






STUDY PROTOCOL

REVISED

Therapeutic drug monitoring for antimicrobial agents for people living with HIV (TAP)

[version 2; peer review: 2 approved, 2 approved with reservations]

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Abstract

Background

Antimicrobial resistance (AMR) is a growing health concern, particularly in Africa, and is predicted to become the leading cause of death after cancer by 2050. Factors like overuse or inappropriate use of antibiotics contribute to this crisis. People living with HIV (PLWH) are particularly vulnerable to AMR with potential drug-drug interactions between antiretroviral and antimicrobial agents against common organisms like *Mycobacterium tuberculosis*. There is limited data on the concentrations of commonly used antimicrobial agents in people living with HIV in resource-limited settings. Therapeutic Drug Monitoring (TDM) offers a promising approach to optimize antibiotic dosing and improve treatment outcomes for those with sub-optimal drug concentrations. TDM has been recommended for PLWH on anti-tuberculosis treatment due to sub-optimal drug concentrations found in a significant proportion of those with TB.



Objectives

The main objectives of this study are to determine the concentrations of selected antimicrobial agents in people living with HIV requiring antimicrobial therapy and to assess the utility of therapeutic drug monitoring in achieving therapeutic targets for PLWH receiving rifampicin and isoniazid for the treatment of tuberculosis.

Open Peer Review

Approval Status ✓ ? ✓ ?

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Any reports and responses or comments on the article can be found at the end of the article.

Methods

This prospective observational study will enroll adult PLWH receiving amoxicillin, azithromycin, ciprofloxacin, rifampicin, isoniazid, or ceftriaxone. Concentrations of these antibiotics will be measured locally using validated liquid chromatography mass spectrometry methods and high-performance liquid chromatography with ultraviolet detection. TDM with dose adjustment will be performed in a subset of participants on TB treatment. Pharmacokinetic parameters will be estimated using non-linear mixed effects models.

Results

This study was reviewed and approved by the research and ethics committee in February 2024. Participant enrolment began in September 2024.

Conclusions

We anticipate that the findings from this research will characterize pharmacokinetic and pharmacodynamics relationships to predict treatment response for optimal antimicrobial therapeutic and anti-tuberculosis dosing among people living with HIV (PLWH).

Clinical registration

The study is registered with Pan African Clinical Trials Registry, registration number PACTR202409710100607, registration date 07 August 2024, pactr.samrc.ac.za/TrialDisplay.aspx?TrialID=31764

Plain Language Summary

Antimicrobial resistance is on the increase and people living with HIV may be at increased risk for this, including those living with HIV due to interactions between antiretroviral and antibiotics. There is scarce data about the concentrations of the commonly used antibiotics in the blood of people living with HIV. Monitoring the drug concentrations and adjusting the dose when needed has been recommended in people living with HIV who develop TB. The main objectives of this study are to determine the concentrations of selected antibiotics in people living with HIV and to assess how useful it is to monitor the amount of the antibiotics in blood while adjusting the dose. This study will enroll adults living with HIV receiving amoxicillin, azithromycin, ciprofloxacin, rifampicin, isoniazid, or ceftriaxone. Concentrations of these antibiotics will be measured, and for those on TB drugs, doses will be adjusted until the participant achieves suitable amounts of the drug in blood. This study was reviewed and approved by the research and ethics committee and we anticipate that the findings from this research will help us to know how much of the antibiotics is in the body and if people respond as expected to the treatment.

Keywords

Therapeutic Drug Monitoring, Pharmacokinetics, Pharmacodynamics, dose adjustment, Antimicrobial Agents, HIV, Tuberculosis

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REVISED Amendments from Version 1

Abstract: The background section has been revised to reflect changes to the main text's background section. These changes include recent evidence on the effect of corticosteroids in community acquired pneumonia that informs the justification for the trial. The methodology section now refers to trial arms as 'control' and 'intervention' arm rather than the previous 'stratum A' and 'stratum B' and does not mention the immunology sub-study. The trial's registration numbers for the Pan-African Clinical Trials Registry and ISRCTN have been added.

Main text: The background section has been shortened and updated with current information on the effect of corticosteroids on mortality in community acquired pneumonia, including the two suggested references by the reviewers, and recent references on the role of corticosteroids in management of severe pneumonia. Other minor changes include; refining the exclusion criteria, changing 'Arm A' and 'Arm B' across the text to 'control arm' and 'intervention arm', replacing 'steroids' with 'corticosteroids' and expanding on the analysis methods for the immunology sub-study.

Study status: Updated to reflect the trial's current status

Any further responses from the reviewers can be found at the end of the article

Introduction

Antimicrobial resistance (AMR) is on the rise and is predicted to become the leading cause of after cancer by 2050, with Africa bearing a significant proportion of this burden¹. In 2019, an estimated 1.27 million deaths were attributed to antimicrobial-resistant infections, and most of these infections were bacterial¹.

Optimal dosing of antimicrobial agents is essential to achieve therapeutic concentration, maximize efficacy, and minimize both resistance and toxicity. Conversely, suboptimal drug levels may cause treatment failure, AMR, and mortality, while excessive exposures may increase toxicity and affect adherence². In sub-Saharan Africa, overlapping diseases lead to multi-morbidity with altered physiologic states, like those seen in infections or inflammation, and obesity³. These require co-treatment with multiple regimens which may result in drug-drug interactions (DDI) that are often inadequately characterized. The SOUTH study found sub-therapeutic levels of rifampicin and isoniazid amongst TB-HIV coinfecting patients, which were associated with delayed sputum conversion⁴ and, as seen in other studies, TB relapse⁵. Such findings have prompted trials of higher anti-TB doses to improve outcomes and prevent resistance⁶⁻⁸. Similarly, suboptimal antibiotic exposure has been associated with poor treatment outcomes⁹ and development of resistance¹⁰.

