQ-resistance which concurs with our first study (**Paper I**) and other (Lee et al., 2019) studies. Genotypic-phenotypic DST results relatively showed a better agreement with LPA results than that of GeneXpert (98.6% vs.75%). The mutation identified by *gyrA* sequencing matched perfectly with the phenotypic analysis, where all the five strains had MIC≥2.0 mg/L. But only 55.6% of the LPA identified resistance for FQs agreed with the sequencing result. Mutations other than *gyrA* may account for the discrepant result. Indeed, among the 102 strains that were recently tested in Uganda for FQ resistance, 70/102 (68.6%) and 01/102 (0.98%) contained mutations at the *gyrA* and *gyrB* loci, respectively, that conferred resistance (Mujuni et al., 2022).

The lower MIC profiles (MIC ≤0.5 mg/L for LFX and MIC ≤0.25 mg/L for MXF) the FQs were associated with a higher proportion of treatment success in our study subjects. This could imply that FQs MIC profiles have an impact on treatment success in MDR/RR-TB patients. As observed in our study, the MIC values ≥0.25 mg/L (23/37, 62.2%) for MXF and ≥0.5 mg/L (20/33, 60.6%) for LFX are at risk of FQ-resistance if optimal doses of these drugs are not administered to patients since most of the values are at the borderline of the critical MIC values for the lower-level resistance. Therefore, larger proportion of these patients may develop acquired drug resistance. It should be noted that we demonstrated that conventional doses of MXF and LFX had inadequate exposure in Ethiopian MDR-TB patients (**Paper II**), and their concentrations in the blood increase proportionately (at least for LFX) with dose. Patients may benefit if the dosage design for both drugs could consider their phenotypic and genotypic DST profiles.

The findings of this study point to the relevance of DST and the conceivable value of further investigation in assessing whether larger doses attain the desired exposure. Evaluating higher starting doses of LFX and MXF, genotypic and phenotypic sensitivity studies, Whole Genome Sequencing (WGS) studies of MTB clinical isolates , and bioassay of the drugs by an appropriate sampling technique at a steady state are some future research directions based on the findings from the present study. Patients who fail to attain the AUC0-24 target or who have mutations indicating low resistance may be prioritized for dose/exposure optimization or replacement of an alternative FQ. TDM is hampered by expensive equipment and keeping the cold chain of the patient specimen. However, new tests, such as those that use dried blood spots or saliva, promise to test drug concentrations nearer to the patient care center (Davies Forsman et al., 2017, Kuhlin et al., 2019, Reynolds and Heysell, 2014).

Finally, a more stringent PopPK study is needed to determine the optimal starting dose that may ensure a favorable treatment outcome. If this can be conducted locally, finding a setting-specific dose is likely and that may decrease the need for TDM on a regular basis (Mohamed et al., 2021, van den Elsen et al., 2020, Vu et al., 2011).

Implications

- A better overall treatment outcome and favorable PK of LFX calls for further controlled studies to prove if treatment advantage with LFX could be utilized.
- Patients with cavitary lesion may benefit from adjustment of treatment duration in TB patients.
- Clinicians should be aware that higher Cr. and ALT levels among MDR-TB patients may result in poor outcomes.
- Inadequate plasma exposure of LFX and MXF may be a challenge for an effective treatment of MXF and LFX in Ethiopian patients, and dosage optimization and TDM may help tailoring doses of LFX and MXF in MDR-TB patients.
- An early drug sensitivity profile of clinical isolates for FQs are useful for the effective treatment of MDR-TB patients. Rapid genotypic tests are helpful here. However, the accessibility and accuracy of the tests in low-income settings are challenges in achieving this goal.
- BMI and Cr. may be useful patient covariates for TDM in Ethiopian MDR-TB patients.

Conclusions and Recommendations

This study found that an LFX-based MDR-TB regimen with the new oral drug background appears to be preferable to an MXF-based regimen in terms of better patient outcomes and reduced risk of adverse outcomes. Early assessment of MDR-TB patients regarding sputum culture conversion

rate, history of alcohol consumption, cavitary lesions, Cr. and ALT levels can help adjust treatment for a better outcome. Ethiopian MDR-TB patients have high IIV in plasma concentrations, exposure, and estimated PK parameters. Drug exposure to MXF and LFX may be inadequate and Cr. and BMI could be key covariates in determining the optimal dose and exposure of FQs. LPA showed a moderate level of drug resistance as evidenced in the clinical strains. Mutation in the *gyrA* gene is probably a dominant mechanism underlying FQs-resistance in the clinical isolates from Ethiopian MDR-TB atients. Logistic constraints restricted assays of the potential mutations in other genes which might also be determinants FQs-resistance in MTB. Genotypic drug sensitivity tests are effective in detecting drug resistance, but there are cases where they do not correlate well with phenotypic results. The actual drug resistance level is likely to be higher than anticipated in the study population. Unfavourable results are associated with higher MIC profiles. In such cases, the use of optimal doses of the drugs may guarantee a successful course of treatment. A more controlled multicenter dosing optimization that takes into account *in vivo* drug exposure and *in vitro* susceptibility profiles of clinical strains is an important area for further studies. Particularly, WGS has a potential that can provide a near-complete information of mutations associated with drug resistance as it includes almost the entire genetic repertoire of agiven clinical isolate of MTB. Also, the effect of pharmacogenetic factors such as UGT1A and ABCB1 genes on LFX and MXF PK is an important area for further investigation.

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Appendices

Appendix I: Scientific papers

Appendix II: Patient Information Sheet (In English).

Appendix III: Patient information Sheet (In Amharic).

Appendix IV: Subject Consent in English and Amharic (A).

Appendix V: Data Abstraction Form (Paper I)

Appendix VI: Ethical approval by the IRB

Infection and Drug Resistance

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ORIGINAL RESEARCH

Explorative Analysis of Treatment Outcomes of Levofloxacin- and Moxifloxacin-Based Regimens and Outcome Predictors in Ethiopian MDR-TB Patients: A Prospective Observational Cohort Study

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Purpose/Background: Although Ethiopia is among the thirty high multi-drug resistant tuberculosis (MDR-TB) burden countries in the world, comparative therapeutic efficacy of moxifloxacin and levofloxacin has not been explored, particularly in MDR-TB patients. We therefore aimed to prospectively compare clinical outcomes and determine potential predictors of the outcomes among patients on moxifloxacin or levofloxacin-based MDR-TB drug regimens.

Methods: We analyzed clinical parameters and laboratory data of eighty MDR-TB patients on moxifloxacin- or levofloxacin-based regimens. The clinical outcomes were compared using the Kaplan–Meier survival functions and the outcome definitions of the 2013 World Health Organization. Monthly sputum culture conversions and a molecular line probe assay results were also assessed. Observed outcomes and patient-related variables between the two groups were compared using chi-square, Wilcoxon Rank and Fisher exact tests. We also determined the potential predictors influencing treatment outcomes of moxifloxacin and levofloxacin using Cox proportional hazard model.

Results: The levofloxacin-based treatment group had a lower failure rate and adverse drug events as well as better treatment success than the moxifloxacin-based group. Overall treatment success was 65%. Disaggregating the data revealed that 53.8% were cured, 11.2% completed treatment, 10.0% died, 11.2% failed, and 13.8% were lost-to-follow-up. The line probe assay result showed that 11.3% of the clinical isolates were resistant to fluoroquinolones and 3.8% were resistant to both fluoroquinolones and injectable anti-TB agents. Treatment regimen type, culture conversion rate, alcohol use, cavity lesion, serum levels of creatinine and alanine aminotransferase were independent predictors of treatment outcome.

Conclusion: The levofloxacin-based regimen group has a better overall treatment success than the moxifloxacin-based group among MDR-TB patients. Clinical parameters and substance use history of the patients influenced treatment outcomes. We recommend further broader clinical studies to substantiate our findings as an input to review MDR-TB treatment guidelines.

Keywords: MDR-TB, moxifloxacin, levofloxacin, line probe assay, treatment outcome, sputum culture conversion, Ethiopia

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Introduction

Drug resistance is a major challenge for tuberculosis (TB) treatment and eradication. It has complicated TB control and undermined the objectives of the World Health Organization (WHO)'s End TB Strategy.[1] The number of new cases of multidrug resistant tuberculosis (MDR-TB), defined as TB resistant at least to

Received: 9 October 2021
Accepted: 30 November 2021
Published: 18 December 2021

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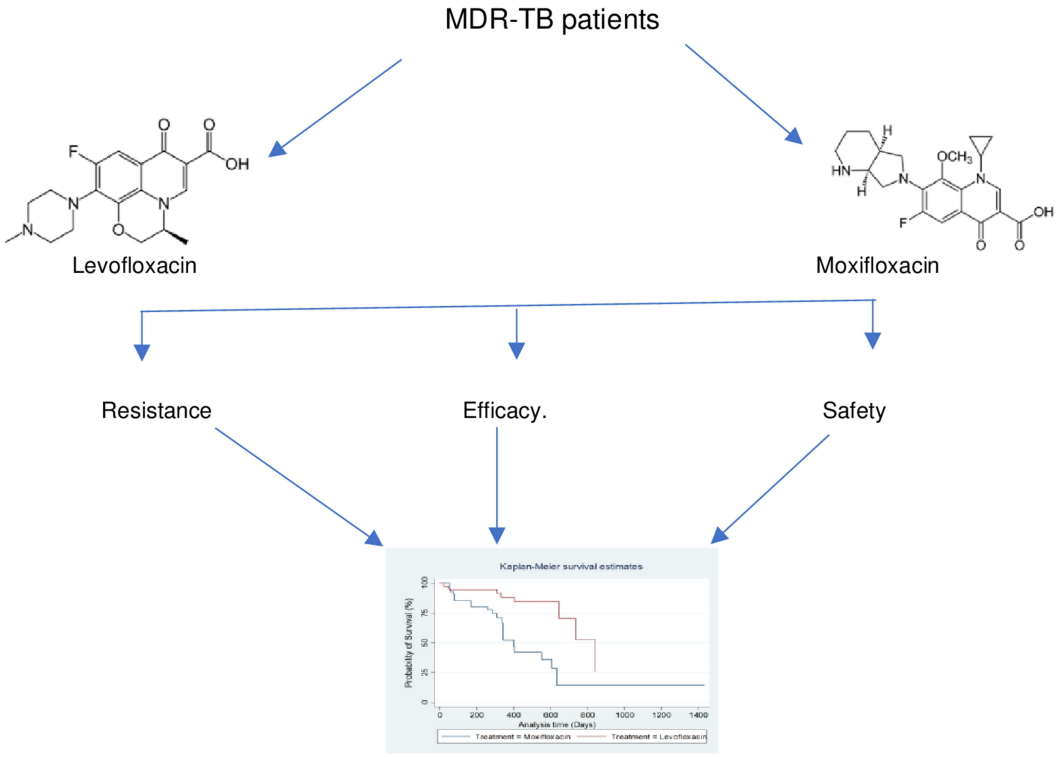
Graphical Abstract



In a prospective observational study, we analyzed clinical parameters and laboratory data of MDR-TB patients and compared the clinical efficacy of moxifloxacin and levofloxacin. Our data showed that levofloxacin-based regimen was associated with greater treatment success and low adverse drug reaction events than moxifloxacin-based regimen.

isoniazid and rifampin, is increasing worldwide.[2] In 2018, there were an estimated 484,000 MDR/RR-TB incident cases worldwide, with an estimated 3.4% of new cases and 18% of previously treated cases.[3] More recently, nearly half a million RR-TB cases occurred in 2019 across the globe.[4]

Treatment outcomes for MDR-TB is poor and its treatment also remains difficult because of high cost,[5,6] long-term treatment, and frequent adverse events (ADRs).[7] The proportion of MDR-TB patients in a 2016 global cohort who successfully completed treatment was only 56%. Only 39% of extensively drug-resistant (XDR)-TB patients successfully completed treatment in 2016.[8] This suggests that the current MDR-TB regimens are suboptimal.

Moxifloxacin and Levofloxacin are the two most potent fluoroquinolones (FQs) currently in use as a core MDR-TB treatment regimens.[9,10] In terms of in-vitro drug susceptibility, moxifloxacin is more potent (critical concentration 0.25 mcg/L) than levofloxacin (critical concentration, 0.5 mcg/mL).[11] Although these drugs have good safety record in long-term administration, their potential to prolong the QT interval (which is more pronounced with moxifloxacin) has raised a concern.[12,13] The injudicious and suboptimal use as well as poor quality of FQs and the accompanying drugs in MDR-TB can also exacerbate the resistance problem.[14–16] Emergence of extensively drug-resistant (XDR) TB has particularly posed a more "complicated" scenario of drug resistance to FQ resistance and MDR-TB and is generally linked with a treatment rate of ≤50%.[17–19]

Inter-individual variabilities among patients and type of drug regimen selected for the treatment of MDR-TB patients can determine the overall treatment success. Important sources of the variabilities may include critical illness, comorbidity, sociodemographic factors, nutritional status, and early bactericidal activity.[20–26] However, in many countries, laboratories are unable to assess drug

resistance and clinical predictors of MDR-TB treatment outcomes, which could have helped tailoring medications use to individual patient needs.[27]

WHO recommends two standardized regimens for treatment of MDR-TB: a short (9–12 months) and a long (18–20 months) regimen. According to the 2016 WHO guideline, the shorter MDR-TB regimen mainly comprised of moxifloxacin, injectable drugs, protionamide, pyrazinamide, clofazimine, and high-dose isoniazid in the intensive phase followed by moxifloxacin, clofazimine, pyrazinamide and ethambutol in the continuation phase. WHO issued a conditional recommendation for the use of this regimen (a no response to extrapulmonary TB, pregnancy, intolerance or risk of potential toxicity, a previous second-line TB medication exposure, or drug resistance to pyrazinamide, ethambutol, kanamycin, moxifloxacin, ethionamide, or clofazimine).[2,28,29] In 2018, the WHO endorsed a fully oral standardized 20-month regimen for MDR-TB, comprising mainly of levofloxacin, bedaquiline, linezolid, clofazimine, cycloserine and others. This later guideline substituted the earlier regimen and excluded the injectable drugs from the regimen. The drugs were selected based on preference of oral above injectable agents, results of drug susceptibility testing (DST), reliability of existing DST methods, population drug resistance levels, history of previous use of medicine in individual patients, drug tolerability and potential drug–drug interactions.[30] However, there are conflicting evidences about preference of either of these regimens in terms of overall treatment success, adverse drug events and the risk of emergence of drug resistance.[31,32]

Recently, the STREAM (Evaluation of a Standardized Treatment Regimen of Anti-Tuberculosis Drugs for Patients with Multidrug-resistant Tuberculosis) Stage I trial compared the shorter vs the longer regimen and reported an overall increased occurrence of ADRs and QT interval prolongation in the shorter than the longer regimen.[33] Two randomized clinical trials conducted in the Korean patient-population to compare culture conversion rates and clinical outcomes between levofloxacin-and moxifloxacin-based regimens reported no apparent difference in the therapeutic advantage between the two groups, although a higher occurrence of ADRs was noted in levofloxacin-than moxifloxacin-based regimen.[34,35]

Ethiopia had been among the 30 high-TB and MDR-TB prevalent countries,[4] although it is now out of the high burden countries list due to the recent low TB or MDR-TB incident rates.[36] National data on MDR-TB treatment outcome in Ethiopia are lacking and those available indicate variable treatment success, ranging from 63% to 78.8%. A recent systematic review and meta-analysis revealed that around 18% of MDR/RR-TB patients treated in Ethiopia had a poor treatment outcome.[37–40] Adopting the WHO guidelines, MDR-TB patients in Ethiopia receive either moxifloxacin-or levofloxacin-based MDR-TB regimen. However, studies comparing their treatment advantages and outcome predictors in Ethiopian patients are rare. We therefore aimed to explore the treatment outcomes prospectively between the two regimens using the 2013 WHO definitions[41] and the outcome predicting factors.

Methods

Study Setting and Design

A prospective observational cohort study was conducted in adult MDR-TB patients enrolled at Butajira, Yirgalem, Arbaminch and Nigist Eleni Mohammed Memorial teaching hospitals between November 2017 and May 2020. These hospitals are among the first four hospitals identified by the Ministry of Health of Ethiopia as treatment initiative centers (TICs) for MDR-TB treatment in Southern Ethiopia.

Inclusion and Exclusion Criteria

All adult patients age 18 years and above (new MDR-TB cases and those with prior treatment history with first-line TB drugs) diagnosed either bacteriologically or clinically for MDR-TB and put on either moxifloxacin- or levofloxacin-based treatment regimen since November 2017, and who can provide a written informed consent were included. Patients with no final treatment outcome (transferred out or still on treatment or treatment outcome missed from data sources), critical illness, prior treatment history with FQs, and extra pulmonary TB cases were excluded.

Accordingly, a total 80 GeneXpert confirmed MDR-TB patients and intended for a therapeutic drug monitoring (TDM) were purposively included in this study. Of these patients, 43 were on moxifloxacin- and 37 were on levofloxacin-based MDR-TB regimen. The patients were ambulatory and had been visiting the hospitals every month. They were followed up prospectively over the range of four years (from 2017 to 2020).

Data Collection and Management

Demographic (age and sex), clinical (radiographic, body-mass index, TB treatment experience, comorbidity, and treatment-regimen), and laboratory (AFB and sputum culture) data were collected using a data abstraction form (Supp. 1) by trained TB nurses and public health specialists (Health officers) in each hospital. The data abstraction form was pilot tested prior to the actual data collection and appropriate modifications were made accordingly. The data collectors were trained and oriented about the study design and its objective and patients' follow-up. Most adverse drug reactions (ADRs) experienced were written in patient charts and taken as stated. Hepatotoxicity and nephrotoxicity were inferred from at least three measurements of liver function test (LFT) and Serum creatinine (Scr.) measurements, respectively, during the treatment period. All ADRs were graded as per the DAIDS criteria and summarized as mild (Grade 1), moderate (Grade 2) and severe (Grade 3 and above).[42] At least five consecutive sputum culture results were also recorded for each patient from the routine monthly culture tests since the beginning of the MDR-TB regimen. A molecular line probe assay (LPA) for second-line TB drugs known as Genotype® MTBDRsl VER 2.0 (Hain Life science, Germany)[43] was also conducted on clinical isolates at the end of the intensive phase for the second-line TB drugs. Collected data were checked for accuracy and consistency.

Operational Definitions

In this study, first sputum culture conversion (FSCC) was defined as "the time in days from the date of initiating MDR-TB treatment to the collection date of the first two consecutive negative sputum culture".[44] Treatment outcomes were classified as per the WHO 2013 guideline and compared between the moxifloxacin and levofloxacin treatment groups at the end of treatment follow-up. Cure and treatment completions were defined as treatment success, whereas death, failure/pre-XDR-TB and lost-to-follow-up (LTFU) cases as unsuccessful/unfavorable outcomes. Treatment completion, as recommended by the guidelines, with no evidence of treatment failure and had at least three to five consecutive negative sputum cultures taken at least 30 days apart during the last months of treatment were defined as "Cured", whereas if patients completed the recommended treatment period with improved clinical symptoms but the required number of culture results could not be obtained/unknown, it was defined as "Treatment completed". Treatment outcome of "Death" was assigned to patients who died during the treatment course for any reason. Patients with two or more positive culture results from the recorded five cultures during the final months of treatment or if the treatment was terminated early because of poor clinical or radiological response or adverse event was declared as "Treatment Failure". Patients whose treatment was interrupted for two or more consecutive months for any reason other than medically approved was declared as LTFU.[41]

Data Analysis

Data were entered into excel sheet and then cleaned, coded and entered into the Statistical Software for Social Science (SPSS) version 25.0. Data analysis was carried out using STATA V.14 (StataCorp, College Station, TX, USA). Kaplan–Meier survival functions and FSCC within 90 days were used to evaluate a successful treatment outcome. Log Rank test was used to explore statistically significant difference between moxifloxacin and levofloxacin treatment groups. A Cox proportional hazards model was used to estimate the association between variables and treatment outcome. Variables associated with univariate analysis ($p<0.20$) were considered for backward multivariable analysis. Association between various potential predictors and treatment outcomes was expressed as adjusted hazard ratio (AHR) and 95% confidence intervals (CIs). Observed outcomes between the two groups were also compared in terms of various factors using chi-square, Wilcoxon Rank and Fisher exact tests. For categorical variables, either chi-square (if the number of observations in both groups is more than 5) or Fisher exact test (if the number observations in both or one of the groups are ≤ 5) was used. Wilcoxon rank-sum test was used for continuous variables. Statistical significance was set at $p < 0.05$.

Ethical Considerations

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from both the Institutional Review Board of College of Health Sciences, Addis Ababa University (Protocol No. 078/17/Pharma) and the National Ethical Review Committee of the Ministry of Science and Higher Education (Reference No. MoSHE//RD/141/2318/19). Written consent was obtained following provision of information to the participants about the objectives, benefits and risks of the study. Confidentiality and anonymity

were assured by restricting data access and removing identifiers.