People living with HIV (PLWH) are at an increased risk of AMR across a range of pathogens and drug classes¹¹. Antibiotics are frequently prescribed in PLWH to manage co-morbidities like other sexually transmitted- and urinary tract infections as well as respiratory tract infection. Prescription rates of these vary widely, ranging from about 40% in Nigeria to as high as 85% on Tanzania¹². While this data was not available for Uganda, a recent general survey showed widespread antibiotic use across hospitals¹³. Outcome data

for common infections indicate an 80% treatment success rate for pneumonia, a major cause of mortality amongst especially children in Uganda. However, resistance to commonly used antibiotics is on the rise in Uganda, with reported rates exceeding 70% for ceftriaxone and ampicillin, and about 43% for ciprofloxacin¹⁴. While it remains unclear to what extent suboptimal drug exposures directly contribute to these resistance patterns, previous studies have reported inadequate antibiotic concentrations to drive development of AMR¹⁰. Other drivers of AMR include genetic mechanisms which also play a significant role in the emergence and spread of resistance to antimicrobials¹⁵.

Despite the potential contribution of inadequate drug exposure to AMR, the pharmacokinetic (PK) profiles of commonly prescribed antibiotics in PLWH are not well characterized. Ceftriaxone and amoxicillin may not exhibit significant DDI with ART but some ARVs like ritonavir-boosted protease inhibitors can inhibit transporters like p-glycoprotein and multi-drug resistance protein 2, potentially increasing azithromycin exposure and raising the risk of arrhythmias¹⁶. However, no clinical PK studies have been conducted to investigate these potential interactions, and dose adjustments are not currently recommended.

Therapeutic drug monitoring (TDM) uses plasma drug concentrations to assess whether a given dose achieves optimal drug exposure¹⁷. It helps evaluate attainment of PK targets for the antibiotics and provides objective data to guide dosing decisions. These PK targets are often based on drug exposure parameters like the area under the concentration-time curve (AUC) or peak/trough concentrations relative to the minimum inhibitory concentration (MIC) of the pathogen. For example, azithromycin and ciprofloxacin require AUC/MIC ratios of ≥ 5 ¹⁸ and ≥ 125 ¹⁹, respectively, while ceftriaxone has a minimum threshold of 8 mg/L²⁰. Amoxicillin efficacy depends on maintaining concentrations above the MIC for at least 40% of the dosing interval²¹. However, MIC testing is rarely done in resource-limited settings due to cost constraints. For important ant-TB drugs like isoniazid and rifampicin, peak plasma concentration of 3 to 6 mg/L and > 8 mg/L are desired, respectively¹⁷.

In resource-limited settings, where antibiotic options are often scarce, optimizing existing treatments is essential to maintain accessibility and prevent AMR. While strategies like regimen intensification, individualized drug combinations, and precision dosing can help prevent AMR, there is limited understanding of which model will be most effective for widespread implementation in low- and middle-income countries (LMICs). TDM has been successfully used for aminoglycosides and anti-TB drugs in special populations, such as PLWH and those with diabetes¹⁷. However, there is limited data on its role in optimizing antibiotic therapy for PLWH in LMICs.

Protocol

Study objectives

1. To determine the concentrations of selected antimicrobial agents in people living with HIV requiring antimicrobial therapy.

- To assess the utility of therapeutic drug monitoring in achieving therapeutic targets for people living with HIV receiving rifampicin and isoniazid for treatment of tuberculosis.
- To develop and validate a population pharmacokinetic-pharmacodynamic model for use during therapeutic drug monitoring for selected antibiotics used among PLWH

Study outcomes

On antibiotics

Primary endpoints

Pharmacokinetic parameters

- Trough concentrations (C_{trough}) for amoxicillin, ciprofloxacin, azithromycin and ceftriaxone

Pharmacodynamic parameters

Clinical response to antibiotics through the time of treatment will be assessed. Clinical response is either clinical cure (resolution of symptoms) or clinical failure (lack of improvement in signs and symptoms of infection OR recurrence of signs/symptoms of infection after initial improvement).

On TB treatment

Pharmacokinetic parameters

- Maximum concentrations (C_{max}) and area under the concentration-time curve (AUC) defined as the total exposure for rifampicin, isoniazid

Pharmacodynamic parameters

- Clinical response will be assessed according to the World Health Organization definitions of cure, completed and treatment failure, which will be extracted from the clinic database.
- Occurrence of adverse drug reactions

Methods

Study site

The study will be conducted at the Infectious Diseases Institute (IDI) which is an out-patient clinic in Kampala, Uganda, where around 8000 PLWH are currently being provided with care, of whom 250 are initiated on TB treatment annually. Participants who are on intravenous medication will be enrolled at Mulago National Referral Hospital which handles both medical and surgical in-patients.

Study participants

Participants will be included if they fulfil the following criteria:

Inclusion criteria

- A personally signed and dated informed consent document
- An adult of 18 years and above living with HIV

- Patients being initiated on or currently receiving antibiotics which may include: amoxicillin (+/- clavulanic acid), azithromycin, ciprofloxacin, rifampicin, isoniazid, ceftriaxone
- Participants who are willing and able to comply with scheduled visits, treatment plan, laboratory tests, and other study procedures.