Results

Patients' Characteristics

Baseline socio-demographic, laboratory and clinical characteristics of the study participants are presented in Table 1. There was a preponderance of the male gender (53.0%). The patients in both moxifloxacin and levofloxacin groups appeared to be matched for almost all socio-demographic and clinical characteristics, as there were no apparent differences observed using a variety of statistical tests. The new cases of MDR-TB constituted 25% of the total MDR-TB subjects, while the rest were with prior TB treatment history. More than half of the patients (47/80) were moderately or seriously malnourished. The

Table 1 Baseline Socio-Demographic, Laboratory and Clinical Characteristics of the Study Participants in Both Moxifloxacin and Levofloxacin Groups

Patient Characteristics	Moxifloxacin Group (n=43)	Levofloxacin Group (n=37)	P-value
Age (years)	25 (19–37)	26 (20–30)	0.34*
Sex Male	22 (51.2)	22 (59.5)	0.46†
Female	21 (48.8)	15 (40.5)	0.46†
Body mass index (kg/m^2)	17.3 (16.0–19.2)	16.7 (15.6–18.4)	0.40*
Past history of TB treatment	35 (81.4)	25 (67.6)	0.15†
Nutritional status			0.21†
Normal	15 (34.9)	18 (48.6)	
Moderately malnourished	28 (65.1)	19 (51.4)	
Smoking			0.75†
No	37 (86.1)	33 (89.2)	
Yes	6 (13.9)	4 (10.8)	
Khat Chewer			0.29†
No	40 (93.0)	31 (83.8)	
Yes	3 (7.0)	6 (16.2)	
Alcohol consumption			0.39†
No	33 (76.7)	32 (86.5)	
Yes	10 (23.3)	5 (13.5)	
Comorbidities			
Peritonitis	1 (2.3)	1 (2.7)	0.91†
HIV	5 (13.5)	6 (16.2)	0.55†
Typhoid	0 (0.0)	1 (2.7)	0.46†
Hypocalcemic tetany	0 (0.0)	1 (2.7)	0.46†
Dyspepsia	1 (2.3)	0 (0.0)	0.54†
DVT	0 (0.0)	3 (8.1)	0.10†
CHF	1 (2.3)	4 (10.8)	0.18†

(*Continued*)

Table 1 (Continued).

Patient Characteristics	Moxifloxacin Group (n=43)	Levofloxacin Group (n=37)	P-value
PUD and abdominal infection	2 (4.7)	1 (2.7)	0.56†
Pneumonia	2 (4.7)	2 (5.4)	0.63†
Hypertension	0 (0.0)	1 (2.7)	0.46†
Radiographic findings (chest X-ray)			0.11†
No cavitary lesion	28 (65.1)	30 (81.1)	
Cavitary lesion	15 (34.9)	7 (18.9)	
Degree of acid fast bacilli (AFB)			0.07†
Scanty	2 (4.7)	7 (18.9)	
1+	14 (4.7)	5 (13.5)	
2+	20 (46.5)	20 (54.1)	
3+	7 (16.3)	5 (13.5)	
Culture test			0.13†
Positive for MTBC (1+)	27 (62.8)	29 (78.4)	
Positive for MTBC (2+)	16 (37.2)	8 (21.6)	
Biochemistry & Hematological characteristics ‡			
Alanine aminotransferase (AST)			0.17*
Mean ± SD	33.4±13.6	32.0±17.9	
Median (IQR)	33.0 (22–42)	29.0 (20–36.5)	
Alanine transaminase (ALT)			0.27*
Mean ± SD	36.5±25.2	27.8±15.8	
Median (IQR)	29.0 (18–48)	22.0 (14–45)	
Hemoglobin (HG)			0.60*
Mean ± SD	14.7±5.8		
Median (IQR)	13.8 (12.4–14.7)		
Serum albumin (ALB)			0.32*
Mean ± SD	3.3±0.4		
Median (IQR)	3.3 (3.1–3.5)		

Notes: Data presented as n (%) or median (IQR); *P value from Wilcoxon rank-sum test. †P value from chi-square test or Fisher exact test, ‡ these are the average measurement at three different occasions.

Abbreviations: DVT, deep vein thrombosis; CHF, congestive heart failure; HIV, human immunodeficiency virus; IQR, interquartile range; MTBC, *Mycobacterium tuberculosis complex*; PUD, peptic ulcer disease; SD, standard deviation.

proportion of patients with substance use history, including smoking, chewing khat and alcohol consumption was 12.5%, 11.3%, and 18.8%, respectively. The most common comorbidity was HIV in both treatment groups. Congestive heart failure (CHF) tended to be more prevalent in the levofloxacin than the moxifloxacin group, probably to avoid exacerbation of cardiac problems due to moxifloxacin-associated QT interval prolongation.

Treatment Regimens

The moxifloxacin-based regimen consists of Moxifloxacin 600–800 mg, Isoniazid 300–600 mg, Ethambutol 800 mg, Pyrazinamide 1200 mg, Prothionamide 750 mg, Cycloserine 500–750 mg, and Clofazimine 100 mg. The levofloxacin-based regimen, on the other hand, included Levofloxacin 750–1000 mg, Cycloserine 500 mg, Delamanid 500 mg, Bedaquiline 200–400 mg, Clofazimine 100 mg, Linezolid 600 mg, and Prothionamide 750 mg. The medications were administered orally as a single dose except for Delamanid and Bedaquiline. Delamanid was administered twice a day, whereas Bedaquiline was administered 400 mg once daily for 2 weeks followed by 200 mg 3 times per week for 22 weeks. Bedaquiline and/or Delamanid were mostly included in the levofloxacin-based regimen, whereas injectable medicines (Amikacin, Kanamycin or capreomycin) were mostly included in the intensive phase in moxifloxacin-based regimen groups (Table 2).

Line Probe Assay

The LPA results performed on clinical isolates obtained from the participants are depicted in Figure 1. The assay revealed that whilst 88.8% of the isolates were sensitive to both FQs and the injectable agents, 3.8% were resistant to both FQs and the injectable second-line drugs (SLDs). The rest (7.5%) were resistant to only FQs, making the overall resistance 11.3%. Chi-square test revealed an association between WHO defined treatment outcome and FQ-resistance ($\chi2=8.18$; $p=0.004$) as well as resistance to both FQ and Injectable agents ($\chi2=5.79$; $p= 0.016$). However, as indicated in Table 3, the overall treatment success rate was higher in levofloxacin-than moxifloxacin-based treatment groups ($p<0.05$).

Treatment Outcome

The detailed description of treatment outcome as per the WHO 2013 guideline is presented in Table 3. Out of the 80 patients, 52 (65.0%) experienced a successful treatment outcome, whereas 28 (35.0%) showed an unfavorable outcome. Disaggregating the data revealed that 43 (53.75%) were cured, 9 (11.25%) completed treatment, 8 (10.0%) died, 9 (11.25%) failed/moved to pre-XDR-TB, and 11 (13.75%) were LTFU. Overall treatment success was

Table 2 Duration of Treatment and Number of Companion Drugs Included in Moxifloxacin-and Levofloxacin-Based Regimens

	Moxifloxacin Group (n=43)	Levofloxacin Group (n=37)	P-value
Duration of treatment, days	297 (169–355)	522 (321–570)	0.007
Duration of fluoroquinolones use, days	297 (169–355)	522 (321–570)	0.007
Drugs used	7 (5–7)	5 (4–6)	0.001
Isoniazid	38 (88.4)	0 (0.0)	0.001
Ethambutol	36 (83.7)	1 (2.7)	0.001
Pyrazinamide	39 (90.7)	23 (62.2)	0.002
Cycloserine	2 (4.7)	36 (97.3)	0.001
Delamanid	1 (2.3)	3 (8.1)	0.331
Clofazimine	41 (95.4)	34 (91.9)	0.524
Bedaquiline	2 (4.7)	17 (45.9)	0.001
Linezolid	2 (4.7)	23 (56.8)	0.001
Prothionamide	35 (81.4)	21 (56.7)	0.055
Injectable agents			
Capreomycin	1 (2.35)	9 (24.3)	0.005
Amikacin	7 (16.3)	0 (0.0)	0.013
Kanamycin	26 (60.5)	2 (5.4)	0.001

Notes: Data presented as n (%) or median (interquartile range). P-values are using chi-square test or Fisher exact test.

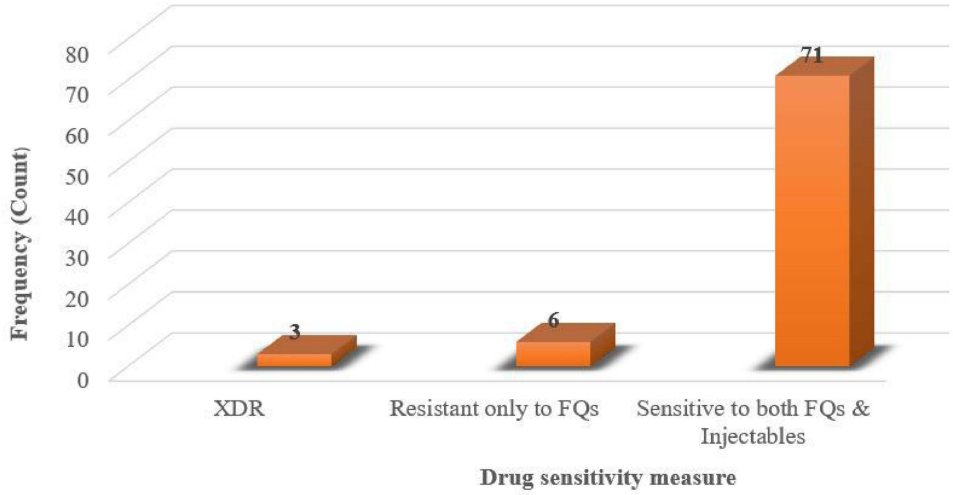


Figure 1 Summary of the Molecular line probe assay for the two core groups of second- line drugs.
Abbreviations: XDR, extremely drug resistant, occurs when there is resistance to both fluoroquinolones and injectable drugs; FQs, fluoroquinolones.

compared between the two treatment groups and found to be higher in levofloxacin- than moxifloxacin-based group ($\chi2$=6.40; p=0.01). However, comparison of each of the WHO defined outcomes between the two treatment groups did not show any statistically significant differences (Table 3).

Nonparametric estimation of the survival distribution comparing the treatment groups using Kaplan–Meier survival analysis is summarized in Figure 2. The overall comparison showed that the risk of unfavorable outcome was lower in the levofloxacin- (Log Rank test ($\chi2$=13.88, P=0.001) than moxifloxacin-based group.

Table 3 Treatment Outcome, Sputum Culture Conversion and Line Probe Assay Results by Treatment Regimen of the Study Subjects with MDR-TB

	Moxifloxacin Group (n=43)	Levofloxacin Group (n=37)	P-value
Treatment outcome			
Cure	19 (44.2)	24 (64.9)	0.06†
Completion	3 (7.0)	6 (16.2)	0.29†
Death	5 (11.6)	3 (8.1)	0.72†
Failed or moved to Pre-XDR-TB	7 (16.3)	2 (5.4)	0.17†
Lost to follow-up	9 (20.9)	2 (5.4)	0.06†
Overall treatment success	22 (51.2)	30 (81.1)	0.01†
First sputum culture conversion			
Within 90 days of treatment started	32 (74.4)	31 (83.8)	0.46†
After 90 days of treatment	11 (25.6)	6 (16.2)	0.41†
Line probe assay result			
Resistance to FQs	7 (16.3)	2 (5.4)	0.17†
Resistance to both FQs and IAs	2 (4.6)	1 (2.7)	0.56†
Duration of follow-up	290 (162–348)	515 (314–563)	0.01*

Notes: n=80; Data presented as n (%) or median (interquartile range); *P value from Wilcoxon rank-sum test. †P value from chi-square test or Fisher exact test.
Abbreviations: FQs, fluoroquinolones; IAs, injectable agents; MDR-TB, multidrug resistant TB; XDR-TB, extremely drug resistant TB.

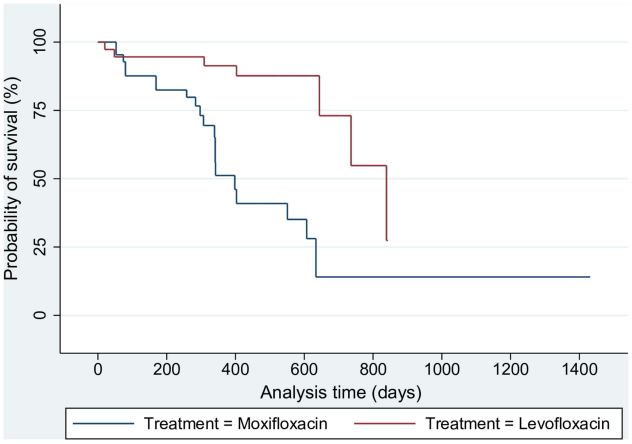


Figure 2 Kaplan–Meier curve showing the probability of survival of MDR-TB patients for moxifloxacin-and levofloxacin-based regimens since commencement to end of treatment follow-up.

Considering treatment group as one of the potential predictor variables, Cox proportional hazard analysis (Table 4) indicated that moxifloxacin treated group had a more risk of (AHR=0.25, 95% CI (0.09–0.71), p<0.05) unfavorable outcome compared to that of levofloxacin group.

The estimated mean survival time ± SE (in days) for moxifloxacin, levofloxacin and overall was 522.73 ±89.90, 716.10±43.80 and 711.05±90.04, respectively. The estimated median survival time ± SE (in days) for moxifloxacin, levofloxacin and overall was 398.00 ± 41.87, 839.00 ± 92.21 and 634.00 ± 20.72, respectively. From the survival plots (Figure 2), it could be observed that the total follow-up time was 1431 days for moxifloxacin and 843 days for levofloxacin. The survival curve for levofloxacin is consistently higher than the curve for moxifloxacin up to 840 days. The median time of survival for levofloxacin treated groups was more than twice that of moxifloxacin treated groups. The probability of survival for moxifloxacin group in the 53rd, 74th, 80th, 169th, 258th, 284th, 297th, 307th, 339th, 341st, 342nd, 398th, 403rd,607th, and 634th days of the follow-up were 95.3%, 92.8%, 87.6%, 82.5%, 79.8%, 76.6%, 73.1, 69.5%, 65.1%, 55.8%, 51.2%, 46.1%, 40.9 and 14.4%, respectively. In the same analysis, the probability of survival for levofloxacin in the 20th, 48th, 309th, 403rd, 644th, 736th, and 839th days of follow-up were 97.3%, 94.6%, 91.3%, 87.7%, 73.1%, 54.8%, and 27.4%, respectively. The cumulative probability of survival at the end of the follow-up period for moxifloxacin treatment group was 14.4%, whereas for that of levofloxacin group was 27.4%. Therefore, the levofloxacin-based regimen had a better survival advantage than that of moxifloxacin-based regimen. The overall treatment success was also better in levofloxacin-than that of moxifloxacin-based regimen as it can be seen from Table 3 (p<0.05). However, there were no statistically significant differences in the WHO defined outcomes such as cure rate (p=0.06), treatment completion (p=0.29), death rate (p=0.72), failure rate (p=0.17) and LTFU (p=0.06).

Culture Conversion Rates

Of the 80 study subjects, 54 (67.5%) had an FSCC within 90 days of treatment. As indicated in Table 4, the FSCC before 90 days of treatment impacted the overall outcome (p<0.05) in both treatment groups. Culture conversion after 90 days was associated with an increased risk of unfavorable outcome. However, the rates of culture conversion between the two treatment groups did not show any significant difference upon time-to-event analysis using the Log Rank (Mantel-Cox) test ($\chi 2$=0.279, P=0.597) (Figure 3). The median (interquartile range) culture conversion estimated days for moxifloxacin, levofloxacin, and the overall were 51 (40–130), 60 (49.5–130) and 60 (49.5–130), respectively. The proportion of culture positivity during the treatment course for moxifloxacin in the 21st, 26th, 30th, 32nd, 33rd, 34th, 35th, 40th, 45th, 47th, 48th, 50th, 51st, 56th, 65th, 66th, 81st, and 85th days was 0.98, 0.95, 0.88, 0.86, 0.81, 0.79, 0.78, 0.74, 0.67, 0.63, 0.56, 0.54, 0.47, 0.44, 0.40, 0.37, 0.35, and 0.33, respectively. On the other hand, it was 0.97, 0.95, 0.92, 0.81, 0.76, 0.70, 0.60, 0.46, 0.43, 0.38, and 0.32 for levofloxacin in the 13th, 14th, 16th, 30th, 48th, 51st, 56th, 60th, 65th, 79th, and 85th days, respectively.

Association Studies

Univariate and multivariate Cox regression analysis was conducted to identify predicting factors for unfavorable treatment outcomes (Table 4). Considering p-value <0.2 as a cut-off point from the univariate regression, potential outcome predicting variables were selected for further multivariate Cox regression analysis. Accordingly, treatment regimen type, prior TB treatment, alcohol consumption, resistance to FQs, FSCC, nutritional status, cavitary lung lesion, Scr., and ALT were selected and the multivariate Cox proportional hazard analysis was carried out (Table 5).

Table 4 Univariate and Backward Multivariate Cox Proportional Hazard Regression to Determine Outcome Predicting Factors in MDR-TB Patients

Univariate Cox Regression					
Patient Variable	**n (%)**	**Outcome**		**P-value**	**HR [95% CI]**
		Success	**Failure**		
Sex, male	44 (55)	28 (53.8)	16 (57.1)	0.38	1.39 [0 0.66–2.97]
Comorbidity	29 (36.3)	18 (34.6)	11 (39.3)	0.88	0.94 [0.62–3.50]
Age (years), >35	15 (18.8)	8 (15.4)	7 (25.0)	0.37	0.94 [0.27–3.22]
Prior TB treatment	60 (75.0)	39 (75.0)	21 (75.0)	0.14*	1.97 [0.80, 4.87]
Body mass index, <18.5	58 (72.5)	38 (73.1)	20 (71.4)	0.55	0.96 [0.83–1.10]
Treatment group, MXF	43 (53.4)	22 (42.3)	21 (75.0)	0.00*	0.22 [0.09–0.53]
Khat consumption	9 (11.3)	3 (5.8)	6 (21.4)	0.29	1.63 [0.46–1.10]
Smoking	10 (12.5)	4 (7.7)	6 (21.4)	0.58	1.30 [0.50–3.38]
Alcohol consumption	16 (20.0)	4 (7.7)	12 (42.9)	0.00*	3.96 [1.83–8.56]
Resistance to FQs	9 (11.3)	3 (5.8)	6 (21.4)	0.09*	3.31 [1.32–6.65]
Resistance to FQs & IAs	3 (3.8)	1 (1.9)	2 (7.1)	0.58	1.42 [0.42–4.83]
FSCC after 90 days	26 (32.5)	5 (9.6)	11 (39.3)	0.01*	2.66 [1.24–5.73]
Malnourished	47 (58.8)	28 (53.8)	19 (67.8)	0.09*	2.07 [0.89–4.81]
Cavitary lung lesion	30 (37.5)	10 (19.2)	20 (71.4)	0.00*	5.11 [2.24–11.65]
Mean AST, ≥32.7†	35 (43.8)	23 (44.2)	12 (42.9)	0.50	2.14 [0.99–1.03]
Mean ALT, ≥32.5†	36 (45.0)	21 (40.4)	15 (53.8)	0.04*	1.58 [0.72–3.49]
Mean ALB, <3.3†	38 (47.5)	24 (46.2)	14 (50.0)	0.57	0.58 [0.58–2.62]
Mean HG, <15.12†	57 (71.3)	37 (71.2)	20 (71.4)	0.69	1.20 [0.96–1.08]
Adverse drug event	60 (75.0)	39 (75.0)	21 (75.0)	0.66	0.82 [0.33–1.99]
Mean Scr. (mg/dL), ≥ 0.87†	31 (38.8)	14 (26.9)	17 (60.7)	0.09*	0.17 [0.030–1.02]

Notes: n=80; *Variables with P-value<0.2.
Abbreviations: CHR, crude hazard ratio; CI, confidence interval; FQs, fluoroquinolones; FSCC, first sputum culture conversion; IAs, injectable agents.

The analysis revealed that the risk of treatment failure was significantly higher in patients with moxifloxacin-based regimen (AHR=0.27, 95% CI=0.10–0.74, p=0.011), FSCC after 90 days (AHR=2.80, 95% CI=1.18–6.66, p=0.02), alcohol consumption (AHR=4.09, 95% CI=1.62–10.34, p=0.003), MDR-TB cases with cavitary lung lesion (AHR=3.09, 95% CI=1.10–8.70, p=0.032), mean Scr ≥0.87 (mg/dL) (AHR=0.27, 95% CI=08–0.88, p=0.029), and mean ALT ≥32.5 (IU/L) (AHR=3.11, 95% CI=1.01–9.54, p=0.019) than their corresponding counterparts.