Exclusion criteria

- Patients who discontinue antibiotics for any reason following initiation
- Patients receiving antibiotics only for prophylaxis

Sample size

We conducted a priori stochastic simulation and estimation to determine the appropriate sample size for our study. Assuming a 30% coefficient of variation (%CV) in drug exposure, we estimated that enrolling 40 participants per antibiotic would provide sufficient power to detect a 30% effect of HIV co-treatment on antibiotic exposure. In alignment with the ICH E9 guidelines on statistical principles for clinical trials and considering an anticipated 10–15% attrition rate due to study withdrawal and loss to follow-up, we increased the sample size by 10 participants per antibiotic. This adjustment results in a total of 50 participants per antibiotic, or 200 participants overall.

For intravenous antibiotics (ceftriaxone) and anti-TB drugs (rifampicin and isoniazid), we assumed a lower %CV of 25% in drug exposure. Based on this assumption and accounting for an anticipated 15% withdrawal rate, we estimate that a sample size of 25 participants will be sufficient to maintain the power needed to detect possible significant changes in drug exposure due to concomitant antiretroviral therapy.

Therefore, the total sample size for this study is 200 participants.

Study design

Participants on antimicrobial drugs

This will be a prospective observational study that will include adult patients with suspected or confirmed bacterial infection receiving amoxicillin (+/- clavulanic acid), azithromycin, ciprofloxacin. The decision to start antibiotics will be left to the managing clinician (based on clinical symptoms, full blood count, urinalysis, depending on the suspected focus) and enrolment will take place when antibiotics are initiated. We will enroll 50 participants on each of the oral antimicrobials (amoxicillin, azithromycin, ciprofloxacin). Patients being initiated on antimicrobial agents will be randomly selected, screened, and enrolled. Prescription of antibiotics will be determined by the managing clinician according to standard clinic guidelines. Participants will be requested to take the antibiotic therapy at the same time daily. At baseline, data will be collected on age, sex, gastrointestinal disorders (e.g., diarrhea and vomiting), weight, height, alcohol use, illicit drug use, and

details of concomitant medications, including antiretroviral regimens. A blood sample will be drawn for measurement of serum creatinine at baseline. [Table 1](#) demonstrates all study procedures.

Participant follow-up

Study visits will be conducted at enrolment, on day 2 for measurement of drug concentrations and the last day of antibiotic use to determine treatment outcome (after 5 – 7 days).

Sparse pharmacokinetic sampling for antimicrobial drugs

Sampling for measurement of antibiotic concentrations will be performed on day 2 of antimicrobial therapy. Directly observed therapy will be performed on the day of the blood draw. For those on oral azithromycin and ciprofloxacin, a pre-dose sample (trough concentrations) will be drawn, while for those on amoxicillin, a pre-dose and 6-hour blood draw will be taken. ([Table 2](#))

Intense pharmacokinetic sampling for antimicrobial drugs

Intensive pharmacokinetic sampling will be conducted after at least 2 days of oral antibiotic use, for 20 randomly selected participants on each of these oral drugs; amoxicillin, ciprofloxacin, and azithromycin. At least 20 participants were chosen based on the target of having less than 20% relative standard error in the estimated coefficient of variation. For amoxicillin, blood will be drawn pre-dose, 0.5 h, 1 h, 1.5 h, 2h, 2.5 h, 3 h, 4h, 6h, and 8 h post-dose. For azithromycin and ciprofloxacin, blood will be pre-dose, and at 1 h, 2 h, 3h, 4h, 5h, 6h, 8h, 12h, and 24h post-dose dosing.

Patients on intravenous antibiotics (Ceftriaxone)

Patients who are acutely ill and admitted in the in-patient wards of Mulago hospital will be enrolled for pharmacokinetic sampling while on ceftriaxone. Blood draws will occur when a patient has received at least 1 dose. A blood draw will be taken prior to dosing (C_{trough}) and again, 2 hours after completion of bolus administration for ceftriaxone. Participants will be followed up to determine treatment outcome at the end of treatment or at discharge.

Participants on anti-tuberculosis treatment

The current tuberculosis (TB) treatment involves fixed dose combinations based on weight bands, including an intensive phase of rifampicin, isoniazid, ethambutol, and pyrazinamide (RHEZ) for 2 months, followed by a continuation phase of isoniazid and rifampicin (HR) for 4 months. Dosing is determined by weight bands, with 3, 4, or 5 tablets of RHZE or HR depending on weight (<55, 55–69, ≥70 respectively). Each tablet contains 150 mg of rifampicin and 75 mg of isoniazid for both combinations. Fixed dose combinations in the intensive phase include concomitant pyrazinamide and ethambutol.

Serum rifampicin and isoniazid concentrations (C_{2hr}, C_{4hr}, C_{6hr}) will be measured in participants when fasting, and following directly observed therapy, after two weeks of treatment (First TDM). Results of blood concentrations will be received within 24–48 hours and assessed to determine if the C_{max} and AUC is within the therapeutic targets. Sub-therapeutic C_{max} concentrations are considered less than 8mg/L and

Table 1. Study procedures.

Protocol Activity	Screen and enrolment	Day 2 +/- 3days	End antibiotic Treatment	Week 2	Week 4	Week 6	Week 8
Informed Consent	x						
Clinical evaluation	x	x	x	x	x	x	X
Laboratory							
Serum creatinine	x						
ALT, total Bilirubin****				x**			
PK blood draw*		x		x**	x**		
Sputum cultures		x		x**	x**	x**	x**

** for participants on TB treatment where applicable

Table 2. TDM for antimicrobials.

Drug	Administration	Time of blood draw
Amoxicillin (+/- clavulanic acid)	Oral	Pre-dose, 6 hours post dose
Azithromycin	Oral	Pre-dose (trough),
Ciprofloxacin	Oral	Pre-dose (trough),

3mg/L for rifampicin and isoniazid, respectively. AUCs for rifampicin and Isoniazid will also be considered and compared with existing published therapeutic ranges²². Participants found to have subtherapeutic drug exposures will have dose escalation and be asked to take one additional pill of the drug that did not achieve therapeutic target concentrations (rifampicin, isoniazid or both) (Table 3). A second blood draw for measurement of drug concentrations will be performed after another two weeks (Second TDM) and dose adjustment performed again based on the guidance in Table 3, if concentrations are still below the target. (Figure 1)

If the participant is still not achieving the therapeutic target concentrations after the second C_{max} , another 1hr and 6hr blood draw will be conducted to assess for the presence of fast or delayed absorption. An additional dose adjustment with 1 additional tablet of the respective drug may be prescribed for these patients at the discretion of the study team and with consideration of observed side effects, treatment response, and concentrations attained. Those who attain therapeutic drug

concentrations during TDM will revert to the previous dose. The final doses provided will be recorded for all participants.

Sputum analysis

For participants with tuberculosis, sputum cultures will be conducted at the Makerere University Microbacteriology laboratory. Cultures using Mycobacterium Growth Indicator tube (MGIT) and Lowenstein-Jensen (LJ) agar will be conducted within 7 days of enrolment and will be conducted every two weeks for the first eight weeks of TB treatment (intensive phase).

Laboratory measurement of drug concentrations and sample handling

After blood sample collection, blood samples will immediately be placed in a dark cooler box and transported to the laboratory within 30 minutes of collection, after which samples will be centrifuged, aliquoted, batched, and frozen at -80°C until quantification of drug concentrations is done. Quantification of the drug concentrations will be performed

Table 3. Dose adjustment table for isoniazid and rifampicin in patients with HIV.

Weight band	Normal drug levels	Sub-target INH <3mg/dl Normal RIF	Normal INH Sub-target RIF <8mg/dl	Sub-target INH <3mg/dl and Sub-target RIF <8mg/dl
<55kg	Continue INH 225 mg and RIF 450 mg (3 FDCs)	Give an additional INH tablet equivalent to: INH 300 mg	Give an additional rifampicin tablet equivalent to 600 mg dose	Give additional rifampicin and isoniazid tablets or FDCs equivalent to 300 mg INH and 600 mg rifampicin
55–69kg	Continue INH 300 mg and RIF 600 mg (4 FDCs)	Give an additional INH tablet equivalent to INH 375 mg dose	Give an equivalent rifampicin tablet equivalent to 750 mg dose	Give additional rifampicin and isoniazid tablets or FDCs equivalent to 375 mg INH and 750 mg rifampicin
$\geq 70\text{kg}$	Continue INH 375 mg and RIF 750 mg (5 FDCs)	Give an additional INH tablet equivalent to 450 mg dose	Give an additional rifampicin tablet equivalent to 900 mg dose	Give additional rifampicin and isoniazid tablets or FDCs equivalent to 450 mg INH and 900 mg rifampicin

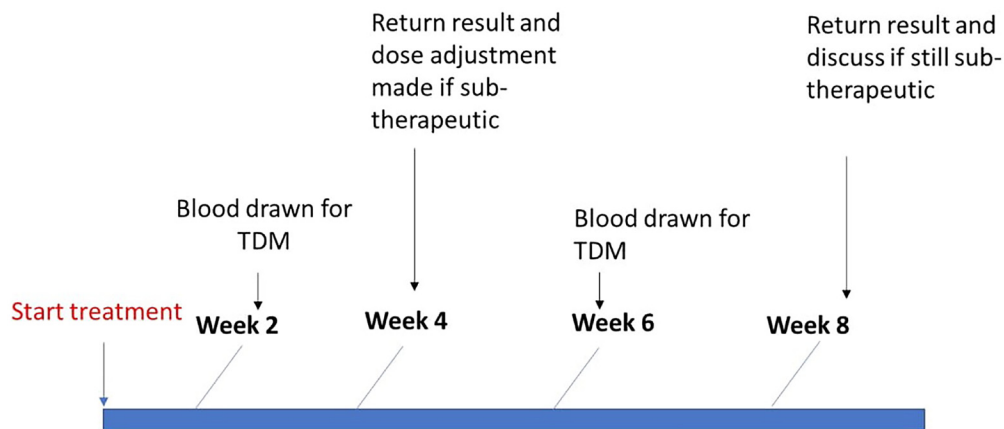


Figure 1. Therapeutic drug monitoring procedure with dose adjustment for participants on TB drugs.

using pre-established validated liquid chromatography mass spectrometry (LC-MS) methods and high-performance liquid chromatography with ultraviolet detection (HPLC-UV).