Adverse Drug Reactions

ADRs noted during the treatment period are summarized in Table 6. Most ADRs occurred were of Grade 1 (mild) or Grade 2 (moderate) and dose or regimen change was not necessary. But in some patients (who were on the moxifloxacin-regimen), ototoxicity with the injectable SLDs was severe (Grade 3) and either doses were reduced or the medications were discontinued. The overall occurrence of ADRs in the study participants was 75.0%. The proportion of ADRs was higher in the moxifloxacin- than the levofloxacin-based group (86.1% vs 62.2%)

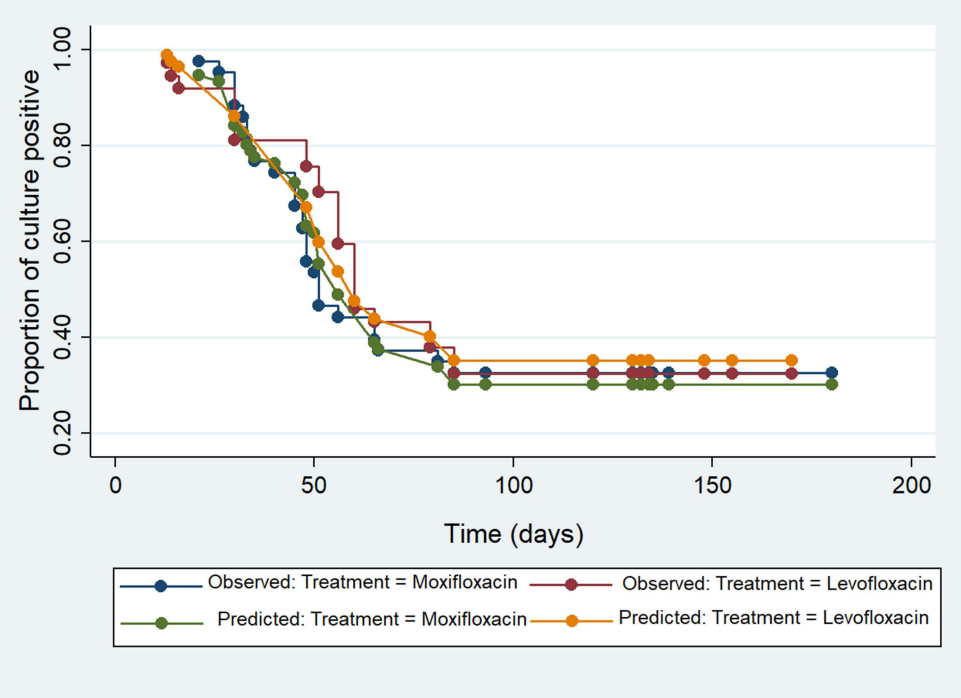


Figure 3 Kaplan–Meier analysis comparing time-to-culture positivity between moxifloxacin- and levofloxacin-based regimens treated MDR-TB patients (n=80).

($\chi2$=6.051; p<0.05). Occurrence of ototoxicity was also higher in the moxifloxacin- than levofloxacin-based group ($\chi2$=6.041; p<0.05). Musculoskeletal abnormalities (myalgia) were the most frequently occurred ADRs in both treatment groups (25.6% versus 29.7%). Occurrence of other ADRs such as myalgia, hematological abnormalities, gastrointestinal upset, hepatotoxicity, psychotic problems, peripheral neuropathy and mild forms of ADRs were not significantly different between the two treatment groups.

Discussion

The results of our study demonstrated a lower risk of treatment failure in levofloxacin- than moxifloxacin-based regimen, as observed in the Kaplan–Meier survival analysis (Log Rank test ($\chi2$=13.88, P=0.001)). Treatment success (cure and treatment completion rates) in the levofloxacin group was also greater than those in the moxifloxacin-group ($\chi2$=7.83; p<0.05). More interestingly, whilst deaths observed in the levofloxacin-based group seemed to be most likely related to the co-existing illnesses (CHF, DVT and pneumonia), deaths were still observed in the moxifloxacin-based group in the absence of other comorbidities and treatment failures. Our finding is concordant with the STREAM Stage I trial, which revealed that shorter regimens were associated with higher risks of treatment failure and relapse compared to longer regimens.[33] This is, however, in contradistinction to the Korean study that reported no apparent difference in treatment outcome between the two groups.[34] On the other hand, time to culture conversion rates between the two groups were not different (Log Rank test $\chi2$=0.279, P=0.597) in our study, which concurs with an earlier Korean study that compared culture conversion rates between the two treatment groups using both liquid and solid media.[35] The overall occurrence of ADRs was also higher in the present study than the Korean study,[34] although no regimen changes were made during the treatment course, as all the ADRs were mild to moderate except for ototoxicity by the injectable agents, which necessitated dose reduction or discontinuation of the offending agents. Moreover, we found more overall ADRs with moxifloxacin- than levofloxacin-based regimen, while the reverse was true for the Korean study. Although musculoskeletal abnormalities were the most frequently cited ADRs in both settings, we did not observe any apparent difference between the two groups, while they were more prevalent in the levofloxacin group in the Korean report.[34] These discrepancies may be due to differences in the background drugs, drug doses, study population and research design. For instance, more proportion of patients in this study received injectable agents as

Table 5 Multivariate Cox Proportional Hazard Regression Analysis of Treatment Outcome Predicting Covariates in MDR-TB Patients

Patient Variable	n (%)	Outcome n (%)		P-value	AHR [95% CI]
		Successful	Unfavorable Outcome		
Treatment group		52 (65.0)	28 (35.0)	0.011*	0.27 [0.10–0.74]
Moxifloxacin	43 (53.8)	22 (42.3)	21 (75.0)		
Levofloxacin	37 (46.2)	30 (57.7)	7 (25.0)		
Prior TB treatment		52 (65.0)	28 (35.0)	0.882	1.08 [0.38–3.08]
None	20 (25)	13 (25.0)	7 (25.0)		
Treated	60 (975)	39 (75.0)	21 (75.0)		
Nutritional Status		52 (65.0)	28 (35.0)	0.652	1.23 [0.50–3.04]
Normal	33 (41.3)	23 (69.7)	10 (30.3)		
Malnourished	47 (58.7)	29 (61.7)	18 (38.3)		
Resistance FQs				0.336	1.75 [0.56–5.46]
FSCC				0.020*	2.80 [1.18–6.66]
Within 90 days	54 (67.5)	42 (77.8)	12 (22.2)		
After 90 days	26 (32.5)	10 (38.5)	16 (61.5)		
Alcohol use				0.003*	4.09 [1.62–10.34]
No	65 (81.2)	51 (78.5)	14 (21.5)		
Yes	15 (18.8)	1 (6.7)	14 (93.3)		
Chest X-ray				0.032*	3.09 [1.10–8.70]
No lesion	58 (72.5)	43 (74.1)	15 (25.9)		
Cavitary lesion	22 (27.5)	9 (40.9)	13 (59.1)		
Mean Scr. (mg/dL)		52 (65.0)	28 (35.0)	0.029*	0.27 [08–0.88]
< 0.87†	49 (61.3)	32 (61.5)	17 (60.7)		
≥ 0.87†	31 (38.7)	20 (38.5)	11 (39.3)		
Mean ALT (IU/L)				0.047 *	3.11 [1.01–9.54]
≤ 32.5†	44 (55)	36 (69.2)	21 (75.0)		
> 32.5†	36 (45)	16 (30.8)	7 (25.0)		

Notes: n=80; *P-value <0.05; †These were the average values considered as a cut-off point below or above which the outcomes were assessed.
Abbreviations: ALT, alanine aminotransferase; IU/L, international units per liter; Scr., serum creatinine; FSCC, first sputum culture conversion (in days); FQs, fluoroquinolones.

companion drugs with the moxifloxacin-regimen, whereas the use of injectable agents in both groups was almost equal in the Korean study.[34] In addition, frequent use of the new drugs (Bedaquiline and Delamanid) with levofloxacin- than moxifloxacin-based regimen might have contributed to the better treatment success and lower risk of treatment failure in the present study. As regards to dose, Korean patients received only 750 mg dose of levofloxacin, whereas patients in the present study received both 750 mg and 1000 mg of levofloxacin. Levofloxacin has the best early bactericidal activity at the dose of 1000 mg/day than at 750 mg/day,[45] which could probably be a reason for the better treatment success observed in the Ethiopian patients. Furthermore, inter-ethnic or inter-individual

Table 6 Adverse Drug Reactions Occurred Among the MDR-TB Patients

	Moxifloxacin Group (n=43)	Levofloxacin Group (n=37)	P-value
Adverse events	37 (86.1)	23 (62.2)	0.014*
Myalgia	11 (25.6)	11 (29.7)	0.679
Hematological abnormalities	7 (16.3)	6 (16.2)	0.994
GI upset	5 (11.6)	2 (5.4)	0.442
Ototoxicity	9 (16.2)	1 (2.7)	0.017*
Hepatotoxicity	9 (20.9)	5 (13.5)	0.556
Psychotic problems	3 (8.1)	1 (2.7)	0.620
Peripheral neuropathy	3 (8.1)	1 (2.7)	0.620
Elevated serum creatinine (nephrotoxicity).	3 (7.0)	2 (5.4)	0.990
Others[†]	9 (11.6)	7 (10.8)	0.823

Notes: n=80, Data presented as n (%). P values are using chi-square test or Fisher exact test. *The P-value is statistically significant (P<0.05); [†]Others refers ADRs like weakness, fatigue, sweating, and chills.

variability, which influence the pharmacokinetics and pharmacodynamics of drugs, maybe a source of variability to the observed drug responses.[46–50] The other probable reason for the observed differences could be the relatively small sample size study sample in the present study. We enrolled a smaller number of patients for the observational follow-up compared to other similar studies and this also might have influenced our study results.

The overall treatment outcome observed in the present study (65%) was relatively lower than a recent national study report (75.7%).[51] Moreover, other studies also indicated a relatively higher treatment success rates in various settings: 82.4% (Taiwan),[52] 75.8% (Pakistan),[53] 72.7% (Korea),[54] 75.7% (Tanzania).[55] However, it is higher than that reported by WHO (57%) in 2017,[56] a meta-analysis (61%)[57] and an Indonesian national (48%) as well as provincial (36%) study.[58] LPA results revealed that 11.3% of the clinical isolates were resistant to FQs, whereas 3.8% were resistant to both FQs and injectable TB drugs. This is much higher than reported recently from a national study (3.4%).[59] A study from Tigray region of Ethiopia reported a rate of FQ resistance much lower (5.3%)[60] than our study. This suggests that the rate of resistance to the essential and most potent MDR-TB drugs (FQs and Injectable agents) may be spreading.

We also determined treatment regimen type, culture conversion rate, alcohol use, cavitary lesion, serum creatinine and ALT levels as predictors of treatment outcome. Late culture conversion was shown to be associated with risk of treatment failure in this study. A negative culture between 2 and 3 months of therapy indicates a successful therapeutic outcome in MDR-TB patients.[61] Outcomes among patients who had a history of alcohol consumption was poor, which concurs with a study report from India.[62] Apart from the probable direct effect of the contemporary and previous alcohol consumption, non-adherence was mentioned to be the main reason for the unfavorable outcome.[62] Cavitary lung lesion was another risk factor related to poor treatment outcome in this study and is in line with a finding of a recent study in Thailand.[63] The possible reason for the association could be related to high bacterial load at the cavities, where drugs may not access and thus unable to eradicate effectively, leading to persistence of the bacteria.[64,65] Patients with a cavity have a bacterial load of up to 10^{11} bacilli/g, making it highly contagious.[66] A study suggested that treatment outcome in TB with cavitary lung lesion may be improved by extending the continuation phase of TB treatment.[67] The other possible reason for poor outcome in cavitary lesion may be due to late stage of the disease which ends in death of the patient.[68]

Mean Scr. level of 0.87 (mg/dL) and above was also associated with unfavorable outcome. The rationale behind this is not very clear. Creatinine, an end product of muscle metabolism, is the most commonly used clinical indicator for renal function.[69,70] It is a frequently used parameter in

hospital wards for the prognosis of diseases and drug dosing. However, it might not be a reliable prognostic parameter in critical illnesses because the pharmacokinetic behavior of drugs in these patients is difficult to predict. Augmented renal clearance is prevalent, even with normal Scr. levels.[71] As a consequence, this results in suboptimal dosing followed by treatment failure and increased mortality.[72] For example, levofloxacin has a linear pharmacokinetics and 80% of it is excreted unchanged via the kidneys. However, renal clearance is 60% higher than creatinine clearance, evidencing the involvement of tubular secretion.[73] In the other scenario, both older and newer FQs are known to elevate Scr. and induce acute interstitial nephritis (AIN), which can cause end stage renal failure that requires hemodialysis.[74] The incidence of elevated Scr. levels is related to FQs range from 0.2 to 1.3%.[75] In the present study, the occurrence of acute elevated Scr. in moxifloxacin- and levofloxacin-based regimen treatment groups was 7.0% and 5.4%, respectively, and is suggestive of AIN. A recent retrospective study of AIN related to FQs use identified that 10% of the study subjects were biopsy-proven AIN cases.[75] Clinicians should be aware of these adverse effects, especially in neutropenic and lymphopenic patients, which might lead to unfavorable outcomes in TB patients.[64–76] Similarly, ALT higher than the mean 32.5 (IU/L) was a predictor of unsuccessful treatment outcome. A study reported that ALT abnormalities were more common in the shorter regimen of 8 weeks.[33] Drug-induced liver injury (DILI) may be the most likely cause of elevated serum ALT.[77] Hepatotoxicity is one of the most frequent and serious ADRs of anti-TB medications like isoniazid and pyrazinamide and may reduce treatment effectiveness by compromising treatment regimens.[78,79] Total occurrence of hepatotoxicity in our evaluation was 17.5%. However, there was no statistically significant difference in the occurrence of hepatotoxicity between the two treatment groups (20.9% verse 13.5%). Early detection of drug-induced elevation of Scr. and ALT levels in MDR-TB patients could help prevent poor treatment outcome due to a possible drug-induced AIN and DILI, respectively.

Limitation of the Study

This study is not a clinical trial but an observational explorative follow-up study on a limited sample of MDR-TB patients in the programmatic treatment course. In addition, the sample size in this study is smaller than related studies. In fact, the total number of patients was expected to be larger than those included in this study. However, difficulties in meeting the eligibility criteria and the COVID-19 pandemic since the beginning of 2020 had significantly affected patients' admission and diagnosis. Nonetheless, the conclusion drawn from this study might be informative for further studies.

Conclusion

Levofloxacin-based MDR-TB regimen with the background new oral drugs seems to be preferable over moxifloxacin-based regimen that includes the injectable SLDs, in terms of better treatment success and lower risk of unfavorable outcomes. Early evaluation of MDR-TB patients for sputum culture conversion rate, history of alcohol use, cavitary lesion, serum Scr. and ALT levels may help tailoring treatment for a better outcome. We recommend further randomized controlled trial in a larger population nationally for a possible MDR-TB treatment program review in the use of these key drugs.

Acknowledgments

The authors would like to appreciate and acknowledge Addis Ababa University and Wolaita Sodo University for their financial support to carry out this study. Moreover, we thank the European and Developing Countries Clinical trails Partnership (EDCTP) for granting monthly stipend to TS.

Disclosure

The authors report no conflicts of interest in this work.

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ORIGINAL RESEARCH

Population Pharmacokinetics of Levofloxacin and Moxifloxacin, and the Probability of Target Attainment in Ethiopian Patients with Multidrug-Resistant Tuberculosis

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Objective: This study aimed to explore the population pharmacokinetic modeling (PopPK) of levofloxacin (LFX) and moxifloxacin (MXF), as well as the percent probability of target attainment (PTA) as defined by the ratio of the area under the plasma concentration-time curve over 24 h and the in vitro minimum inhibitory concentration (AUC0-24/MIC) in Ethiopian multidrug resistant tuberculosis (MDR-TB) patients.

Methods: Steady state-plasma concentration of the drugs in MDR-TB patients were determined using optimized liquid chromatography-tandem mass spectrometry. PopPK and simulations were run at various doses, and pharmacokinetic parameters were estimated. The effect of covariates on the PK parameters and PTA for maximum mycobacterial kill and resistance prevention was also investigated.

Results: LFX and MXF both fit in a one-compartment model with adjustments. Serum-creatinine (Cr) influenced ($p = 0.01$) the clearance of LFX, whereas body mass index (BMI) influenced ($p = 0.01$) the apparent volume of distribution (V) of MXF. The PTA for LFX maximal mycobacterial kill at the critical MIC of 0.5 mg/L with the simulated 750 mg, 1000 mg, and 1500 mg doses were 29%, 62%, and 95%, respectively, whereas the PTA for resistance prevention at 1500 mg was only 4.8%, with none of the lower doses achieving this target. At the critical MIC of 0.25 mg/L, there was no change in the PTA for maximum bacterial kill when the MXF dose was increased (600 mg, 800 mg, and 1000 mg), but the PTA for resistance prevention was improved.

Conclusion: The standard doses of LFX and MXF may not provide adequate drug exposure. PopPK of LFX is more predictable for maximum mycobacterial kill, whereas MXF's resistance prevention target increases with dose. Cr and BMI are likely important covariates for dose optimization in Ethiopian patients.

Keywords: population pharmacokinetics, probability of target attainment, moxifloxacin, levofloxacin, MDR-TB patients, Ethiopia

Introduction

Fluoroquinolones (FQs) are used off-label for the treatment of multidrug-resistant tuberculosis (MDR-TB), and for evaluation in shortening the duration of drug susceptible TB in recently prioritized regimens.[1] Within the class, levofloxacin (LFX) and moxifloxacin (MXF) play a substantial role in ensuring success in treatment outcomes and are important components of the World Health Organization (WHO) Group A regimen.[2–5] However, pharmacokinetic (PK) variability within and between individuals is very common and may lead to suboptimal plasma levels and undesired treatment outcomes.[6–8] The observed FQs PK variability may be due to comorbidities (diabetes mellitus or HIV) that contribute to malabsorption, concomitant medications, dietary intake, medication adherence, or host pharmacogenetics,

Received: 17 September 2022
Accepted: 11 November 2022
Published: 28 November 2022

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and can impact other PK drivers such as drug metabolism.[9–11] Dosage optimization based on an understanding of plasma exposure profile of FQs in patients (PK factor) and in vitro drug susceptibility patterns (PD factor) of *Mycobacterium tuberculosis* (Mtb) may improve treatment outcomes.[12,13]

The ratio of the 24 h area under the concentration-time curve (AUC) and in vitro minimum inhibitory concentration (MIC) (AUC0-24/MIC) has been accepted as the strongest predictor of FQs exposure and favorable clinical outcome.[14–16] A hollow fiber bioreactor system (HFS) experiment has established that an unbound AUC0-24/MIC ratio of 53 for MXF substantially decreases the total population of Mtb by more than 3 log10 CFU/mL and suppresses the emergence of drug resistance. Moreover, an AUC0-24/MIC ratio of ≥100 for MXF completely kills FQ-sensitive population of Mtb without development of drug resistance.[17,18] Another HFS experiment with LFX recently showed that the AUC0-24/MIC associated with maximal Mtb killing was 146, while the ratio associated with resistance suppression was 360. The experiment suggested that up to 1500 mg LFX doses are needed for best microbial killing if adverse effects are not an issue.[19]

Even though Ethiopia has recently transitioned out of the MDR-TB/Rifampicin resistant (RR)-TB world countries list, it remains on the list of 30 high TB/HIV burden countries.[20] People of diverse ethnic background live in Ethiopia, although one-size-fits-all scenario is practiced in TB therapeutics.[21,22] Evidence of interindividual variability (IIV), drug exposure profiles in MDR-TB patients and its impact on treatment outcomes are lacking. To the best of our knowledge, the PK of LFX and MXF among Ethiopian patients treated for MDR-TB is not yet studied. Furthermore, whether adequate in vivo serum exposure is attained with the current standard dose to achieve maximal bacterial kill and prevent resistance is not yet explored. The aim of this study was therefore to determine the population pharmacokinetic modeling (PopPK) of LFX and MXF and the probability of target attainment (PTA) for maximal mycobacterial kill and prevention of resistance in a group of people treated for MDR-TB in Ethiopia.

Materials and Methods

Study Setting and Design

This is a follow-up of our previous prospective cohort study,[23] enrolling MDR-TB/RR-TB patients in four hospitals of Southern Ethiopia, namely, Butajira, Yirgalem, Arbaminch, and Nigist Eleni Mohammed Memorial teaching hospitals. These hospitals were among the first four hospitals identified by the Ministry of Health of Ethiopia as treatment initiative centers for MDR-TB treatment in Southern Ethiopia.

Participant Eligibility

As recommended by the recently integrated WHO Treatment Guidelines,[24] eligible study participants were outpatients who visited the center monthly for drug refills and treatment response monitoring. MDR-TB patients aged 18 years and older with or without co-infection with HIV, receiving LFX (750 or 1000 mg/day) – or MXF (600 mg/day)-based regimens for at least 8 days (see Ref 23 for details of regimens) and provided written informed consent were included. Those severely ill and anemic defined by a hematocrit less than 25% (most recent value, measured within 30 days of the PK study) were excluded. All patients included in this study received standard TB treatment according to the National Guidelines for Tuberculosis and Leprosy, which is adopted from the WHO Guidelines.[25,26]

Variables, Sample Collection and Bioanalysis

Demographic, clinical and laboratory data were collected using a data abstraction form by trained public health specialists in each hospital. Upon giving informed consent, the patients were asked to stay for 48 h at the centres for blood sampling during one of their monthly visits. About 4 mL blood was drawn using a vacutainer (Becton–Dickinson Biosciences, San Jose, CA, USA) in EDTA tubes at 0 (pre-dose sampling), 2, 4, 6, 9, 12, and 24 h following drug intake. After mixing the blood with the anticoagulant, plasma was immediately separated, and the test tubes were appropriately labeled. The samples were transported to Wolaita Sodo University Hospital, stored at −80°C and then shipped to Inje University College of Medicine, South Korea for bioanalysis. Plasma concentrations of LFX and MXF were determined using a high-performance liquid chromatography-electrospray ionization–tandem mass spectrometry previously developed and validated by the Inje University research group.[27]

Determination of Minimum Inhibitory Concentration

MICs of the second-line antitubercular drugs were determined from patients for whom pre-treatment clinical isolates of Mtb were available. MIC was determined using the broth microdilution method in Middlebrook 7H9 broth supplemented with 10% Oleic Albumin Dextrose Catalase (OADC) and 0.05% tyloxapol.[28] *M. tuberculosis* H37Rv (ATCC 27294) was used as the reference strain and its targeted MIC values were in the range of 0.0625–8.0 mg/L for LFX and 0.0625–16.0 mg/L for MXF.