Safety assessment

A creatinine level will be measured in patients who undergo intensive pharmacokinetic blood draws, acutely ill in-patients and out-patients without a recent measurement. All other safety testing will be performed according to standard of care. Participants who are found to be pregnant during follow-up will not be excluded, however, pharmacokinetic data will be censored at that point.

Adverse event (AE) assessment and reporting will be conducted for participants undergoing dose adjustments for tuberculosis treatment. All observed or volunteered AEs, regardless of suspected causal relationship, will be reported. Adequate information will be obtained to evaluate the causality and severity of the AE using the Naranjo score and Division of AIDS (DAIDS) table respectively. Following adverse events, follow-up will be conducted until the event or its sequelae resolve or stabilize. Participants who suffer harm will be managed by the study which has provided trial insurance for those receiving dose adjustment.

Statistical analysis

Analysis of data on antimicrobial drugs: sparse pharmacokinetic data

Sparse pharmacokinetic data for antimicrobials will be analysed as medians with standard deviations. We assess for the effect of covariates like age, sex, body-mass index, CD4 cell counts on drug concentrations

Analysis of data on antimicrobial drugs: intensive pharmacokinetic data

Non-linear mixed-effects models will be used to calculate the pharmacokinetic parameters of the drugs and this will include the maximum plasma concentration (C_{max}), time to maximum plasma concentration (T_{max}), and area under the plasma concentration-time curve (AUC). We will also explore the effects of covariates like age, sex, concomitant medications, and body-mass index on the pharmacokinetic parameters.

Pharmacodynamic parameters

We will use pharmacokinetic and pharmacodynamic models to assess the effect of the pharmacokinetic parameters on clinical response. For sparse pharmacokinetic data, we will use drug concentrations as a covariate in the linear regression models predicting clinical response. Clinical response will be defined as either clinical cure (resolution of symptoms) or clinical failure (lack of improvement in signs and symptoms of infection or recurrence of signs/symptoms of infection after initial improvement or death considered at least possibly due to the infection).

Analysis of data on anti-tuberculosis drugs

For participants on anti-tuberculosis drugs, we will evaluate whether dose adjustment leads to the achievement of therapeutic targets.

As an exploratory objective, using Kaplan Meier survival analysis, time to sputum culture negativity will be compared for those who received dose adjustment and those who did not receive dose adjustment within this study. We will also utilize the SOUTH dataset where drug concentrations of rifampicin and isoniazid were measured (without dose adjustment), where 78% and 84% of participants had rifampicin and isoniazid concentrations below the therapeutic targets respectively.

Data Monitoring Committee

A Data Monitoring Board (DMB) will be established to evaluate data for participants undergoing TDM with dose adjustment. The board, consisting of an independent chair and members with expertise in clinical trials, will be responsible for safeguarding participant safety and ensuring study integrity. Periodic data reviews will be conducted by the DMB to monitor toxicities, and any concerns identified will be communicated to the study team. The following events, among others, may prompt a request to analyze available safety data for participants receiving dose adjustment and to generate a recommendation on termination of TDM; 1) three participants receiving dose adjustment experience a grade 4 or 5 adverse event in the same system organ class or considered similar (excluding non-clinically significant laboratory abnormalities) that is assessed as probably related or as related to the experimental treatment and 2) one subject receiving dose adjustment experiences a Grade 5 adverse event that is assessed as related to the experimental treatment.

Dissemination of results

These results will be presented to both local and international stakeholders through meetings and international conferences. The raw dataset can be made available to other researchers upon request and signing data sharing agreements, after final results have been published.

Confidentiality

Clinical data will be entered into the study specific dataset by designated staff on a regular basis. Case Record Forms and other source documents will be kept in locked cabinets. No participant identifying information will be disclosed in any publication or at any conference activities arising from the study.

Discussion

Obtaining optimal drug concentrations is vital in treatment success whereas sub-optimal drug concentrations can lead to consequences such as AMR, treatment failure, and adverse outcomes.

This study will increase our understanding of the concentrations of commonly used antibiotics including amoxicillin, azithromycin, ciprofloxacin, ceftriaxone in PLWH, who are at high risk of recurrent infections such as urinary and respiratory tract infections. Understanding the pharmacokinetics and pharmacodynamics of these drugs will increase our understanding of the rising burden of AMR in PLWH, and possibly pave way for interventions that directly impact drug administration.

Therapeutic drug monitoring is increasingly being used for antibiotic dose optimization in the attempt to improve the attainment of pharmacokinetic and pharmacodynamics targets and outcomes of severe infections including in critically ill patients. Although there is a great need of TDM for monitoring selected antibacterial use, there is limited data on its applicability in ensuring appropriate pharmacokinetic and pharmacodynamics outcomes in resource-limited settings. Data from this study will contribute to informing clinicians and researchers about the practicability of using TDM in real-life settings for monitoring the achievement of target concentrations, and the utility of dose adjustment following TDM in different sub-groups of patients such as those with TB and patients with HIV on ART.

This study has some limitations. We cannot ascertain adherence to all treatment provided since directly observed therapy is not conducted throughout treatment, however, we will conduct directly observed therapy on the days when blood samples are drawn for measurement of drug concentrations. In addition, there are several factors that affect pharmacokinetics that we may not be able to assess during this study, for example pharmacokinetics during pregnancy, drug interactions with unknown medications provided over the counter, and comorbidities that occur less frequently and may lead to physiological changes.