Pharmacokinetic Model Development

Structural Model

Individual PK profiles for both LFX and MXF were estimated using a non-compartmental approach in Phoenix WinNonlin version 8.0 (Certara, Princeton, NJ, USA) and maximum plasma concentration (Cmax) and AUC0–24 were calculated and reported as mean ± S.E and median (IQR). The PopPK was developed using nonlinear mixed effect modelling (Phoenix NLME) and analysis of covariates was performed. First-order conditional estimation algorithms were used for model development. Various structural models were tested, including one or two compartment distributions with first-order absorption and elimination (with or without absorption lag time). Additive, proportional, and combined residual error models were also tested. Model selection was based on model fit and visual inspection of diagnostic plots, the precision of parameter estimation, minus two log likelihood (−2LL) value, and Akaike information criterion (AIC) and Bayesian information criterion (BIC). The reliability of the final model was assessed by the 95% confidence intervals (CIs) of the 2.5th and 97.5th percentiles of the simulated concentration-time profile. The PK parameters of a typical population along with their IIV were estimated using an exponential function. The PopPK estimated PK parameters were reported in terms of geometric mean (GM) and percent relative standard error (%RSE) while IIV was reported in terms of percent coefficient of variability (%CV).

Covariates

Based on previous similar studies and scientific interest, we included the following covariates in the current PopPK: nutritional status, gender, serum creatinine (Cr), adverse drug effects, comorbidities, alanine aminotransferase (AAT), aspartate aminotransferase (AST), bilirubin, and body mass index (BMI). The covariate selection was performed by forward addition (p = 0.05) and backward subtraction (p = 0.01). Visual predictive checks (VPCs) based on 1000 Monte Carlo simulations were performed to assess robustness of the final model. For continuous covariates, a power function was used using Equation 1:

$$\mathrm{TVPi} = \theta_1 \times (\mathrm{COVi}/\mathrm{COV}_{\mathrm{mean}})^{\theta 2} \quad (1)$$

Where TVPi is the typical value of a PK parameter (P) for an individual i with a COVi value of the covariate, while θ_1 is the typical value for an individual with a mean covariate value of $\mathrm{COV}_{\mathrm{mean}}$ and θ_2 is the power coefficient describing covariate parameter relationship.

For binary covariates, the fractional change in the typical parameter values was determined according to Equation 2:

$$\mathrm{TVPi} = \theta_1 \times \theta_2{}^{\mathrm{N}} \quad (2)$$

where TVPi is as defined in Equation 1 above, θ_1 is the typical value for an individual in whom the covariate takes the value 0 (N= 0), and θ_2 is the fractional change in the typical value when the covariate takes the value 1 (N = 1).

Interindividual Variability

Distributions of IIV were assumed to be log-normal and described by the following exponential error model (Equation 3):

$$\mathrm{Pi} = \mathrm{TVP} \times \exp(\eta\,\mathrm{Pi}) \quad (3)$$

Where Pi is the parameter value for an individual i, TVP is the typical population value of the parameter, and ηPi (ETAs) are individual-specific IIV for an individual i and the parameter P. ETAs were assumed to be normally distributed with a mean of 0 and variances of ω^2: $\eta \sim \mathrm{N}(0, \omega^2)$. For all PK parameters, IIV was estimated and described as percent coefficient of variability (%CV).

Residual Error

The residual error model was described by both combined additive and proportional error models for individual and population predicted plasma concentrations as depicted in Equations 4 and 5, respectively:

$$DV_{ij} = IPRED_{jj} + IPRED_{jj} \times \varepsilon 1_{ij} + \varepsilon 2_{ij} \quad (4)$$

$$DV_{ij} = PRED_{jj} + PRED_{jj} \times \varepsilon 1_{ij} + \varepsilon 2_{ij} \quad (5)$$

Where DV_{ij} is the observed plasma concentration of the individual i at time j, IPRED is the corresponding model predicted concentration, and PRED is the population predicted plasma concentration, $\varepsilon 1_{ij}$ and $\varepsilon 2_{ij}$ are proportional and additive components, respectively, of the residual random error. The residual error components were assumed to be normally distributed with a mean of 0 and variances of σ^2: $\varepsilon \sim N(0, \sigma^2)$

Drug Exposure and the Percent Probability of Target Attainment

The final model was used to construct simulated PK profiles for individual patients at various doses (using individual PK parameters and covariates, assuming dose-independent PK) and to calculate % PTA. The simulated doses of LFX were 750 mg, 1000 mg and 1500 mg, while those of MXF were 600 mg, 800 mg, and 1000 mg. C_{max} and AUC0-24 were calculated using the individual simulated profiles. Box plots were used to show simulated concentrations and patient exposure (C_{max} and AUC0-24) for each drug.

Using the assumed MIC based on those values determined for some patients involved in this study, and excluding those values well above the typical cut-off for resistance, the %PTA as defined by AUC0-24/MIC ratio for each MIC value for the original and simulated doses of LFX and MXF was analyzed descriptively. The number of patients achieving a specific target AUC0-24/MIC was described in % PTA against the total observations. The targets selected for the maximal mycobacterial kill and resistance suppression in the mutant strains for LFX were 146 and 360, respectively.[19] Similarly, the two targets selected for MXF were 53 and 100, respectively.[17,18] The formula we used for the PTA calculation was:

$$PTA(\%) = \frac{\text{number of patients attained the required AUC0} - 24/\text{MIC}}{\text{Total number of patients}} \times 100\% \quad (6)$$

Ethical Considerations

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was obtained from both the Institutional Review Board of College of Health Sciences, Addis Ababa University (Protocol number 078/17/Pharma) and the National Ethical Review Committee of the Ministry of Science and Higher Education (Reference number MoSHE//RD/141/2318/19). Written informed consent was obtained after participants were informed about the purpose, benefits, and risks of the study. Confidentiality and anonymity were ensured by restricting access and removing identifiers.

Results

Patient Characteristics

There were 62 MDR/RR-TB patients for a potential PK sampling, out of which 7 were excluded due to critical illness. Plasma samples were therefore obtained from 55 patients. Of these, a total of 39 patients (21 for LFX and 18 for MXF) had adequate sample collection and plasma concentration with acceptable concentration-time profiles. Samples with lower than 5 time-point concentrations over 24 h and those with erratic concentration profiles were excluded from the analysis. Demographic, laboratory, and clinical characteristics of the patients are summarized in Table 1. There were no statistically significant differences in baseline characteristics between the LFX and MXF groups. Of the 39 patients included in the analysis, 19 patients (9 from LFX-and 10 from MXF-based regimen treatment group) had encountered at least one ADRs during the follow-up.

Table 1 Demographic and Clinical Characteristics of Ethiopian MDR-TB Patients Recieving the Standardized LFX- (n=21) and MXF- (n=18) Based Regimen

Characteristics	Total N=39	LFX-Regimen (n= 21)	MXF-Regimen (n=18)
Age	26 (20–32)	26 (20–30)	26 (20–37)
Male	20 (51.3)	12 (57.1)	8 (44.4)
Weight (Kg)	49 (43.0–52.4)	45 (42.5–52.3)	51 (46.6–65.0)
Height (cm)	165.5 (160–172)	168 (160–174)	165 (161.5–171.5)
BMI (kg/m^2)	17.1 (15.6–18.9)	16.7 (15.6–18)	17.3 (16.2–19.2)
Co-existing illness	13 (33.3)	8 (38.1)	5 (27.8)
Prior TB treatment	30 (77)	15 (71.4)	15 (83.3)
Smoking	5 (12.8)	3 (14)	2 (11.1)
Malnourished*	19 (48.7)	9 (42.9)	10 (55.6)
Cavitary disease	18 (46.2)	9 (42.9)	9 (50)
Cr. (mg/dL)	0.9 (0.8–1.3)	1 (0.8–1.3)	1 (0.8–1.4)
AST (**IU/L**)	29 (20–38)	25 (18.0–34.6)	31 (21.0–40)
ALT (**IU/L**)	23 (15–45)	19.2 (14.0–35)	28 (17.8–50.2)
Hgb (g/dl)	13.8 (12.3–15.1)	12.8 (12.1–14.3)	13.4 (12.2–14.3)

Notes: Data presented as n (%) or median (IQR); *Patients with BMI less than 16 kg/m^2 were considered as malnourished.

Abbreviations: AST, alanine aminotransferase; ALT, alanine transaminase; ALB, albumin; BMI, body mass index; cm, centimeter; g/dl, grams per deciliter; Hgb, hemoglobin; IU/L, international units (IU) per liter.

The Population PK Model

Whilst the LFX dataset fits into a one-compartment model that includes both additive and multiplicative error models with lag time, the MXF dataset fits into a one-compartment model with additive error models without lag time. Residual variation was taken into account for all parameters. Table 2 shows the PopPK estimates in GM and %RSE, and IIV as %

Table 2 Overall and Final One-Compartment Model for LFX (n=21) and MXF (n=18), and Covariates Included in the Model in Ethiopian MDR-TB Patients

Drug	PK Parameter	Final Model	
		GM (%RSE)	%CV (Shrinkage, %)
LFX	Ka (1/h)	0.2 (4.0)	14 (60)
	V (Liter)	122 (21.5)	26 (30)
	Cl (L/h)	10.4 (7.7)	66 (60)
	Tlag (h)	0.2 (6.1)	3.4 (60)
	Cr (mg/dL)	1.1 (10)	14.7 (NA)
	−2LL	449.2	
	AIC	473.2	
	BIC	507.2	(9)
MXF	Ka (1/h)	0.5 (18.8)	59 (50)
	V (Liter)	102.2 (10.0)	10 (40)
	Cl (Liters/h)	20 (3.5)	2.7 (70)
	BMI (Kg.m2)	17.8 (1.1)	4.5 (NA)
	−2LL	184.2	
	AIC	202.2	
	BIC	225.7	(3)

Note: Lag time (Tlag) is the time taken for the drugs to appear in circulation following the drug dosing.

Abbreviations: AIC, Akaike's information criterion; BIC, Bayesian information criterion; Cl, clearance; Percent coefficient of variation (% CV= 100 x IIV/parameter estimate); Ka, absorption constant; GM, geometric mean; IIV, interindividual variability; −2LL, minus two log likelihood; LFX, levofloxacin; MXF, moxifloxacin; NA, not applicable; PK, pharmacokinetics; %RSE, percentage of relative standard error (%RSE = 100 x standard error/parameter estimate); Cr, serum creatinine; Tlag, lag time for absorption; V, apparent volume of distribution.

CV of the PK parameters for both groups. The Goodness-of-fit of the final model for LFX and MXF is summarized in Figure 1. VPCs for both models showed that most of the data are within the 90% prediction interval and around median values (Figure 2). Upon performing a stepwise search for covariates, the −2LL value was significantly reduced in scenarios, where Cr was used as a covariate for clearance (CL) of LFX data set. Therefore, this was selected as the final model for LFX. In a similar covariate screen, BMI as the apparent volume distribution (V) covariate, the −2LL score was significantly reduced and this was selected as the final model for MXF.

Total Drug Exposure

The individual participants' mean ± standard error and median (Interquartile range) values of C_{max} and AUC0-24 from the non-compartmental analysis of the original and simulated drug doses are summarized in Table 3. Figure 3 depicts box plots of C_{max} and AUC0-24 for LFX and MXF.

MIC Distribution of the Clinical Isolates

The MIC of LFX and MXF were successfully determined from the clinical isolates of 28 patients. However, the MICs for the remaining clinical isolates could not be determined as they did not grow from subculture after transportation to the referral laboratory. The individual MIC values (percent) for LFX were 0.25 mg/L (46.4%), 0.50 mg/L (42.8%), 2.0 mg/L (3.6%), 4 mg/L (3.6%), and 8 mg/L (3.6%); whereas they were 0.0625 mg/L (25%), 0.125 mg/L (53.6%), 0.25 mg/L (10.7%), 2.0 mg/L (7.1%), and 8.0 mg/L (3.6%) for MXF. Given that the WHO recommended critical susceptibility value for Mtb is 0.5 mg/L for LFX and 0.25 mg/L for MXF, 7.2% of the clinical isolates were resistant to LFX and 10.7% were resistant to MXF.

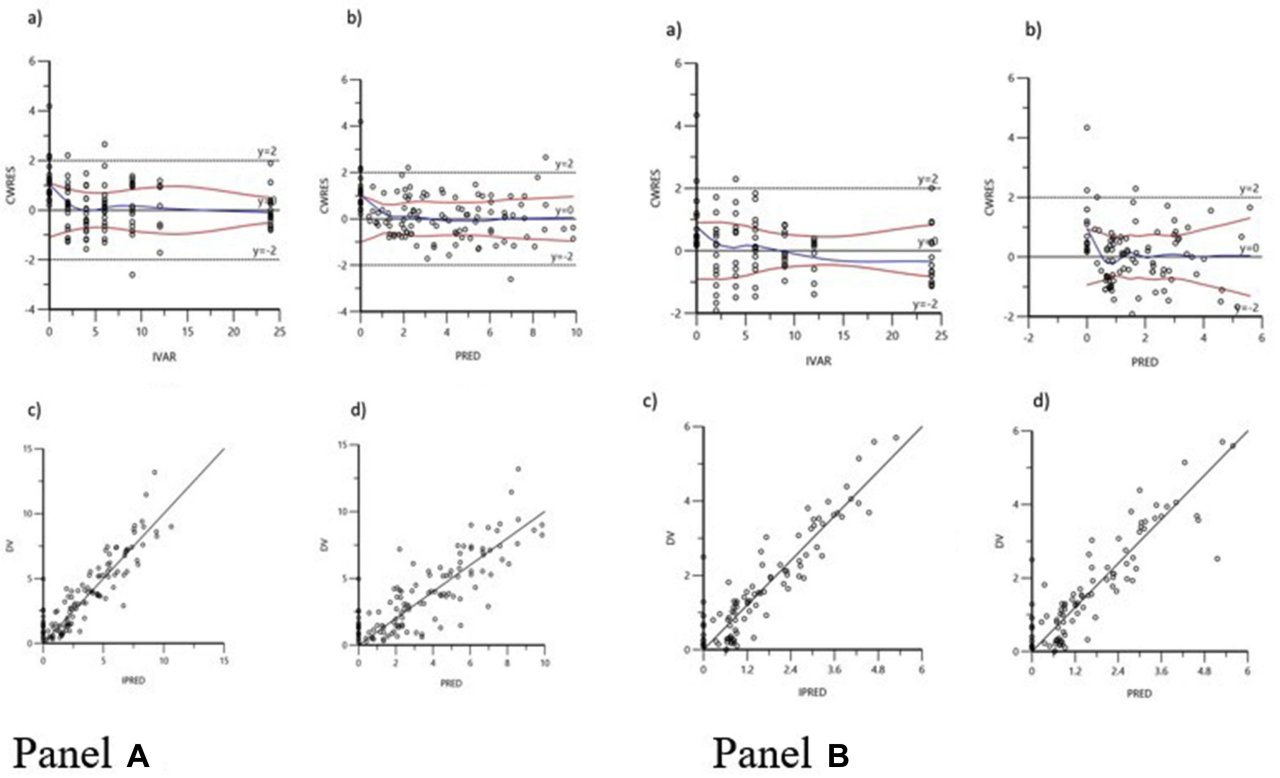

Figure 1 Goodness-of-fit plots for the final population pharmacokinetics of LFX (**A**) and MXF (**B**): conditional weighted residuals versus time (CWRES versus IVAR) (a); conditional weighted residuals versus population predicted concentrations (CWRES versus PRED) (b); observed versus individual predicted concentrations (DV versus IPRED) (c); observed versus population predicted concentrations (DV versus PRED) (d). The red lines in the panels represent smoothed regression lines.

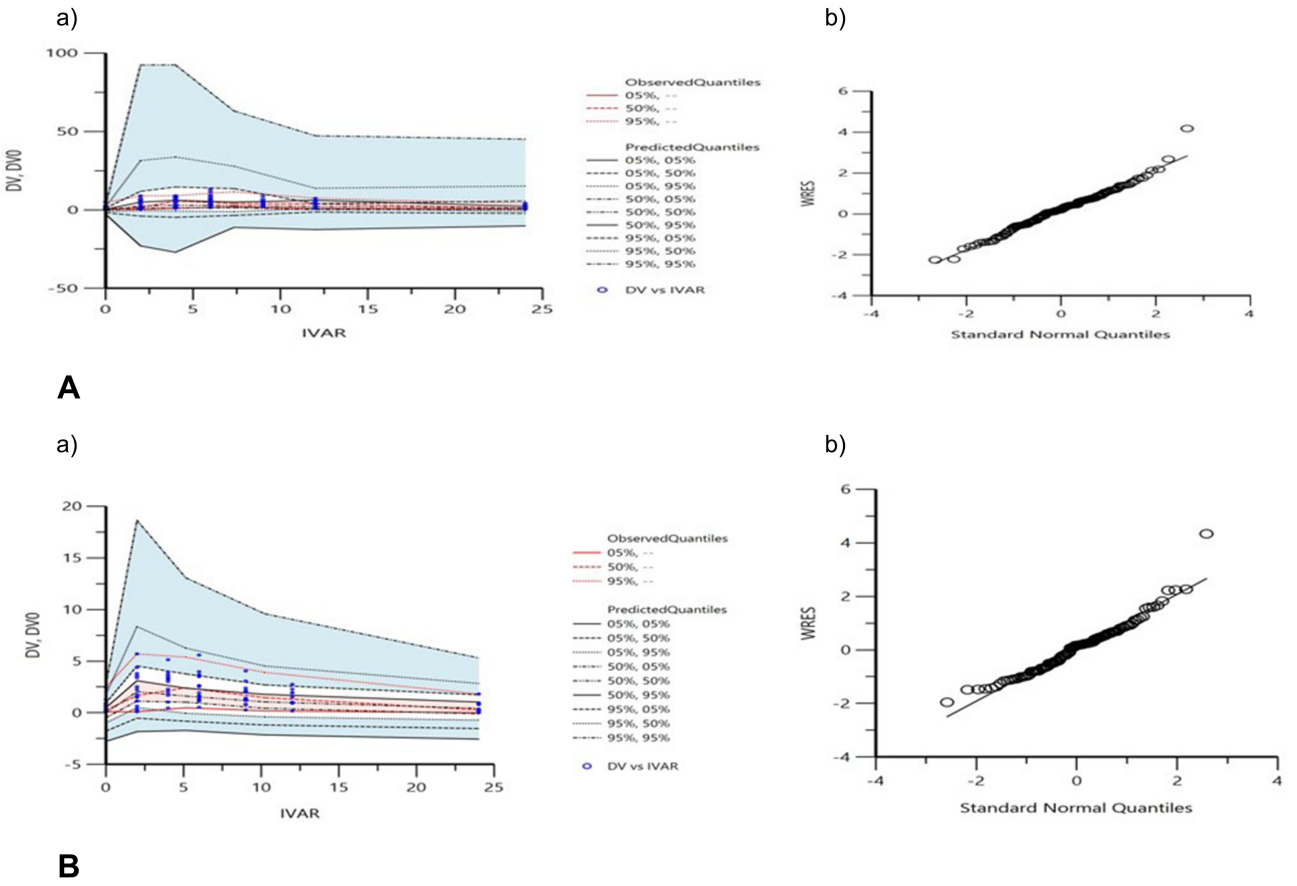

Figure 2 Visual predictive checks (a) and WRES versus Standard Normal Quantiles (b) for the final model of LFX (**A**) and MXF (**B**): red lines represent the 5th, 50th, and 95th percentiles of the observed concentrations; the shaded areas represent the 90% confidence intervals of the 5th, 50th, and 95th percentiles of the simulated concentrations, respectively; the dots represent the observed data; DV represents observed concentration; DV0, observed concentration at zero time; IVAR, time; WRES, weighted residuals.

The Probability of Target Attainment

The % PTA for the simulated doses of 750 mg, 1000 mg, and 1500 mg of LFX for the two targets (AUC0-24/MIC ≥ 146, for the mycobacterial kill and AUC0-24/MIC ≥ 360, for resistance suppression) is depicted in Figure 4. At the WHO recommended critical MIC of 0.5 mg/L, the PTAs for the maximal bacterial kill were 29%, 62%, and 95% for the simulated doses of LFX, respectively. As regards to resistance suppression, whilst the 750 mg and 1000 mg LFX did not achieve the PTA needed for resistance suppression, only 4.8% attained the PTA needed with the 1500 mg LXF. Only the 1500 mg LFX achieved 24% PTA for the maximal bacterial kill when the MIC was changed to 1 mg/L. Interestingly, none of the doses were able to attain the PTA for resistance suppression at MIC values ≥1 mg/L.

In a similar fashion, the PTA for the simulated doses of 600 mg, 800 mg, and 1000 mg of MXF at the two targets (AUC0-24/MIC ≥ 53, for the mycobacterial kill and AUC0-24/MIC ≥ 100, for resistance suppression) is depicted in Figure 5. At the WHO recommended critical MIC of 0.25 mg/L for MXF, the PTA needed for the maximal mycobacterial kill was the same (94.4%) for all doses. However, the PTA needed for resistance suppression varied with doses, 50%, 77.8%, and 94.4%, respectively. The PTAs calculated for the mycobacterial kill decreased with increasing the MIC value to 1 mg/mL and were 11.1%, 27.8%, and 38.9%, respectively. However, none of the doses achieved the PTA needed for resistance suppression at the MIC value of 1 mg/L and above.

Table 3 Descriptive Summary of the Non-Compartmental Analysis of the Estimated Individual PK Parameters for the Original and Simulated Doses of LFX (n=21) and MXF (n=18) in Ethiopian MDR-TB Patients

Drug	Dose (mg)	C_{max}±SE (mg/L)	C_{max} (IQR) (mg/L)	AUC0-24±SE (h. mg/L)	AUC0-24 (IQR) (h. mg/L)
LFX*	750 1000 1500	7.8 ±0.6 10.3 ±0.8 15.4 ±1.2	7.5 (5.5–9.7) 10.0 (7.3–12.8) 15.1 (10.8–19.2)	63.8 ±6.4 85.3 ±8.6 128 ±13.0	60.9 (40.0–81.8) 81.5 (51.9–109.2) 122.7 (77.8–164.1)
LFX†	750 1000	5.8 ±0.5 8.2 ±0.7	5.3 (4.6–6.7) 7.4 (6.1–9.4)	66.5 ±9.5 97.9 ±9.3	58.1 (45.0–80.5) 103.4 (71.5–108.6)
MXF*	600 800 1000	2.7 ± 0.4 3.5 ± 0.4 4.40 ± 0.6	2.6 (1.5–3.9) 3.5 (2.0–5.0) 4.4 (2.5–6.3)	31.5 ± 4.1 40.6 ± 4.8 50.8 ± 6.0	25.1 (19.0–47.0) 33.4 (25.2–62.7) 41.8 (31.6–78.4)
MXF†	600	2.9 ± 0.4	3.2 (1.4–3.9)	33.4 ± 4.2	28.2 (19.3–50.8)

Notes: *Simulated doses and †Observed doses.
Abbreviations: AUC0-24, area under the concentration-time curve over twenty-four hours; C_{max}, maximum serum concentration; IQR, interquartile range; mg/L, milligram per liter; SE, standard error.

Discussion

In this first PopPK of LFX and MXF in Ethiopian patients treated for MDR-TB, we importantly demonstrated that current doses of both medications led to poor probability of attaining PK targets that have previously been associated with microbial kill and prevention of acquired resistance. While multiple factors may have influenced this underexposure, the observed IIV in PK/PD parameters may result in poor treatment outcomes.[29] We also demonstrated that the LXF

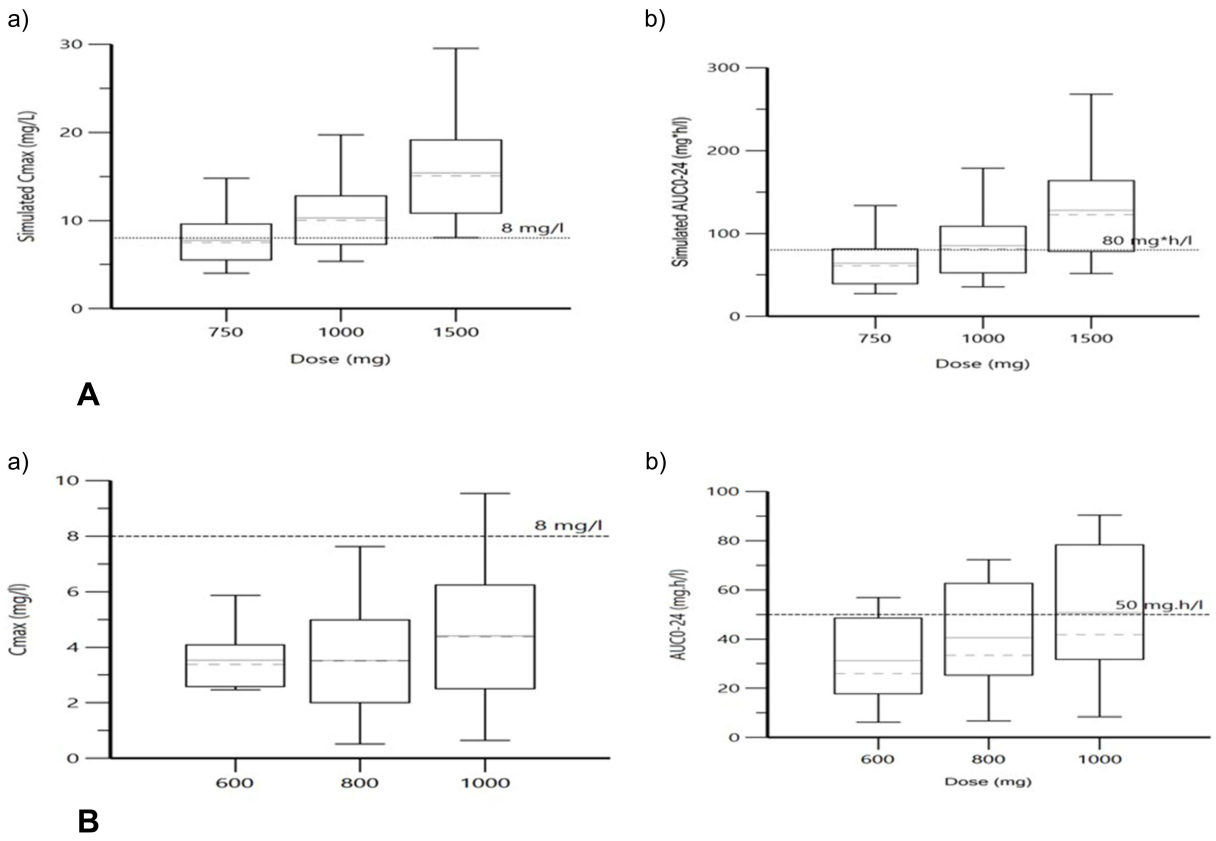

Figure 3 Box plot for simulated C_{max} (a) and AUC0-24 (b) of LFX (**A**) and MXF (**B**): the dashed line indicates the minimum threshold for C_{max} and AUC0-24.

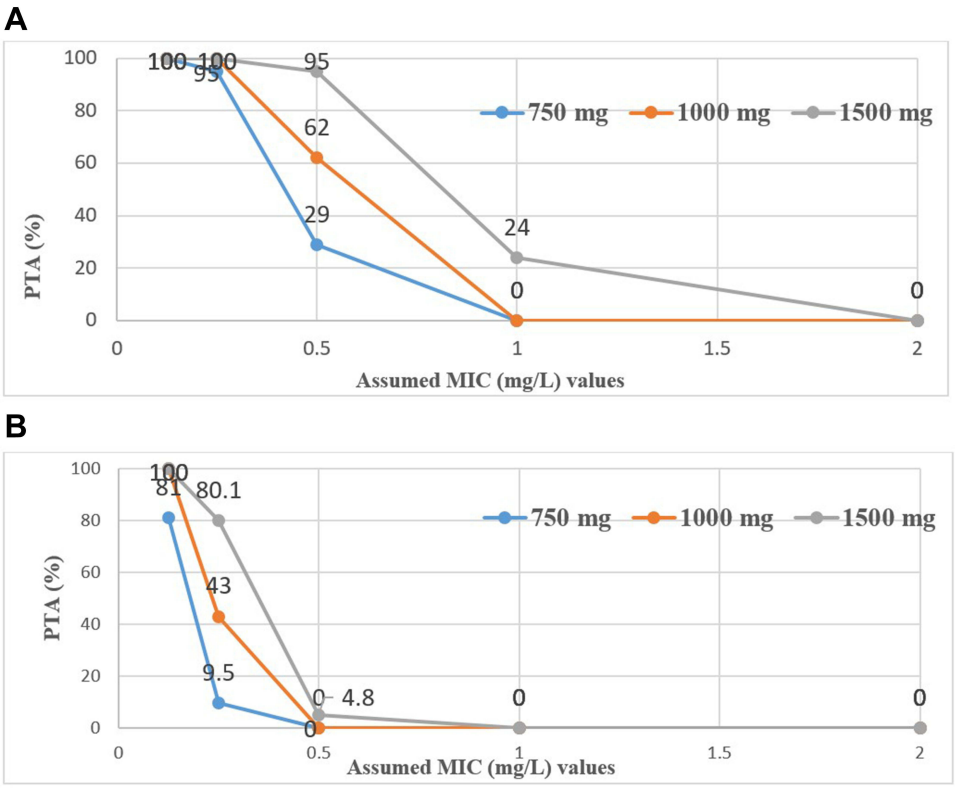

Figure 4 Percent probability of target attainment, PTA (%) for the maximal mycobacterial kill (AUC0-24/MIC ≥146) (**A**) and resistance suppression (AUC0-24/MIC ≥360) (**B**) of the simulated doses of LFX (750 mg, 1000 mg, and 1500 mg) against the assumed MIC values in Ethiopian MDR-TB patients (n=21).
Note: The formula used for the calculation of the percent probability of target attainment (PTA) was: $\text{PTA } (\%) = \frac{\text{\# of patients attained the required AUC0}-24/\text{MIC}}{\text{Total \# of patients}} \times 100\%$.

concentration dataset was described best by a one-compartment model with both additive and multiplicative combination error model with lag time, whereas that of MXF was described best by a one-compartment additive error model with no lag time. MXF and LFX data for MDR-TB patients have been described by a one-compartment model in some previous studies[30,31] and by a two-compartment model in others.[32,33] The reason for this variation is unclear. The differences in the total sample size and number of plasma samples collected during the elimination phases in the studies could be a reason for the observed variations. For example, MXF PopPK was described by a two-compartment model in 241 MDR-TB patients from South Africa.[33] Plasma samples on 10 different occasions were collected over 50 h while in the present study 5 to 7 plasma samples were collected over 24 h. We found that the relative IIV (%CV) of the population estimated Ka was higher in the MXF group than in the LFX group, and the IIV of V and CL was higher in the LFX group than in the MXF group. High shrinkage was observed for some model estimated PK parameters in this study. This might be associated with fewer number observations per subjects as it is consistent with other previous studies.[34]

Overall, higher doses were predicted to result in improved maximal bacterial kill target for LFX but not for MFX, although MXF achieved resistance prevention targets better than LFX. The AUC0-24 for MXF corrected to a 400 mg standard dose ranged from 10.2 to 79.1 mg.h/L in a study by Van den Elsen et al,[35] which compared MXF with and without rifampin. The corresponding AUC0-24 values for the 600 mg in our study ranged from 3.5 to 72.2 mg·h/L. Given that no rifampin was used in our cohort, we expected even higher MXF exposure. At the in vitro critical concentration of 0.25 mg/L, which is currently recommended by WHO to determine susceptibility,[35] there was no difference in the PTA (94.4%) needed for the maximal bacterial kill between the lower and higher MXF simulated doses. However, significant improvements in PTA were reached for the target of resistance prevention with the highest simulated MXF dose (1000 mg). Nevertheless, when utilizing an MIC value just a dilution higher at the borderline of susceptible (0.5 mg/ liter) and the target AUC0-24 of 50–65 mg.h/liter,[36,37] the PTA needed for the resistance suppression with the 600 mg dose of MXF in Ethiopian patients would be only 22.2%. Yet, at higher simulated doses of 800 mg and

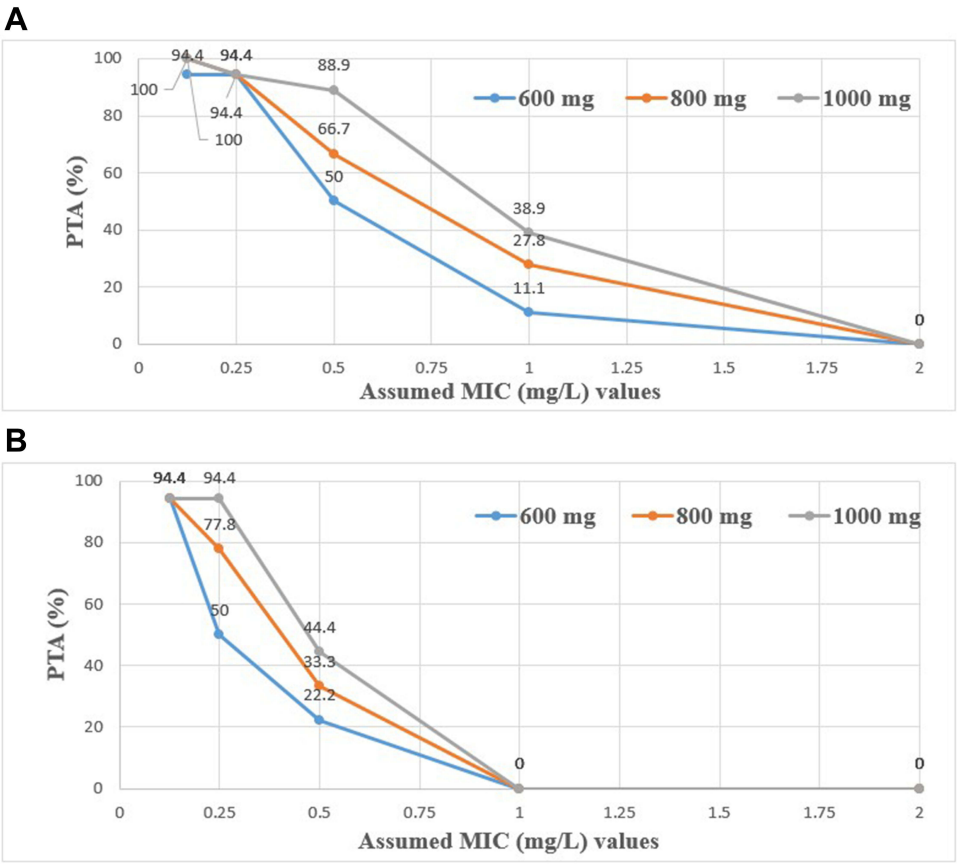

Figure 5 Percent probability of target attainment, PTA (%) for the maximal mycobacterial kill (AUC0-24/MIC≥53) (**A**) and resistance suppression (AUC0-24/MIC ≥100) (**B**) of the simulated doses of MXF (600 mg, 800 mg, 1000 mg) against the assumed MIC values in Ethiopian MDR-TB patients (n=18).
Note: The formula used for the calculation of the percent probability of target attainment (%PTA) was: $\text{PTA } (\%) = \frac{\#\text{ of patients attained the required AUC0}-24/\text{MIC}}{\text{Total \# of patients}} \times 100\%$.

1000 mg, this was improved only to 33.3% and 44.4%, respectively. Ethiopian MDR-TB patients are rarely given a dose of 800 mg MXF based on body weight. Therefore, the current WHO recommendation of a critical concentration of 0.25 mg/L is likely to be accurate for this population, and well-powered interventional studies would be needed to determine if higher doses of MXF improve PTA.

Despite the appeal of a higher starting dose of MXF for optimizing outcomes, our findings may explain a study in Indian MDR-TB patients in a tertiary care center in Mumbai, which found that an increased MXF dosing did not improve treatment outcomes in the presence of low-level resistance to MXF.[38] More interestingly, another recent study by Yun et al[32] suggested a twice 400 mg instead of a single 800 mg MXF is an optimal dosing regimen for MDR-TB patients because it provides superior efficacy and safety. BMI was shown to have a statistically significant influence on V for MXF. The fact that BMI affected V may justify weight-based dosing of MXF in Ethiopian patients. It is important to note that the median CL and V values of MXF in the Ethiopian MDR-TB patients are greater than previously reported values from European patients.[8] This higher CL and V of MXF may explain the lower AUC0-24 of MXF in this study compared to previous studies.[35]

Genetic diversities in drug transporters in African populations has been reported to have implications for conventional therapies, notably in TB and HIV.[39] The study reported the prevalence of genetic variation in UGT1A and ABCB1 genes in black South African TB patients and assessed the effect of these on MXF PK. The study revealed that genetic variation in UGT1A rs8175347 reduced MXF clearance by 20.6%, whereas rs3755319 increased by 11.6%, and the ABCB1 rs2032582 SNP reduced the bioavailability by 40%. Therefore, our findings fit with other recommendations that more rigorous PK and pharmacogenetic studies may be of further benefit in settings such as Ethiopia.[40,41]

The overall drug exposure of the standard LFX doses (750–1000 mg) in Ethiopian patients was also inadequate. Specifically, the conventional 750 mg LFX dose neither achieved an adequate maximal bacterial kill nor a resistance prevention targets. Moreover, the PTA for the resistance prevention with the highest simulated dose (1500 mg) was only 4.8%, whereas none of the lower doses achieved this target. However, the PTA needed for the maximal mycobacterial kill with the simulated LFX doses (750 mg, 1000 mg, and 1500 mg) was increased more proportionately and predictably with a dose increase compared to that of MXF. For instance, the PTA required for a maximal bacterial kill at a critical MIC of 0.5 mg/L is more than tripled for 1500 mg LFX compared to 750 mg LFX dose. Peloquin et al[30] in their earlier PopPK study of LFX, Gatifloxacin, and MXF revealed that the most favorable PK results were seen with LFX in the study population. This observation, the evidence that LFX has less risk for QT interval prolongation[42] than MXF, and the findings of less LFX resistance when using the WHO critical concentrations, suggest LFX-based regimens are more preferable in the treatment of MDR-TB in Ethiopia. Deshpande et al[19] recently identified that 1500 mg LFX confers equivalent effect to MXF 800 mg/day using HFS-TB in tandem with Monte Carlo Experiment, artificial intelligence-based analyses, and Probit models of clinical data. Cr, which affected CL of LFX, may be an important covariate in the therapeutic drug monitoring (TDM) of LFX. This is expected since LFX undergoes significant renal clearance and hence the renal function can affect drug clearance.[43] Cr clearance was shown to be a covariate that influences LFX clearance in other studies as well.[30]

Delayed drug absorption and erratic concentration-time curves for some concentrations were observed in our study. It may be related to the food and drink intake behavior of patients before and during the investigation, as the patients were ambulatory and there was no food and drink restrictions. On the day of PK sampling, pre-dose blood samples were drawn in a fasting state while they received their daily dose after breakfast (under direct observation of the medical staff). However, the drug adherence information prior to the PK testing day was dependent on self-report of the patients. Therefore, non-adherence to the drug regimen could be a likely reason for some of the observed erratic concentration-time curves. Variable patterns of adherence to prescribed treatment are common and yet underestimated factors to sub-therapeutic plasma concentrations of TB drugs. Adverse drug effects and individual patient-related factors (psychiatric illness, substance use, and homelessness) increase the risk for variation in patterns of adherence.[44] The other possible reason for the sub-therapeutic plasma concentrations may be a long storage time before the bioanalysis. It has been suggested that plasma sample storage time should also be regarded as an equally prominent covariate as age or gender and need to be included in epidemiological studies involving drugs in plasma. Repeated freeze-and-thawing of a sample during sample storage could affect data quality by interfering with peak detection.[45,46]

These findings support the importance of testing drug susceptibility and the potential benefit of follow-up TDM to determine if higher doses achieve targeted exposures. A further clinical study based on this work could be designed around higher starting doses of LFX and MXF, gyrA sequencing to determine no/low/high level resistance more rapidly than MIC testing, and measurement of serum drug concentrations by a limited sampling strategy performed at steady-state. Those patients not achieving target AUC0-24 or those with mutations consistent with low-level resistance could be triaged for a further dose/exposure optimization or exchange of the FQ drug class. Barriers to TDM include higher costs of specialized equipment and preserving the cold chain for analysis,[47–54] yet assays under development, such as those using dried blood spots or saliva, promise to deliver drug concentration testing closer to the point-of-care. Lastly, more rigorous PopPK analyses to determine the optimal starting dose, if performed at local or regional levels, may be able to determine a setting-specific dose that could mitigate the routine need for TDM.[55–57]

Limitations

This was an observational study of MDR-TB patients in a programmatic treatment course. Many aspects which may affect the study result were not strictly controlled in the present study unlike in an interventional trial. Moreover, although our sample size is adequate for PopPK for a population with similar covariate distribution, it may not be representative of all Ethiopian MDR-TB patient populations. Incomplete MIC profile of the clinical isolates from the study subjects is also a limitation of this study. Bacterial killing and suppression of resistance depend on the concentration of unbound drug. However, for PTA calculations, raw drug concentrations were used to calculate the area under the concentration-time curve over 24 h.

Conclusion

The total plasma exposure in Ethiopian MDR-patients for both LFX and MXF is relatively lower than reports from previous studies in other settings. LFX demonstrated a more proportionate increase in the PTA required for the maximum mycobacterial kill with dose, whereas resistance prevention target was better achieved by higher dose of MXF. Cr and BMI are likely important covariates to consider for further interventional studies of dose increase or TDM to establish the optimal dose and plasma exposures of both drugs in Ethiopian patients.

Acknowledgments

We acknowledge Wolaita Sodo University and Addis Ababa University for their financial support in this study, and European and Developing Countries Clinical Trials Partnership (EDCTP) for granting a monthly stipend to TS for one year (grant Number: TMA2016-1778). This work was also supported by the National Research Foundation of Korea (NRF) grant (No.2018R1A5A2021242) funded by the Korean government (MSIT). We would like to thank the Inje University College of Medicine, Busan, Korea for granting TS access to its laboratory and generously providing all materials needed to conduct the bioassay and microbiological analysis. We also thank Pham My Tung, Seong Hyeon-Jeong and Sunyoung Lee for their technical assistance. We would also like to thank the Clinical Research staff of Inje University for their generous assistance in handling the data and study samples. Finally, we thank Dr. Getnet Yimer for mentoring TS during this study.

Disclosure

The authors report no conflicts of interest in this work.