In conclusion, the findings from this study will provide information on drug concentrations of frequently used antimicrobial agents in PLWH and guide clinical practices for optimizing doses of rifampicin and isoniazid in order to work towards appropriate dosing and improved treatment outcomes among PLWH.

Ethics and consent

This study has been approved by the Infectious Diseases Institute Research and Ethics Committee (IDI REC) (IDI-REC-2023-83) on 26th February, 2024 and approvals for these changes will be sought prior to implementation. Written informed consent will be obtained by the study team before any study-related activities are conducted. The study will adhere to the Declaration of Helsinki. This publication adhered to the SPIRIT guidelines for reporting study protocols.

Data availability

Underlying data

No data are associated with this article.

Extended data

The extended data associated with this study are available in the Open Science Framework (OSF) titled “Therapeutic drug monitoring for antimicrobial agents for people living with HIV (TAP)”, <https://doi.org/10.17605/OSF.IO/3QE4M>¹.

This project contains the following extended data:

- Participant information sheet and consent form.
- SPIRIT checklist.

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC0 1.0 Public domain dedication).

Sponsor

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Author contributions

CSW contributed to the conceptualization of this study, CSW, ANK, FWO, EALO, AB, NO and CW contributed to the development of the methodology this study, IK, HM, FK, AK contributed toward project administration and coordination, AK, FK, CSW contributed towards the acquisition of financial resources leading to this project, CSW, IK, RK, wrote the original draft, AK and all authors contributed towards the review and editing of this manuscript.

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Version 2

Reviewer Report 30 May 2025

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? **Collins Iwuji** 

University of Sussex, Brighton, England, UK

The authors plan to investigate the concentrations of selected antimicrobial agents in people living with HIV requiring antimicrobial therapy and assessed the utility of therapeutic drug monitoring in achieving therapeutic targets for PLWH receiving **rifampicin** and **isoniazid** for the treatment of tuberculosis. This is an important study that will increase our understanding of the pharmacokinetics of commonly prescribed antimicrobial agents in people living with HIV.

My comments are summarised below

1. The title of the study should indicate it is a study protocol
2. The word 'death' is missing from the sentence - leading cause of death after cancer - first paragraph of the introduction
3. Sample size. The last sentence in the first paragraph should read " This adjustment results in a total of 50 participants per antibiotic, or 150 participants overall. This ties in better with the last sentence in the second paragraph which states "Therefore, the total sample size for this study is 200 participants (taking into account 25 patients on Ceftriaxone and another 25 on Rifampicin/Isoniazid
4. How will clinical cure be ascertained in those with culture positive TB who have no symptoms? The investigators should consider using outcomes such as culture conversion and microbiological cure, Treatment completed could be used in those without culture results
5. Will individuals on combinations of antimicrobial agents being investigated be eligible for inclusion?
6. What is the underlying study hypothesis? Are suboptimal drug levels driven by drug-drug interactions used to treat HIV or by HIV itself? Would the participants be restricted to those on specific ARV regimens or will all regimen types be allowed? An HIV negative control would make the study design much stronger
7. Perhaps including people with HIV with no chronic comorbidities and ongoing chronic medications might make it easier to explain study findings and potential attributions
8. The researchers should consider making the raw dataset available in an easily accessible repository following indexing of study results

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: HIV treatment strategies, drug resistance, sexual and reproductive health, climate and health

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 26 May 2025

<https://doi.org/10.21956/wellcomeopenres.26588.r122582>

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Jessica Cusato 

University of Turin, Turin, Italy

The study aims to generate data to support personalized antimicrobial and anti-TB dosing in PLWH.

In fact, it is a prospective study addressing antimicrobial resistance in PLWH, focusing on antibiotic exposure and drug-drug interactions, particularly during TB treatment. The authors measured plasma concentrations of selected antibiotics (e.g., amoxicillin, rifampicin, isoniazid) using validated chromatographic methods and apply TDM with dose adjustments in a TB-treated subgroup.

To the Authors

1. The article deals with the TDM of antimycobacterials in PWH with tuberculosis. I suggest

revising the title accordingly (it should be clearly stated the study will focus only on antimicrobial agents used for the treatment of TB).

2. Table 2 is not acceptable in the present form. Please add information on the time of blood draw for all the 6 antibiotics that will be subjected to TDM

3. Table 3 provides dose adjustment advice for isoniazid and rifampicin based on TDM results for PLWH with lower drug exposure. What about dose adjustments in patients with rifampicin or isoniazid concentrations above the upper therapeutic threshold (i.e. rifampicin > 24 mg/L)?

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Pharmacology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 05 February 2025

<https://doi.org/10.21956/wellcomeopenres.25009.r116607>

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Lambert Assoumou

INSERM, Pierre Louis Institute of Epidemiology and Public Health,, Sorbonne University, Paris, France

The main aims of this study are to determine the concentrations of selected antimicrobial agents (amoxicillin, azithromycin, ciprofloxacin and ceftriaxone) in people living with HIV (PLWH) who

require antimicrobial therapy, and to assess the usefulness of therapeutic drug monitoring (TDM) in achieving therapeutic goals in PLWH receiving rifampicin and isoniazid for the treatment of tuberculosis. Two primary endpoints are planned: a pharmacokinetic endpoint and a pharmacodynamic endpoint (resolution of signs/symptoms). The study will also explore the effects of covariates such as age, gender, concomitant medications and body mass index on pharmacokinetic parameters.