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Phenotypic and genotypic characterization of resistance to fluoroquinolones and other antitubercular drugs, and their correlation with clinical outcomes in Ethiopian patients with multidrug-resistant tuberculosis

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Running title: Characterization of resistance to fluoroquinolones.

Abstract

Introduction: Characterization of drug resistance for WHO Group A drugs like Fluoroquinolones is crucial to worldwide tuberculosis eradication efforts. This study aimed to characterize drug resistance to fluoroquinolones and other anti-TB drugs, and determine factors associated with resistance to fluoroquinolones and correlation between resistance and clinical outcomes.

Methods: Clinical isolates were prepared from baseline sputum culture of a cohort of 80 MDR-TB patients who were prospectively enrolled in our previous follow up study. Molecular line probe assay was performed for fluoroquinolones and second-line injectables using GenoType® MTBDR*sl* VER 2.0 and *gyrA* sequencing was performed on some of the clinical isolates (n=14). Moreover, minimum inhibitory concentration (MIC) was determined using the broth microdilution assay for fluroquinolones (n=70) and other anti-TB drugs (n=28). The correlation between clinical and demographic factors, fluoroquinolones-resistance and clinical outcomes was assessed using appropriate statistical tests.

Results: Of the 70 clinical isolates, 9 (12.9%) were resistant to fluoroquinolones, and 3 (4.3%) of which were resistant to both fluoroquinolones and injectable second-line drugs. The MIC data of 21 (75%) were concordant with the Xpert MTB/RIF assay result for the other anti-TB drugs, whereas 94.6% and 84.8% MIC values were concordant with the line probe assay results for Moxifloxacin and Levofloxacin, respectively. *GyrA* sequencing identified mutations in the D94 (Asp94) loci for 5/14 (35.7%) of the clinical isolates for the fluoroquinolones. The five mutations identified by *gyrA* sequencing were at D94N (n=1, 7.1%), D94H (n=1, 7.1%), and D94A (n=3, 21.4%). The lower MIC profiles of levofloxacin and moxifloxacin had association with with treatment outcomes (62% vs 38%, $\chi 2=18.7$; p=0.001 and 78% vs 22%, $\chi 2=23.6$; p=0.001, respectively). Baseline cavitary lesion (AOR=1.2; 95%CI=0.1-2.4, p=0.01) and serum creatinine (AOR=11.3; 95%CI=1.2-14.1, p=0.03) had association with fluoroquinolones-resistance.

Conclusions: Genotypic test identified a moderate level of fluoroquinolones-resistance but it may not always reflect the actual level of resistance. Optimized dosage of fluoroquinolones may better prevent risk of resistance. *GyrA* mutation is identified in the drug resistant-strains. Phenotypic drug

sensitivity test results and clinical variables correlate with clinical outcome and fluoroquinolones-resistance.

Keywords: Clinical isolates, Line probe assay, Minimum inhibitory concentration, Fluoroquinolones, *gyrA* sequencing, MDR-TB, Ethiopia

Introduction

Drug-resistant tuberculosis (DR-TB) is a global public health threat because treatment is less effective than for drug-sensitive TB [1]. Early drug resistance detection and characterization are critical for predicting clinical outcomes and global TB eradication efforts [2,3].

According to the latest World Health Organization (WHO) update, DR-resistant TB is classified into 5 categories: isoniazid-resistant TB, rifampicin-resistant (RR)-TB and MDR-TB (TB resistant to isoniazid and rifampicin), pre-extensively drug-resistant TB (pre-XDR-TB, which is MDR-TB with resistance to fluoroquinolones (FQs)), and finally XDR-TB (MDR-TB and TB-resistant to FQs plus at least bedaquiline or linezolid as per the revised WHO definition) [4]. Highly resistant infections are difficult to treat and can lead to poor clinical outcomes [5].

MDR-TB possesses an enormous epidemiological effect, with 480,000 identified cases and 190,000 fatalities in 2014, 10% of which cases qualify the definition for XDR-TB [6]. With increasing resistance to drugs, the epidemic persists and will necessitate considerable focus and commitment to eradicate it [7]. In 2018, around 484,000 MDR/RR-TB incidents were reported globally, accounting for 3.4% of new infections and 18% of already treated cases [8]. Even though there was a decreasing trend in MDR-TB in previous years, there was an increase (up to 3.1%) in the estimated MDR-TB incidence globally between 2020 and 2021 [1]. This is likely due to a

general increase in TB from 2020 to 2021, which is presumed to be due to the effect of COVID-19 on TB diagnosis [1, 9].

There is a dearth of evidence on the prevalence of MDR-TB in Ethiopia. In 2018, roughly, 0.71% of new TB cases and 7.2% of TB cases treated in the past contributed to DR-TB, with an approximate 1,600 cases emerging as MDR/RR-TB [10]. According to a recent systematic review, the prevalence of MDR-TB was 2.6% in newly diagnosed cases and 11.5% in retreated TB patients, while the overall pooled prevalence of MDR-TB was 10.8% [11].

FQs are broad-spectrum antibiotics shown to be useful in the treatment of TB in 1984 and have since become an essential component of TB treatment, especially in drug-resistant diseases [**5**]. Levofloxacin (LFX) and moxifloxacin (MXF) are highly effective FQs that have a significant role in treatment success [12]. The drugs are also key WHO Group A components, as they are the most effective drugs used to treat MDR-TB [13]. Therefore, the emergence of FQ-resistant TB has a significant implication in a weak healthcare system [14]. Globally, resistance rates to FQs has been predicted to vary between 0.15% to 30% [15]. These proportions are greater among individuals who were exposed to FQs prior to a diagnosis of TB or in patients who developed resistance through repeated exposures [16]. However, in certain locations in which FQ consumption is very substantial, resistance could be due to the spread of FQ-resistant pathogen in the population [15]. In more than 100 nations with adequate information, 20% of individuals with MDR/RR-TB additionally have FQ resistance (pre-XDR-TB), of which 8.5% were XDR-TB cases [17].

The main target of FQs in *Mycobacterium tuberculosis* (MTB) is the DNA gyrase encoded by *gyrA* and *gyrB.* FQs interfere with DNA replication of MTB by binding to the enzyme. Mutations in DNA gyrase confer MTB resistance to FQs. Mutations in the *gyrA* gene are associated with high

FQ resistance and mutations in *gyrB* are associated with low resistance. In particular, mutations occur in two short regions known as quinolone resistance-determining regions (QRDRs) [18, 19].

Mycobacterial resistance to drugs may be characterized by phenotypic and genotypic methods. Molecular testing is considered to be the most promising means to achieve rapid and universal drug susceptibility testing (DST) [20]. However, the minimum inhibitory concentration (MIC), defined as the lowest concentration of antimicrobial agent that inhibits the visible growth of a microorganism after incubation, is considered the 'gold standard' for determining susceptibility of organisms to antimicrobials against which the performance of other methods can be judged [21].

Ethiopia remains in the list of the 30 high TB/HIV burden countries although it recently transitioned out of the MDR-TB/RR-TB list [22] due to an achieved WHO End TB strategy milestone [23]. There is, however, little information on resistance characteristics of MTB against FQs and other anti-TB drugs. In our previous exploratory study [24], we determined treatment outcomes between the LFX-and MXF-based regimens, and the outcome predictors in a cohort of the MDR-TB patients in Ethiopia. In the present study, we report the resistance characteristics of MTB isolates obtained from the same cohort against the FQs (LFX and MXF) and other anti-TB drugs that the patients received, and their association with treatment outcomes as well as patient characteristics reported earlier [24].

Materials and Methods

Patients and samples

This is a follow-up study of a previous prospective cohort study that enrolled MDR/RR-TB patients from four hospitals in Southern Ethiopia [24]. The study was conducted between

November 2017 and May 2020. Details of participants' eligibility, demographic and clinical characteristics, the study setting, and drug treatment are reported in that study. All study participants received either Levofloxacin (LFX)- or Moxifloxacin (MXF)-based therapy according to recently consolidated WHO treatment guidelines [25].

The clinical isolates of MTB extracted from the baseline sputum cultures of the cohort were used for determination of MIC and *gyrA* gene sequencing at the Armauer Hansen Research Institute (AHRI) (Addis Ababa, Ethiopia) and Inje University College of Medicine (Busan, Republic of Korea).

Culture Identification of the Clinical Isolates

Sputum samples were decontaminated with an equal volume of 4% sodium hydroxide solution for 15 min. The decontaminated samples were centrifuged for 15 min at 3000 rpm. The supernatant was discarded, a drop of phenol red solution was added as an indicator, and the sediment was neutralized drop by drop with 2N hydrochloric acid solution [26]. Egg-based LJ-pyruvate and LJ-glycerol media were prepared, aliquoted, and stored in a refrigerator at 2 - 8^0C for a maximum of two months [27]. To enhance the growth of MTB lineages, the stored isolates were inoculated into conventional LJ media slants supplemented with 0.4% sodium pyruvate and 0.3% glycerol, and incubated at 37^{o}C for at least 8 weeks, and the presence of mycobacterial colonies was observed weekly [26]. To identify AFB-positive isolates, the colonies were examined under the microscope with the Ziehl-Neelsen stain. Loop full colonies were harvested into 2 mL cryovials containing 500 µL sterile nuclease-free water for heat inactivation and used for the line probe assay (LPA) earlier [24]. The remaining colonies were collected and processed for determination of MIC and sequencing of the *gyrA* gene in this study.

MIC determination

MIC determination was performed in 96 U bottom-shaped polystyrene microtiter using the EUCAST broth microdilution [28]. Briefly, 7H9 broth-OADC and stock anti-tuberculous agents were prepared based on the protocol. Except for column 1, other wells were filled with 50 µL of 7H9-OADC media, and column 1 was filled with 50 µL of the working solution (containing anti-TB drugs) (Supplementary Table 1). Column 2 was also filled with 50 µL of the working solution. Using a multichannel pipette, 1:2 dilutions were made by mixing and adding 50 µL of column 2 to the following column. Serially 2-fold dilution to column 11 was made and finally, 50 µL was discarded from the last row. A 0.5 McFarland standard was prepared from fresh colonies (within 2 weeks from visible growth), and 1:100 and 1: 10,000 bacterial suspensions were subsequently prepared in 7H9-OADC media.

A 50 µL 1:100 inoculum of 0.5McFarland was added to antibiotic-containing wells. Growth controls (1:100 inoculum, GC100% and 1: 10,000 inoculum, GC1%) were then inoculated, and the plates were covered with a plastic lid and then kept in O_2-/CO_2- permeable plastic bags and incubated at 37°C for a maximum of 21 days. G1% and G100% represented suspensions of clinical isolates at 1% and 100%, respectively, to which the test drugs were not added during inoculation. G1% was established as the critical bacterial concentration, below which strains were classified as sensitive, and above which they were categorized as resistant. To validate the test, the negative control should exhibit no growth. In cases where GC100% showed a positive result, we can subsequently examined GC1%. If GC1% also exhibited visible growth (typically of weaker intensity compared to GC100%), the MIC was determined as the lowest concentration of the agent at which no visible growth is observed.

158 *M.tuberculosis* H37Rv (ATCC 27294) was used as a drug-sensitive reference strain, and its target
159 MIC values ranged from 0.008-8.0 mg/L for LFX and 0.016-16.0 mg/L for MXF. Standard LFX
160 (Sigma Aldrich 28266-10G-F) and MXF Hydrochloride (Sigma Aldrich SML1581-50MG) were
161 used for the determination of MIC in the clinical isolates. The targeted ranges for the rest of the
162 drugs for which MIC was determined are provided in the Supplementary file. The plate was read
163 using an inverted mirror after 7 and 14 days of incubation, and the MIC value was reported in
164 mg/L.

165 *GyrA* sequencing

166 *GyrA* sequencing was conducted at the microbiology laboratory of the Inje University College of
167 Medicine. Sequencing of *gyrA* (this gene was purposely selected based on previous information
168 for the most prevalent mutation for FQs) was performed for the strains identified as resistant in the
169 LPA and additionally suspected strains for resistance. One loop of selected clinical isolates was
170 dispensed in Tris-EDTA buffer (pH 8.0) and incubated at 95°C for 30 min to obtain genomic DNA.
171 The DNA fragment of the *gyrA* gene was PCR amplified with primer sets (Table 1) and SolgTM
172 Pfu-X (Solgent, Republic of Korea). Sequence of *gyrA* was confirmed by Sanger sequencing
173 (Macrogen, South Korea). Single nucleotide polymorphism of each strain's *gyrA* sequence was
174 analyzed by UGENE (Unipro, Russia).

175 Correlations between drug resistance and clinical outcome

176 WHO-defined clinical outcomes and culture conversion rates, determined previously, were
177 evaluated for their correlation with the MIC values. The association between the total days of
178 culture conversion and the MIC values as well as factors predicting FQ-resistance were evaluated.

179 Data analysis

The data were entered into Excel and analyzed descriptively using SPSS software, version 25.0 (IBM, Armonk, NY, USA). The association between MIC values and treatment outcome was evaluated using the Chi-Square test. Predictors of FQ resistance were also evaluated using univariate and multivariate binary logistic regression. The backward multivariate binary logistic regression analysis included variables with p-values ≤0.2 in the univariate analysis. The results were described as numbers (frequency) and percentages and compared using the Chi-square test for categorical variables. On the other hand, continuous variables were compared using the Mann-Whitney test. P value <0.05 was considered statistically significant.

Ethical considerations

Ethical approval was obtained from both the Institutional Review Board of the College of Health Sciences of Addis Ababa University (protocol number 078/17 / Pharma) and the National Ethical Review Committee (Reference number MoSHE//RD/141/2318/19). Written informed consent was obtained after participants were informed about the study's purpose, benefits, and risks. Restricting access and removing identifiers ensured confidentiality and anonymity.

Results

Resistance characteristics

The phenotypic and genotypic studies used to characterize drug resistance in clinical isolates from patients are depicted in Fig 1. Initially, 80 clinical isolates extracted from a baseline sputum cultures of the cohort diagnosed as RR-TB by Cepheid GeneXpert MTB/RIF (Xpert) were obtained from the regional laboratory in Southern Ethiopia. An LPA was performed on these strains using GenoType® MTBDR*sl* VER 2.0 (Hain Life science, Germany), and 9 (11.3%) were

found to be resistant to FQs (Pre-XDR-TB), while 3 (3.8%) were resistant to both FQs and injectable second-line drugs (XDR-TB) [24].

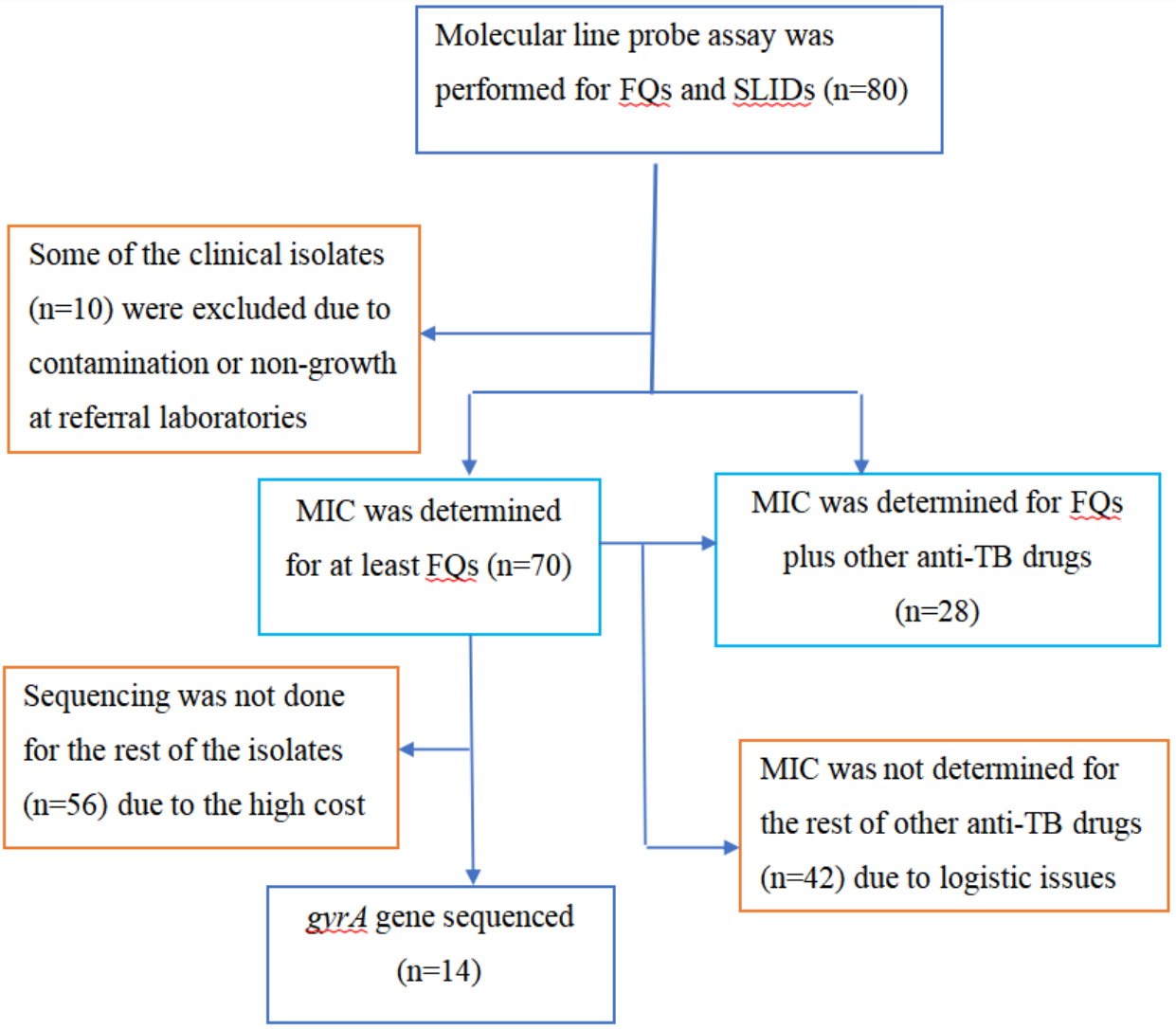

Figure 1: Summary of phenotypic and genotypic experiments on the clinical isolates of *Mycobacterium tuberculosis*. **Abbreviations:** FQs, fluoroquinolones; LPA, line probe assay; MIC, minimum inhibitory concentration; SLIDs, second-line injectable drugs

Complete MIC profiles for 28 (35.4%) of the 80 clinical isolates were determined for other anti-TB drugs (Table 2), whereas complete MIC profiles of FQs were obtained for 70 strains (LFX, n=33 and MXF, n=37). MIC for the rest of other anti-TB drugs (n=42) were not determined due to logistic reasons, while the MIC of FQs were not determined for some isolates (n=10) due to contamination or non-growth at the referral laboratories. Based on LPA results, 61 (87.1%) of the 70 clinical isolates included in the final analysis were FQ-sensitive, while 9 (12.9%) were FQ-resistant. The frequency (number count) of MIC distributions of clinical isolates for the FQs is depicted in Fig 2. The MIC profiles for both LFX and MXF ranged from 0.0625 mg/L to 8.0 mg/L. MIC values ≥1.0 mg/L constituted 9 (24.3%) for MXF and 7 (21.2%) for LFX. As a result, the total number of clinical isolates with MIC profiles of ≥1.0 from both groups of patients was 16/70 (22.9%). According to the MIC profiles, 12/37 (32.4%) and 13/33 (39.4%) of the MIC profiles correspond to the WHO critical concentration for lower resistance (0.25 mg/l for MXF and 0.5 mg/l for MXF, respectively) (Fig 2).

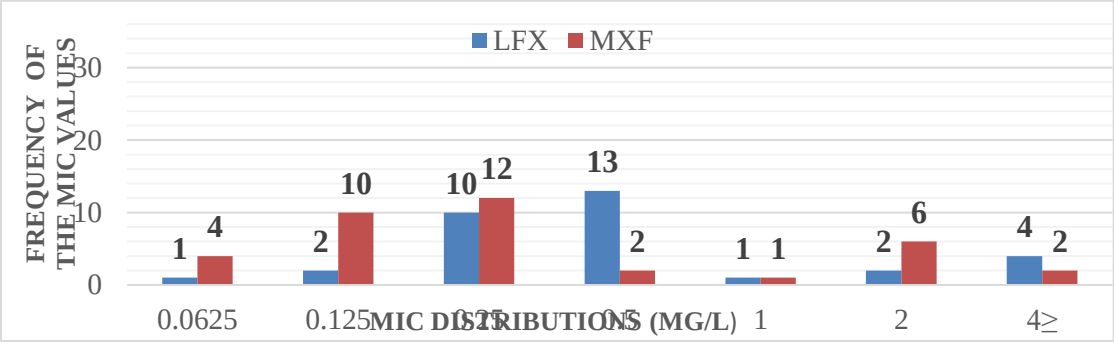

Figure 2: Frequency of the minimum inhibitory concentration distributions of the clinical isolates tested for the fluroquinolones (n=70) sensitivity (MXF=37 and LFX=33). **Abbreviations:** LFX, levofloxacin; MXF, moxifloxacin; MIC, minimum inhibitory concentration

In this study, the genotyping results from GeneXpert and LPA as well as the phenotypic results from the MIC values were compared and contrasted. Although they had previously been reported to have RR-TB based on GeneXpert testing, 7 (25%) of the 28 clinical isolates for which MICs were determined, were susceptible to isoniazid and rifampicin. As a result, the GeneXpert test results correlated with 21/28 (75%) of the phenotypic DST (MIC values of the other antitubercular drugs) from the total of 28 clinical isolates (Table 2). The MIC values for MXF and LFX, on the other hand, were concordant with the LPA results of the clinical isolates in 35/37 (94.6%) and 28/33 (84.8%) of the cases, respectively (Table 3).

The 9 resistant cases in the LPA and 5 additional clinical isolates (14 clinical isolates in total, due to high sequencing cost) suspected of resistance (based on previous clinical findings in the patients) were sequenced to look for mutations in the QRDR's *gyrA* gene. Five (5/14, 35.7%) of the sequenced strains had a *gyrA* 94 mutation. As a result, 5/9 (55.5%) of the LPA results matched the sequencing results, and the five mutant isolates (100%) had MIC values ≥2 (Table 4).

Association Studies

Between MIC values and treatment outcomes

In our previous study [24], we evaluated the WHO-defined treatment outcomes [29] of MDR-TB patients (n=80). Of these, the present study successfully determined the MIC profiles of 70 (87.5%) (37 on MXF- and 33 on LFX-based regimen) clinical isolates. Next, we investigated the relationship between clinical strain MIC values and treatment outcomes. The overall treatment success (cured or treatment completed) rate was 50/70 (71.4%), with the remaining 20/70 (28.6%)

cases having unfavorable outcome (death, treatment failure, and lost-to-followup). Of the 50 treatment success cases, 42 were cured and 8 completed treatments. Among those that had unfavorable outcome, 9/20 (45%) were FQ resistant as per the LPA evaluation, whereas 11/20 (55%) were FQ sensitive, the unfavorable outcome for the latter was due to lost-to-follow-up (7) and death (4). Three of the nine FQ-resistant cases were resistant to both FQs and injectables. Treatment outcome and MIC values were positively correlated for LFX at ≤0.5 and MXF at ≤0.25 (62% vs 38%, $\chi 2=18.7$; p=0.001 and 78% vs 22%, $\chi 2=23.6$; p=0.001, respectively).

Similarly, the association between the MIC values and culture conversion within 3 months for LFX and MXF was statistically significant ($\chi 2 =14.7$, p=0.03 and $\chi 2=12.5$; p=0.04, respectively). In this case, the culture conversion within 3 months and beyond 3 months were compared. Overall, 45/70 (64.3%) of MDR-TB patients receiving both LFX- and MXF-based regimens had culture conversion within 3 months, while 25/70 (35.7%) converted after 3 months. The Mann-Whitney test revealed a statistically significant ($p<0.05$) association between the MIC values and the total number of days for culture conversion for both LFX and MXF.

Predictors of Fluoroquinolone Resistance

Table 5 summarizes the results of the univariate and multivariate binary logistic regression analyses. Variables including median age, gender, body mass index (BMI), comorbidity, prior TB treatment, smoking, khat chewing, alcohol consumption, baseline AFB, cavitary lesion, nutritional status, and median values of serum creatinine (Cr.), albumin (ALB), alanine transaminase (ALT), and alanine aminotransferase (AST) were screened in the univariate analysis. The variables included in the multivariate analysis were smoking, chewing tobacco, alcohol consumption,

cavitary lesion, median Cr., and ALT. Baseline cavitary lesion (AOR=1.2; 95% CI=0.1-2.4; p=0.01) and Cr. (AOR=11.3; 95% CI=1.2-14.1; p=0.03) were found to predict FQ-resistance.

Discussion

In this study, we used phenotypic and genotypic methods to characterize resistance level and type, as well as their relationship with treatment outcomes in MDR-TB patients. The MIC of the drugs was determined using the broth microdilution method for the phenotyping study, and the genotype of the drugs was evaluated using LPA and gyrA sequencing. FQ resistance observed in this study (12.9%) was lower compared to other settings, including, India (36%) [30] and South Korea (30.2%) [31]. FQ resistance was found to be predicted by Cr. and cavitary lesions in this study. It is of note that cavitary lesion and Cr. were among the independent predictors of treatment outcome in our previous study [24], and cavitary lesion is also demonstrated to be a determinant of FQ resistance in other studies [25]. However, whether Cr. has a direct impact on FQ resistance is unclear. It has been hypothesized that the drug-creatinine interaction is responsible for the increase in Cr. levels in patients receiving FQs [32].

The correlation between genotypic and phenotypic DST results for LPA was higher than that of GeneXpert (94.6%,MXF and 84.8%, LFX vs. 75%). Clinical isolates from 7/28 (25%) of the previously diagnosed RR-TB cases (false positive) were found to be sensitive to both isoniazid and rifampin after their MIC was determined. Furthermore, MIC tests revealed that 2/37 (5.4%) and 5/33 (15.2%) of the LPA-defined sensitive (false negative) cases of MXF and LFX, respectively, were resistant. The susceptibility status might have changed during culture storage prior to the MIC test, which could explain the latter cases. Because the patients were already

289 exposed to MDR-TB drug regimens (including FQs) based on the geneXpert screening, acquired
290 drug resistance is likely in this case.

291 The gyrA gene mutation, previously identified as the most common mutation type for FQ-
292 resistance in other studies [33], was also identified in the current study's sequencing experiment
293 (for the 5/14, 35.7% of strains sequenced). The five mutations identified by *gyrA* sequencing were
294 at D94N (n=1, 7.1%), D94H (n=1, 7.1%), and D94A (n=3, 21.4%) (Table 4). D94N showed the
295 highest MIC values (8 mg/L) for both LFX and MXF. The MIC values for the rest of the *gyrA*
296 mutations ranged between 2 mg/L and 8 mg/L. A90V and D94G in the *gyrA* genes were mostly
297 responsible for the FQs' resistance among MTB isolates from Bangladesh even though the MIC
298 values were comparable with our finding [34]. The discrepancy may be due to the small number
299 of the strains sequenced in the present study.

300 Of the 102 strains recently tested for FQ resistance in Uganda, 70/102 (68.6%) and 01/102 (0.98%)
301 had resistance-conferring mutations at the gyrA and the gyrB loci, respectively [36], indicating
302 that the role of *gyrB* in FQ-resistance is minor. Although the rate observed is different between the
303 two studies due to sample size differences, both studies (the present and Ugandan) show that *gyrA*
304 mutations largely account for FQ-resistance. Other resistance mechanisms could have explained
305 the remaining strains that did not show mutation in the *gyrA* sequencing.

306 Furthermore, it should be noted that the MIC values determined by the broth microdilution were
307 ≥2.0 mg/mL for all the five cases of mutant strains identified by the *gyrA* sequencing method,,
308 indicating a strong concordance between the two methods. Our study showed that there was a link
309 between MXF and LFX MIC values and WHO-defined treatment outcomes. The major proportion
310 of treatment success was positively correlated with the critical MIC values ≤0.5 mg/L for LFX

(80% vs 20%; $\chi 2=14.12$; p=0.04) and ≤0.25 mg/L for MXF (86% vs 14%; $\chi 2=12.5$; p=0.003). This could imply that FQs MIC profiles reflect treatment outcomes in MDR/RR-TB patients. According to our findings, clinical isolates with MIC values of 0.25 mg/L for MXF (12/37, 32.4%) and 0.5 mg/L for LFX (13/33, 39.4%) would be at risk of FQ-resistance because majority of the values were on the borderline of the critical MIC values for lower-level resistance. Furthermore, the overall proportion of clinical isolates with ≥1.0 (16/70, 22.9%) for both MXF and LFX may reflect that a greater proportion of patients are already at risk of developing acquired resistance to FQs due to the use of non-optimized doses of these drugs. We demonstrated that standard doses of MXF and LFX may not provide adequate serum exposure in Ethiopian MDR-TB patients [35], and their serum drug concentrations increase proportionately (at least for LFX) with dose. Patients may benefit if the dosage design for LFX and MXF takes their phenotypic and genotypic DST profiles into account.

Limitations

Sample size with insufficient statistical power to find a significant difference between FQ-resistant and FQ-sensitive groups might be the main limitation of this study. The Sequencing experiment also relied on small number of isolates due to logistic issue.

Conclusions

Ethiopian patients have a moderate level of FQ resistance compared to other settings. Although genotypic tests are effective in detecting drug resistance, they do not always correlate well with phenotypic results since a single or two genes may not explain all the potential FQ-resistances fully. The actual drug resistance level could be higher than anticipated in TB patients. In resistant

strains, the *gyrA* 94 mutation was identified, and more than half of the *gyrA* sequencing results matched the LPA-identified mutations. Cavitary lesion and Cr. may aid in the prediction of FQ resistance. Furthermore, the MIC profiles of clinical isolates were linked to treatment outcomes. These findings may aid in improvement of treatments. MIC profiles higher than the critical values observed in the study population may increase the risk of drug resistance and poor outcomes. Optimized LFX or MXF doses may be required to avoid the risk of further drug resistance. Investigations involving other potential genes associated with FQ-resistance as well as whole genome sequencing are essential areas of further studies to generate more evidences and substantiate the findings of the present study.

Supporting information

Supplementary File 1

Acknowledgments

We acknowledge the AHRI and Inje University College of Medicine (Busan, South Korea) for permitting TS access to their laboratory and generously providing all materials needed for the laboratory experiment. We also would like to acknowledge the support of Dr. Kidist Bobosha from AHRI. Finally, we would like to thank Dr. Getnet Yimer and Dr. Workineh Shibeshi, who guided TS through this research.

Author Contributions

Conceptualization: TS & EE; Data curation: TS, MA, MT, SL, YP; Formal analysis: TS;

Funding acquisition: JGS, EA, EE; Investigation: TS, MA, MT, YP, SL, EA, YC, JGS, EE; Methodology: TS, YP, SL, MA, MT; Supervision: EE, YP, JGS, EA, YC; Writing original draft: TS; Writing – review & editing: YP, EE, JGS, EA.

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Legend to Figures

Figure 1: Summary of phenotypic and genotypic experiments on the clinical isolates of *Mycobacterium tuberculosis*. **Abbreviations:** FQs, fluoroquinolones; LPA, line probe assay; MIC, minimum inhibitory concentration; SLIDs, second-line injectable drugs

Figure 2: Frequency of the minimum inhibitory concentration distributions of the clinical isolates tested for the fluroquinolones (n=70) sensitivity (MXF=37 and LFX=33). **Abbreviations:** LFX, levofloxacin; MXF, moxifloxacin; MIC, minimum inhibitory concentration

Table 1. Primer sets for *gyrA* sequencing

gyrA1_F	GATGTCTAACGCAACCCTGCGTTCGAT
gyrA1_R	AGGTACGACCGCGGGAATCCTCTTCTA
gyrA2_F	CCGACGCGGTGTTCTGG
gyrA2_R	TCGATTTTGGCCAGGTCGTC
gyrA3_F	GTCGGAGACCGTCGATA
gyrA3_R	GAACCTGATGGACTGCCC
gyrA4_F	GCAACGGGCTGGTGAAAAAG
gyrA4_R	GACCAAGCCATCCGCATTC

Table 2: Summary of minimum inhibitory concentration and line probe assay results of the clinical isolates from the multi-drug resistant tuberculosis patients (n=28)

Isolate code	*LPA result*	*MIC (mg/L) Distribution of MDR-TB regimen*											
		BDQ	CFZ	CS	DLM	EMB	INH	KNM	LFX	LZD	MXF	PTA	RIF.
H37Rv	s/s	0.12	0.5	4	0.005	0.5	0.05	2	0.5	0.5	0.125	0.25	0.06
44	r/s	-	-	-	-	-	-	-	0.5		2.0	-	-
45	s/s	0.24	0.5	4	-	-	>3.2	-	0.5	0.25	0.125	-	>4
61	r/s	-	-	-	-	-	-	-	0.5	-	2.0	-	-
64	s/s	0.24	0.5	8	-	-	>3.2	-	0.25	0.5	0.06	-	>4
68	s/s	-	-	8	-	-	0.025	-	0.25	-	0.06	1	0.03
73	s/s	-	-	-	-	-	3.2	-	0.25	-	0.06	-	>4
121	s/s	-	0.25	-	-	0.5	>3.2	2	0.25	-	0.13	16	>4
160	s/s	-	0.25	-	-	0.5	>3.2	2	0.25	-	0.13	>16	>4
168	s/s	-	-	4	-	-	>3.2	-	0.5	-	0.13	2	>4
180	s/s	-	0.25	-	-	1	0.8	-	0.5	-	0.13	-	>4
190	r/r	-	-	-	-	4	>3.2	-	8	-	8	0.5	>4
223	s/s	-	-	-	-	-	>3.2	-	0.5	-	0.25	-	>4
278	s/s	0.24	0.25	8	-	-	>3.2	-	0.25	0.25	0.06	-	>4
284	s/s	0.24	0.5	8	-	-	>3.2	-	0.25	-	0.06	-	>4
302	s/s	-	-	-	-	-	>3.2	-	0.5	-	0.13	-	>4
314	s/s	-	-	-	-	-	>3.2	-	0.5	-	0.13	0.25	>4
318	s/s	-	-	-	-	-	>3.2	-	0.5	-	0.13	-	>4
325	s/s	-	0.25	-	-	2	>3.2	2	0.25		0.13	0.25	>4
328	s/s	-	-	-	-	-	0.05	-	0.25	-	0.13	-	0.13
329	s/s	0.12	0.5	4	-	-	0.05		0.5		0.5	0.13	0.06
342	r/s	-	-	-	-	-	-	-	2.0	-	2.0	-	-
380	s/s	-	-	-	-	-	3.2	-	0.25	-	0.06	-	>4
392	s/s	-	-	-	-	-	-	-	0.06	-	0.13	-	-
405	r/s	0.96	1		0.005		0.025		4.0	0.5	2.0		0.06
407	s/s	-	-	-	-	-	-	-	0.5	-	0.25	-	
450	s/s	-	-	-	-	-	0.05	-	0.25	-	0.13	-	0.06
491	s/s	0.24	0.5	4	-	-	>3.2	-	0.5	0.3	0.06	-	>4
511	s/s	-	0.25	-	-	4	3.2	2	0.5	-	0.125	0.25	>4
512	s/s	-	0.13	-	-	0.5	0.03	2	0.25	-	0.125	0.25	0.06
563	r/s	-	-	-	-	-	-	-	1.0	-	1.0	-	-
702	r/r	-	-	-	-	-	-	-	8.0	-	2.0	-	-
705	s/s	-	-	-	-	-	-	-	0.5	-	0.25	-	-
725	s/s	-	-	-	-	-	3.2	-	0.5	-	0.25	-	>4
728	s/s	0.12	0.25	8	-	-	3.2	-	0.5	0.5	0.25	-	>4
739	r/s	-	1	-	-	0.5	0.05	4	2	-	2	0.25	0.06
745	s/s	-	-	-	-	-	>3.2	-	0.25	-	0.125	-	>4
1008	r/r	-	-	-	-	-	-	-	2.0	-	4.0	-	-

Note: LPA results were described as s/s, sensitive to both FQs and inectable second line drugs; r/s, sensitive to FQs but sensitive to injectable secondline drugs; and r/r, resistatnt to both FQs and injectable secondline drugs.. Abbreviations: BDQ, bedaquiline; DLM, delamanid; CFZ, clofazimine; CS, cycloserine; EMB, ethambutol; INH, isoniazid; KNM, kanamycin; LZD, linezolid; LPA, line probe assay; LFX, levofloxacin; MXF, moxifloxacin; PTA, prothionamide; RIF, rifampicin; the hyphen (-) indicates cases for which the MIC profile was not determined.

469 **Table 3**. Genotypic and phenotypic analysis and concordance levels between the two drug
470 sensitivity testing methods for the clinical isolates from the multi-drug resistant tuberculosis
471 patients

Drug	N, strains tested	n (%), resistant genotype	n (%), resistant phenotype	n (%), Concordance
INH	28	UNK	21 (75)	UNK
Rif	28	28 (100)	21 (75)	21 (75)
MXF	37	7 (18.9)	9 (24.3)	35/37 (94.6)
LFX	33	2 (6.1)	7 (21.2)	28/33 (84.8)

472 **Note:** n=33 for MXF and n=37 for LFX; Abbreviations**:** INH, Isoniazid; Rif. Rifampicin; MXF, Moxifloxacin,
473 LFX, Levofloxacin; UNK, Unknown; N, the total number of strains tested, n (%), number of strains and percent.

Table 4: *gyrA* sequenced clinical isolates with their corresponding line probe assay and minimum inhibitory concentration values.

Clinical isolate no	*LPA result*	*MIC (mg/L) values of LFX and MXF*		*Mutation in gyrA sequence*	
				D94 (Asp94)	
		LFX	MXF	280 (GAC)	281(GAC)
H37Rv	s/s	0.5	0.125	No	No
44	r/s	0.5	2.0	No	no
61	r/s	0.5	0.25	No	No
68	s/s	0.25	0.0625	No	No
121	s/s	0.25	0.125	No	No
190	r/r	8.00	8.00	A(AAC)-D94N	No
325	s/s	0.25	2.00	No	No
405	r/s	4.00	2.00	No	C(GCC)-D94A
739	r/s	4.00	2.00	No	C(GCC)-D94A
702	r/r	8.00	2.00	C(CAC)-D94H	No
1008	r/r	2.00	4.00	No	C(GCC)-D94A
342	r/s	2	0.25	No	No
392	s/s	0.0625	2.00	No	No
407	s/s	0.5	0.25	No	No
705	s/s	0.5	0.25	No	No
563	r/s	1.00	1.00	Not sequenced	

Note: n=14: Abbreviations**:** LPA, line probe assay; LFX, levofloxacin; MXF, moxifloxacin; upper case alphabets represent single letter naming of amino acids (A, alanine; D, aspartic acid; H, histidine; N, Asparagine)

Table 5. Predictors of fluoroquinolones resistance in multi-drug resistance tuberculosis patients, Ethiopia

Univariate binary logistic regression			
Variables	No FQ-sensitive/ Total (%)	FQ-R /Total (%)	p value, OR [95%CI]
Median age, ≤26.5	55/61 (90.2)	8/9 (88.9)	0.9, 1.2 [0.1-10.8]
Sex, Male	32/61 (52.5)	5/9 (55.6)	0.9, 0.9 [0.2-3.6]
Median BMI, ≥17.1	19/61 (31.2)	2/9 (22.2)	0.6, 1.6 [0.3-8.4]
Comorbidity, yes	25/61 (41.0)	2/9 (22.2)	0.3, 2.4 [0.5-12.7]
Prior TB treatment, yes	48/61 (78.7)	6/9 (66.7)	0.4, 1.9 [0.4-8.4]
Smoking, yes	7/61 (11.5)	3/9 (33.3)	0.1*, 0.3 [0.05-1.3]
Alcohol consumption, yes	6/61 (9.8)	3/9 (33.3)	0.07*, 0.2 [0.04-1.1]
Chewing khat, yes	2/61 (3.3)	3/9 (33.3)	0.01*, 0.07 [0.01-0.5]
Baseline AFB, positive	54/61(88.5)	7/9 (77.8)	0.4, 0.5 [0.1-2.6]
Cavitary lesion, yes	19/61(31.1)	8/9 (88.9)	0.01*, 0.1 [0.01-0.5]
Malnourished‡, yes	40/61 (65.6)	6/9 (66.7)	0.9, 0.9 [0.2-4.2]
Median Cr.(mg/dL), ≥0.87	23/61(37.7)	6/9 (66.7)	0.11*, 3.3 [0.8-14.5]
Median AST (U/L), ≥33.75	31/61(50.8)	3/9 (33.3)	0.33, 0.3 [0.1-2.1]
Median ALT (U/L), ≥34.0	35/61(57.4)	3/9 (33.3)	0.19*, 0.4 [0.1-1.6]
Median ALB (g/dL), ≥3.27	31/61 (50.8)	5/9 (55.6)	0.80, 1.2 [0.3-4.9]
Multivariate binary logistic regression			
Variables	Frequency/total encounter (%)	FQ-R /Total (%)	p value, AOR (95%CI)
Smoking, yes	7/61 (11.5)	3/9 (33.3)	0.7, 0.4 [0.0-49.1]
Alcohol consumption, yes	6/61 (9.8)	3/9 (33.3)	0.08, 0.05 [0-1.4]
Chewing khat, yes	2/61 (3.3)	3/9 (33.3)	0.9, 1.6 [0.01-78.1]
Cavitary lesion, yes	19/61(31.1)	8/9 (88.9)	0.01†, 1.2 [0.1-2.4]
Median Cr.(mg/dL), ≥0.87	23/61(37.7)	6/9 (66.7)	0.03†, 11.3 [1.2-14.1]
Median ALT (U/L), ≥34.0	35/61(57.4)	3/9 (33.3)	0.11, 0.2 [0.02-1.5]

Note: n=70; Abbreviations: AOR, adjusted odds ratio; ALB, albumin; ALT, alanine transaminase; AST, alanine aminotransferase; AFB, acid fast bacilli; Cr, Serum creatinine; CI, Confidence interval; FQ-R, fluoroquinolone resistant; BMI, body mass index; OR, crude odds ratio; *Variables with p-value ≤0.2 in univariate analysis; †Variables with p-values <0.05 in multivariate analysis; ‡ Patients with BMI less than 16 kg/m^2 were considered as malnourished.

Appendix II: Patient Information Sheet (In English)

Introduction

⇒ We are inviting you to take part in MDR-TB resistance and pharmacokinetic (PK) study of tuberculosis (TB) medicines in your blood**.**

Why is this study being done?

⇒ This study will help us to learn how drug resistance in TB develops and how well the amount of moxifloxcin or levofloxacin that you are taking works against TB.