The study will recruit adult PLWH, aged 18 or over, with suspected or confirmed bacterial infection receiving at least one of the following antibiotics: amoxicillin, azithromycin, ciprofloxacin, rifampicin, isoniazid or ceftriaxone. For participants on anti-tuberculosis treatment, a dose adjustment will be made for those who have not reached target therapeutic concentrations of rifampicin, isoniazid or both at any time during the follow-up period based on TDM.

50 participants on each of the oral antimicrobials (amoxicillin, azithromycin, ciprofloxacin) and 25 participants for intravenous antibiotics (ceftriaxone) and anti-TB drugs (rifampicin and isoniazid) were expected.

Here is my comments :

The study has two primary endpoints, however, the sample size calculation was based solely on the pharmacokinetic endpoint. With such a sample size for each antibiotic, what would be the power to achieve the pharmacodynamic endpoint (sign/symptom resolution)?

The method used to diagnose bacterial infections and tuberculosis must be indicated.

The authors should explain why patients who discontinue antibiotics during the study should be excluded from the analysis. In my opinion, only participants who discontinue antibiotics without follow-up assessment should be excluded from the analysis. In addition, for women who become pregnant during the course of the study, pharmacokinetic data should be censored at the date of pregnancy, but pharmacodynamic data should be evaluated throughout the duration of the study.

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Partly

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 09 Apr 2025

Isabella Kyohairwe

Comment: The method used to diagnose bacterial infections and tuberculosis must be indicated. **Response:** TB is diagnosed using GeneXpert and CXR however only those who are GeneXpert positive are enrolled. For bacterial infections, those with suspected or confirmed bacterial infection, based on the managing clinicians decision, were enrolled when antibiotics were started. Bacterial infection is largely diagnosed using clinical symptoms and a full blood count, urine dip stick, depending on the suspected focus – line 125

Comment: The authors should explain why patients who discontinue antibiotics during the study should be excluded from the analysis. In my opinion, only participants who discontinue antibiotics without follow-up assessment should be excluded from the analysis. In addition, for women who become pregnant during the course of the study, pharmacokinetic data should be censored at the date of pregnancy, but pharmacodynamic data should be evaluated throughout the duration of the study. **Response:** Yes, data from the participants who get pregnant will be analysed and only the PK data will be censored from the point of pregnancy. Line 211 The decision to exclude those who discontinued antibiotics was made so as to have complete PK data for the majority of the patients and avoid having data with no matching PK parameters. We cannot change the exclusion of those whose antibiotics were discontinued at this point since the study is almost completed. However, we have not had to exclude anyone for this reason up to this point.

Competing Interests: No conflict of interest to disclose.

Reviewer Report 13 January 2025

<https://doi.org/10.21956/wellcomeopenres.25009.r115966>

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Rubeshan Perumal

Centre for the AIDS Programme of Research in South Africa, KwaZulu Natal, South Africa

The investigators propose an important and timely interrogation of the pharmacokinetics and dose-optimisation of commonly used antimicrobial and anti-tuberculous agents in people living with HIV receiving ART. Overall, the proposal is well-written, describes the project in sufficient detail, and advances the important scientific and public health goals of mitigating antimicrobial resistance and optimising the treatment of infections (including TB) in people with HIV. The following comments may help to strengthen the manuscript/protocol:

Abstract

The objective, as stated in the Abstract, needs to be rewritten for greater clarity. At present, it reads as though the concentrations of antimicrobial drugs will be assessed in people living with HIV and TB (receiving ART and rifampicin-based TB treatment). However, the study aims to evaluate the pharmacokinetics of commonly used antimicrobials (amoxicillin, azithromycin,

ciprofloxacin, ceftriaxone), rifampicin, and isoniazid) in people with HIV receiving ART.

“This study was reviewed and approved by the research and ethics committee in February 2024. Enrolment is projected to begin by August 2024.” Has the trial already started recruiting? Perhaps this needs to be updated to reflect the present status.

Introduction

The introduction is well-written and provides a clear rationale for pursuing the proposed work on therapeutic drug monitoring for people with HIV on TB treatment. However, a stronger rationale for evaluating the PK and drug-drug interactions of the commonly prescribed antimicrobial drugs is necessary. Suggestions include:

1. The scale of prescription of common antimicrobial agents in people living with HIV in Uganda and sub-Saharan Africa.
2. The known population PK profiles of these commonly prescribed antimicrobial agents. This should include outcomes for the most common indications (e.g. community-acquired pneumonia) in this population and any evidence to suggest that suboptimal drug concentrations may be contributory to the suboptimal outcomes. Please also include whether PK/exposure targets exist for these drugs.
3. Since the central theme of this project is the prevention/mitigation of antimicrobial resistance, it would be helpful for the introduction to include some empirical data on the drivers of resistance to the specific antimicrobial agents of interest in this study. To what extent is the problem known to be driven by PK/PD mismatch compared to other mechanisms of AMR?
4. The known and potential drug-drug interactions between common antimicrobials and ART should be presented.

Protocol and methods

The overall study design and methods reflect high levels of scientific rigour and appropriateness.