⇒ Mr. Temesgen Sidamo (a PhD candidate) and his advisors Prof. Ephrem Engidawork and Dr. Workineh Shibeshi, from Addis Ababa University (Ethiopia), and Prof. Eleni Akllilu from Karolinska Institutet (Seweden) in collaboration with Nigist Eleni Mohamed Memorial referral and Arbaminch, Butajira and Yirgalem general Hospitals administration are working together on this study.

How many people will take part in this study?

⇒ Up to 80 people will participate in this study.

How long will I be in the study?

⇒ You will be in the study for about two days.

What will I have to do to be in the study?

⇒ To be in this study, you must be taking TB medicines as your normal course of treatment for MDR-TB (patients).

⇒ Immediately after you are diagnosed and confirmed for MDR-TB, your sputum will be taken. Also your sputum may be taken within one week from the day you started your MDR-TB medications. The sputum will be used to study different ways that TB develops drug resistance. After growing culture drug susceptibility (DST), line probe assay (LPA) and genetic sequencing will be performed.

⇒ Blood sampling will be done after you take at least 8 doses of study treatment i.e. One week after the initiation of your MDR-TB medication.

⇒ The blood samples will be used to study different variables and factors affecting the amount of drugs in your blood. You will be asked to not drink any alcohol for 48 hours before starting the PK study (withdrawing blood) until after the last blood draw is done.

What is involved in the PK study?

⇒ On the morning (6 AM) you begin the study. We will measure your height, weight, blood

pressure, pulse, breathing rate, and temperature.

⇒ You will be asked a few questions about your health including questions about:

- Your medical history
- Any recent problems such as stomach upset or diarrhea
- Any medicines you are taking.
- Any other illnesses you may have.
- If you drink alcohol or take drugs.

⇒ To answer these questions will take about 15-30 minutes. You do not have to answer these questions if you do not want to.

- As part of the study your medical records will be reviewed.
- A thin tube (sometimes called an "IV") will be put in your vein and left in place until the blood draws are completed. This IV will allow us to get blood samples at different times without many needle sticks,
- We will draw your blood from the IV before you take the TB medications.
- You will then take your usual dose of TB medicines.
- Over the next 24 hours we will draw your blood from the IV tube 6 more times.
- The blood draws will be done at 0 (pre-dose), 2, 4, 6, 9, 12 and 24 h after you take your TB medications.
- About 3-4cc will be taken at each of the blood draws.
- The blood will be sent to outside laboratories for genetic analysis and testing the levels of medicine in your blood. The tubes that contain your blood will be labeled with a study number only. Your name will not be on the tubes.
- We may do other blood tests as well [using a little more than 12cc of blood drawn from the IV. We will not take more blood if we can get results for these blood tests done within the 30 days before you start the PK study. These tests are to:
- See how well you liver and kidneys are working.
- See how well your blood clots (bleeding time)
- measure your blood count and the amount of inflammation in your body (C-reactive protein)
- We won't repeat these tests if we can get results of these tests done the last 12 months.

What are the risks of the study?

⇒ There are a few small risks from having blood drawn or having an IV line placed. These include brief pain from the needle stick, lightheartedness, bruising, bleeding, and rarely, infection where the needle enters the vein.

Benefits

⇒ There is no direct benefit to you for taking part in this study. Your taking part in this research may help us to improve TB treatment in the future.

⇒ There is no any payment to you in involving in this study. However, a special meal and fluids will be provided on the date we draw blood from you for this study.

Your Privacy

⇒ Everything we learn about you in the study will be kept private. If we publish the results of the study in a scientific journal or book, we will not identify you in any way.

⇒ We will not use your name in any speech or paper about the study. We will keep all information from your medical records private as much as the law allows.

In case of Injury

⇒ It is unlikely you will be harmed because of taking part in this PK study, thus you will be treated by the hospital as usual in case of any unlikely events**.** However, by signing this consent form and agreeing to be in this study, you are not giving up your legal rights.

⇒ If you believe that you have been harmed, please contact **Mr. Temesgen Sidamo** (*Addis Ababa University*) by calling +251911093567 to learn about your rights and advice about what to do.

Right to refuse and reasons for withdrawal

⇒ Taking part in this study is your choice. You do not have to be a part of this study if you do not want to. If you choose to be a part of this study, you may change your mind and choose to stop taking part in this study at any time.

⇒ We will tell you if we learn new things during the study that might change your decision to stay in the study.

Appendix III: Patient information Sheet (In Amharic)

<u>መድሃኒት በተላመደዉ ቲብ (MDR-TB) ምርምር ለሚሳተፉ ታካሚዎች መረጃ መቀበያ ቅጽ መግቢያ</u>

ዉድ ታካሚ

በደም ዉስጥ ያላዉን የቲብ መድኃኒት መጠን ለመለካትና የመድኃኒቱን ፍቱንነት ለማወቅ ወይም ለመለካት በሚደረገዉ ምርምር ተሳታፊ እንድሆኑ በትህትና እንጋብዝዎታለን።

ይህ ምርምር ለምን አስፈለገ?

ይህ ምርምር በደምዎ ዉስጥ የሚገኛዉ ሞክሲፍሎክሳስን (moxifloxacin) ወይም ልቮፍሎክሳስልን (levofloxacin) የተባላዉ መድሃኒት መጠን ምን ያህል እንደሆነና ይህም ለመድሃኒቱ ፍቱንነት ምን ያህል አስተዋጽኦ እያደረገ እንደሆነ፣ በደምዎ ዉስጥ የምገኘዉ የባክቴሪያዉን ዝሪያና ዘረመል (Genetic analysis) እንድሁም ለመድሃኒት የሚሰጠዉን ምላሽ (Drug Susceptibility) ለማጥናት ታስቦ የሚደረግ ምርምር ነዉ።

ምርምሩን የሚያካሄዱት በአድስ አበባ ዩኒቬርስቲ የዶክትሬት ጥናት እያደረጉ ያሉት አቶ ተመስገን ሲዳሞና የምርምሩ አማካሪዎች ኤፍሬም እንግዳወርቅ (ፕሮፌሰር)፣ ወርቅነህ ሺበሺ(ተባባር ፕሮፌሰር)፣ እና እሌኒ አክሊሉ (ፕሮፌሰር) ናቸዉ። እንዲሁም የንግስት እሌኒ ሙሐመድ መታሰቢያ ሪፈራል ሆስፒታልና የአርባምንጭ፤ የቡታጅራና የይረጋ-ዓለም አጠቃላይ ሆስፒታሎች የአስተዳደር አካላት የዝህ ጥናትና ምርምር ተባባሪ ናቸዉ።

በአጠቃላይ 80 ታካሚዎች በዝህ ምርምር እንደሚሳተፉ ይጠበቃል።

በዝህ ምርምር ለመሳተፍ ምን ያስፈልግዎታል?

የዚህ ምርምር አካል ለመሆን መደበኛዉን መድሃኒት የተላመዳ ቲብ (MDR-TB) ህክምና/መድሃኒት የሚከታታሉ/የሚወስዱ መሆን ይኖርብዎታል።

መድሃኒት የተላመደ ቲብ በደምዎ መኖሩ ከተረጋገጠ በኋላ ህክምናዎን ከመጀመሪዎ በፊት 3-5 ሲሲ የሚሆን አክታ ይሰጣሉ (እንወስዳለን)።ነገር ግን መድሃኒት መዉሰድ ከመጀመርዎ በፊት አክታ ያልሰጡ እንደሆነ መድሃኒት በጀመሩ ሠምንት ግዜ ዉስጥ አክታ እንዲሰጡ ልጠየቁ ይችላሉ።

ህክምናዉን ከጀመሩ ቢያንስ ከ8 ቀን በኋላ ደግሞ በደምዎ ዉስጥ ያለዉን ሞክስፍሎክሳስንና ለቮፍሎክሳስን የተባሉ መድሃነቶችን መጠን ለመለካት የሚያስችል ደም በ24 ሰዓታት ዉስጥ ለሰባት ግዜ ይወሰዳል (የደም ናሙና ስለሚወሰድባቸዉ ሰዓታት ከታች ይመልካቱ)። የመድሃኒቱን ፍቱንነት የሚወስኑ ከእርስዎ በተወሰዱ የደም ናሙናዎች ለይ የመድሀንቱን ፍቱንነት የሚቀይሩ ሁኔታዎች ምርምር ይካሄድባቸዋል። ስለዚህ ምርምሩ ከመጀመሩ በፊትና በኋላ ቢያንስ ለ48 ሰዓታት ያህል ግዜ ከአልኮል መጠጦች እንዲታቀቡ እንጠይቅዎታለን።

የደም ናሙና በሚወሰድበት ቀን ምርምሩ/ጥናቱ ምን ምን ያካትታል?

ጥናቱ በጠዋቱ 12፡00 ሰዓት ላይ ይጀምራል። የርስዎ ቁመት፣ የደም ግፍት፣ የልብ ምት፣ የአተነፋፈስ ሁኔታ እንዲሁም የሙቀት መጠን ይለካል። አንዳንድ ጥያቄዎችን ስለ አጠቃላይ የጤናዎ ሁኔታ ይጠየቃሉ፡ ለምሳሌ፡-

- ከመነሻዉ የነበረ የህክምና/የጤና ሁኔታዎ (Medical history)
- በቅርቡ የተከሰቱ ችግሮች እንደ የሆድ ህመም ወይም ተቅማጥ
- እየወሰዱት ያለዉ የመድሃኒት ዓይነት
- ከቲብ በሽታ ዉጭ ያለዎት ማንኛዉም ህመም/በሽታ
- አልኮል ይጠጡ የነበረ እንደሆነ

እናዚህን ጥያቄዎች ለመመለስ በአጠቃላይ ከ15-30 ደቂቃ በቂ ነዉ። እናዚህን ጥያቄዎች ለመመለስ ከልፈለጉ አይገደዱም።

- ✓ ተመዝግቦ የሚገኛዉን የእስከሁኑን የጤና ሁኔታዎን ዳሳሳ (ወይም ክለሳ) ማድረግ የጥናቱ አካል ነዉ።
- ✓ የደም ናሙና ከርስዎ በሚወሰድበት ወቅት ቀጭን ፕላስቲክ (ወይም አይቭ ካኑላ) በደም ስርዎ ገብቶ ይቆያል። ይህም ደም ስርዎን በመርፌ በተደጋጋሚ መዉጋት ሳያስፈልግ የደም ናሙና ለመዉሰድ ያስችላል።
- ✓ በእለቱ የቲብ መድሃኒቶችን ከመዉሰድዎ በፍት የደም ናሙና ይወሰዳል።
- ✓ ከዚያም በየእለቱ የሚወሰዱ መደበኛዉን የቲብ መድሃኒት ይወስዳሉ።
- ✓ ቀጥሎም በ24 ሰዓታት ዉስጥ 6 ግዜ ያህል የደም ናሙና ይወሰዳል።

- ✓ በጥቂሉ የደም ናሙና የሚወሰድባቸዉ ሰዓታት ዜሮ ሰዓት (በእለቱ መድሃኒት ከመዉሰድዎ በፍት) ከዚም መድሃኒት ከወሰዱ በሉት 2፣ 4፣ 6፣9፣ 12 እና 24 ሰዓታት ወስጥ ይሆናል።
- ✓ በአንድ ግዜ የሚወሰደዉ የደም ናሙና መጠን ከ3-4 ሲሲ ብቻ ነዉ።
- ✓ ከርስዎ የተወሰደዉ የደም ናሙና የቲብ ባክቴሪያ ዘረመል ምርማራ ለማካሄድና በደምዎ ዉስጥ የሚገኝዉን የመድሃኒት መጠን ለመለካት ወደሚያስችሉ ለቦራቶሪዎች ይላካል። የርስዎ የደም ናሙና ያለበት ብልቀጥ የኮድ ቁጥር ብቻ የሚጸፍበት ሲሆን ስምዎ በፍጹም በብልቀጡ ላይ አይጸፍም።
- ✓ ምናልባት ላሌሎች ጠቀሚ ምርማራዎች 9-12 ሲሲ መጠን ያለዉ ደም ከርስዎ ልወሰድ ይችላል። ይህም፡-

-የጉበትና የኩላልትዎን የጤና ሁኔታ

- የደም መርጋት ሁኔታዎችን

- ጠቅላላ የደም መጠንዎንና ሌሎች የጤና ችግሮች ለመመርመር ያስችላል።

እናዚህ ምርመራዎች በለፉት 30 ቀናት ዉስጥ የተደረጉና ዉጤቱን ማግኘት የሚቻል ከሆነ ተጨማሪ የደም ናሙና አያስፈልግም ወይም አይወሰድም።

- ✓ ሌላ 3-4 ሲሲ ያህል የደም ናሙና ለሄፓታይቲስ ብ እና ሲ ምርማራ ልፈለግ ይችላል። ለዚህም በለፉት 12 ወራት ዉስጥ ምርመራዉ የተደረገና ዉጤቱን ማግኘት የሚቻል ከሆነ፤ ይህም ተጨማሪ የደም ናሙና አያስፈልግም።
- ✓ ኤች አይ ቭ በደምዎ ዉስጥ ያለ ታካሚ ከሆኑ 11 ሲሲ ተጨማሪ የደም ናሙና የሲዲ ፎር መጠንና (CD4 count) አጠቃላይ የቨይረሱን ቁጥር (Viral Load) ለመለካት ያስፈልጋል። ነገር ግን በለፉት 30 ቀናት ይህንን ምርመራ ያካሄዱና ዉጤቱም የሚገኝ ከሆነ ተጨማሪ የደም ናሙና አይወሰድም፡

በጥናቱ ወቅት ምን ችግር ልያገጥም ይችላል?

የደም ናሙና ከመዉሰድ ጋር ተያይዞ መርፌ በደም ስርዎ በምገባበት ግዜ ጥቅት ህማምና መድማት ልከሰት ይችላል። ሌሎች እንደእንፌክሽን ያሉ ችግሮች የመከሰት እድል አነስተኛ ነዉ።

በጥናቱ ላይ በመሳተፌ ምን ጥቅማጥቅም አገኛለሁ?

በጥናቱ ላይ በመሳተፍዎ የተለየ ቀጥተኛ ጥቅማጥቅም የለም። ነገር ግን እርስዎ በጥናቱ ላይ በመሳተፍዎ ወደፊት የቲብ ህክምና እንድሻሻል አስተዋጽኦ ያደርጋል።

በዚህ ምርምር በመሰተፍዎ የሚከፈልዎት ምንም ዓይነት ክፍያ የላም።ነገር ግን የደም ናሙና በሚወሰድበት ዕለት በዓይነቱ ለየት ያለ የምግብና ፈሳሽ አቅርቦትይኖራል።

የግል ምስጥርዎን በተመለከተ

ማንኛዉም እርስዎን የሚመለከት መረጃ በምስጥር ይያዛል። የምርምሩ ዉጤት በሳይንሳዊ ጆርናሎች ላይ ስታተም የርስዎ ስም አይገለጽም። ማንኛዉም እርስዎን የሚመለከቱ የግልና የህክምና መረጃዎች ምስጥራዊነት በጥብቅ የተጠበቀ ነዉ።

በጥናቱ ግዜ እርስዎ ላይ ጉዳት ቢያገጥምስ?

በዚህ ጥናት በመሳተፍዎ ጉዳት በጣም አነስተኛ ሲሆን፣ ማንኛዉም ጉዳት ቢደርስብዎ ግን በነጻ ይታከማሉ። ነገር ግን በዚህ ጥናት ላይ ለመሳተፍ በመወሰንዎና በመፈረምዎ ማንኛዉም ህጋዊ መብትዎ አይከለከልም ወይም አይገደብም።ምናልባት በጥናቱ ምክንያት ጉዳት እንደደረሰብዎ ቢሰማዎት ለተመስገን ሲዳሞ (አድስ አባባ ዩንቨርስቲ) በ0911093567 በመደወል ስለ ህጋዊ መብትዎ ማዎቅ ወይም ማማከር ይችላሉ።

በጥናቱ ጨርሶ የአለመሳተፍ ወይም የማቆዋረጥ መብትዎ

በዝህ ጥናት መሳተፍ ወይም አለመሳተፍ የርስዎ የግል ምርጫ ነዉ።መሳተፍ ከልፈለጉ አይገደዱም፤ ለመሳተፍ ከወሰኑም በኖዋላ ሃሳብዎን ቢቀይሩ በማንኛዉም ግዜ ማቆዋረጥ ይችላሉ።

Appendix IV: Subject Consent

A. In English

I_____ have read or well informed about the research being conducted on MDR TB patients and its general aim as it is given on the patient information sheet (Appendix A and B). Now I have understood that this research will help to learn how drug resistance in TB develops and how well the amount of Moxifloxacin in blood works against TB. Therefore, I give my consent by signature here-below that I am willingly participating in this research.

Name: _____Signature: _____

B. In Amharic

<u>መድሃኒት በተላመደዉ ቲብ (MDR TB) ምርምር ለሚሳተፉ ታካሚዎች ፈቃደኝነት መጠያቂያ ቅጽ</u>

እኔ አቶ/ወ/ሮ/ወ/ሪት፡ ----------

መድኃኒት በተላመደዉ ቲቢ ላይ ስለምደረገዉ ምርምር በቂ ግንዛቤ አግኝቻለሁ። ይህ ምርምር ለቲቢ በሽታ መነሻ የሆነዉ ባክቴሪያ እንዴት መድሃኒትን እንደሚላመድ፤ በክቴሪያዉ ለመድሃኒቶቹ ያላዉን ምላሽ እንድሁም በደም ዉስጥ የሚገኘዉ የመድሃኒት መጠን ለህክምናዉ ፊቱንናት ምን ያህል አስተዋጽኦ እንዳለዉ ለማወቅ የታለመ ምርምር እንደሆነ ተረድቻለሁ። ስላዝህ በዚህ ምርምር ለመሳተፍ ፋቃደኝናቴን ከታች በፊርማዬ አረጋግጣለሁ።

ስም፡ ----------ፊርማ፡----------

Appendix V: Data Abstraction Form (Study I)

Please fill the required items, numbers, or mark (√) in each blank space where it applies

Site (Hospital): _____

Name: _____

Hospital ID: _____

Contact No: _____

Age: _____

Sex: _____

Weight: _____

Diagnosis:

GeneXpert result: Negative Positive

Chest X-ray: Presence cavitary lesion No Yes

Baseline AST_____ ALT_____ Cr. _____

Was there any comorbidity:

Specify_____

Prior TB drugs:

New_____; Treated with FLDs_____; Treated with SLDs_____

MDR-TB Regimen type (mark √ in the spaces):

FQ administered (specify the dose): Moxifloxacin _____Levofloxacin _____

List other companion drugs other than FQs

TB-drug name	Dose	Route	Frequency (QD or BID)
_____	_____	_____	_____
_____	_____	_____	_____

_____ _____ _____ _____

_____ _____ _____ _____

_____ _____ _____ _____

_____ _____ _____ _____

_____ _____ _____ _____

Treatment Evaluation (mark √ in the spaces)

Cured_____; Completed_____; Dead_____; Failed/Pre-XDR TB; _____LTFU; _____

Date of Evaluation: _____

LPA result (Indicate as RR, resistant to both FQs & IAs, RS, resistant to only FQs, SS, susceptible for both drugs)

At base line _____At intensive phase: ___ At continuation phase_____

Did adverse event occur during the treatment? No Yes

Specify the type of the ADR: _____; _____; _____;

_____

If the answer is "Yes" for the above question, was the initial treatment regimen or dose changed due to the ADR occurred? No Yes

ADDIS ABABA UNIVERSITY, COLLEGE OF HEALTH SCIENCES (IRB)
አዲስ አበባ ዩኒቨርስቲ፡ ጤና ሳይንስ ኮሌጅ
Institutional Review Board

ANNEX 3
Form AAUMF 03-008

IRB's Decision

Meeting No: 04/2023
Date: April 19, 2023
Protocol number: 078/17/SoP

Protocol Title: Genetic analysis of resistance and PK/PD model-based study of moxifloxacin and levofloxacin in MDR-TB patients attending hospitals in Southern Ethiopia		
Principal Investigator:	Temesgen Sidamo Summoro	
Institute:	College of Health Sciences, AAU	
Elements Reviewed (AAUMF 01-008)	☑ Attached ☐ Not Attached	
Review of Revised Application ☐ Yes ☐ No	Date of the Previous review:	
Decision of the meeting:	☑Approved ☐Approved with Recommendation ☐Resubmission ☐ Disapproved	

I. Elements approved-
1. Protocol Version No: 05
2. Protocol Version Date:
3. Informed consent Version No.
4. Informed Consent Version Date: 05

II. Obligations of the PI-
1. Should comply with the standard international & national scientific and ethical guidelines
2. All amendments and changes made in protocol and consent form need IRB approval
3. The PI should report SAE within 10 days of the event
4. End of the study, including manuscripts and thesis works should be reported to the IRB
5. The PI should report non-compliance and unanticipated events

III. TO NERC ☑

Institution Review Board (IRB) Approval: Period from August 02, 2023, to August 01, 2024
Follow-up report expected in 3 Months ___ 6 months ___✔___ 9 months____ one year____

Chairperson, IRB
Dr. Adamu Addissie
Signature
Date: August 2, 2023

ADDIS ABABA UNIVERSITY Institutional Review Board Office (IRB) Faculty of Medicine

Director of Research & Technology Transfer, CHS
Professor Senbeta Guteta
Signature:
Date August 2/2023

Addis Ababa University College of Health Sciences Directorate of Research and Technology Transfer