1. Study objective 2 should include “impact on clinical outcomes” – this could be sputum culture conversion or WHO-defined end-of-treatment outcomes.
2. A brief justification for using trough concentrations as the PK parameter endpoint for each antimicrobial drug is necessary.
3. Using clinical responses to the commonly used antimicrobial agents is pragmatic but may be heavily confounded by the indications for the treatment and the appropriateness of the prescription. The authors should consider whether focusing their attention and effort on the most common indication/s for these drugs would be more useful.
4. While the study makes provision for people receiving intravenous medication, it may be best to limit this study to ambulatory patients receiving oral treatment for all drugs except ceftriaxone.
5. Studying the pharmacokinetics, drug-drug interactions, and PK/PD in the context of an acute diarrheal illness may be very challenging. If this is an exclusion criterion, it should be included in the exclusion criteria. If it is not an exclusion criterion, a brief section on how this will be handled in interpreting the data is necessary.
6. The sample size section should include a final statement on the proposed sample size. The total sample size appears to be 200 participants (50 for amoxicillin, 50 for ciprofloxacin, 50 for azithromycin, 25 for ceftriaxone, and 25 for TB treatment).
7. Co-treatment with more than one of these agents will likely occur (e.g. ceftriaxone plus azithromycin, or amoxicillin-clavulanate plus azithromycin). Would this be permissible?

8. At present, the antiretroviral therapy regimen is not specified. Allowing *any* ART regimen is not practical and will limit the ability to study drug-drug interactions, in particular. Would it be possible to restrict this study to one (or at most, two) ART regimen/s to improve the methodological rigour?
9. Have the investigators considered a control group of people who are HIV-negative and, therefore, not receiving ART? Without this group, it will be challenging to determine how any antimicrobial PK data relates to co-administration with ART (i.e., drug-drug interaction).

Therapeutic drug monitoring with dose-adjustment

The proposed study of therapeutic drug monitoring for optimising the treatment of tuberculosis is an attractive application of TDM for clinical impact and the mitigation of AMR.

1. In people with HIV, rifampicin C_{max} has been shown to occur later than expected (C_{4hr} or C_{6hr}) in a significant proportion of people. It would be helpful if a justification for the C_{2hr} timepoint is provided for this particular setting.
 1. What proportion of people with HIV from this setting has been shown to have T_{max} at 2 hours vs 4 hours vs 6 hours?
 2. What is the risk of increasing the dose based on a low C_{2hr} in a patient with C_{max} at 4 or 6 hours, for example? It may be worthwhile to mention some of the rapidly accumulating data on the safety of higher doses of rifampicin. The use of a DMC and stopping rules, as proposed, does strengthen this study,

Once again, the investigators are congratulated for proposing this highly relevant and timely study. The TDM component, in particular, responds to a significant gap in the present therapeutic approach to TB.

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Yes

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Clinical Pharmacology; TB/HIV Epidemiology; Drug-resistant tuberculosis; TB/HIV Treatment Research

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Author Response 09 Apr 2025

Isabella Kyohairwe

We are grateful to the reviewers whose comments will improve the description of this study.

Reviewer: The objective, as stated in the Abstract, needs to be rewritten for greater clarity. At present, it reads as though the concentrations of antimicrobial drugs will be assessed in people living with HIV and TB (receiving ART and rifampicin-based TB treatment). However, the study aims to evaluate the pharmacokinetics of commonly used antimicrobials (amoxicillin, azithromycin, ciprofloxacin, ceftriaxone), rifampicin, and isoniazid) in people with HIV receiving ART.

Response: In the abstract, the objective of this study is to determine the concentrations of selected antimicrobial agents in people living with HIV requiring antimicrobial therapy and to assess the utility of therapeutic drug monitoring in achieving therapeutic targets for PLWH receiving rifampicin and isoniazid for the treatment of tuberculosis. This implies that we shall evaluate the PK of antibiotics in participants living with HIV. Those with TB will enrolled in the TDM arm.

Comment: "This study was reviewed and approved by the research and ethics committee in February 2024. Enrolment is projected to begin by August 2024." Has the trial already started recruiting? Perhaps this needs to be updated to reflect the present status.

Response: This is true. We have adjusted the statement to indicate that enrollment began in September 2024.

Reviewer: The scale of prescription of common antimicrobial agents in people living with HIV in Uganda and sub-Saharan Africa.

Response: Antibiotics are commonly prescribed for prophylaxis and management of other conditions among people living with HIV. The rates of prescription may vary across different countries in the region and range from 40% in Nigeria to as high as 85% in Tanzania (1). In Uganda, this data remain limited but antibiotic prescription is highly prevalent as shown by a recent survey conducted across 13 hospitals in the country (2). This has been added on line 25-28 in the revised manuscript.

Reviewer: The known population PK profiles of these commonly prescribed antimicrobial agents. This should include outcomes for the most common indications (e.g. community-acquired pneumonia) in this population and any evidence to suggest that suboptimal drug concentrations may be contributory to the suboptimal outcomes. Please also include whether PK/exposure targets exist for these drugs.

Response: Thank you for this important comment. The PK profile of these antibiotics in our study population in Uganda has not previously been described, and we have added a section about known PK targets for the antibiotics covered in this study starting on line 41. According to the Ugandan Ministry of Health data, at least 25 children die of pneumonia every day. Among adults, pneumonia remains a major cause of morbidity and mortality, particularly in people living with HIV. For example, in Uganda, studies at Mulago Hospital have shown that a microbial cause was identified in over 90% of HIV-positive adults with pneumonia, with tuberculosis and pneumococcus being the most common pathogens. Outcome data for urinary tract infections, which are also a common indication for antibiotics, is also limited. However, at Mulago National Referral Hospital, 30% of people who received urine cultures were resistant to ampicillin (line 34). It is not yet established

whether this could have been attributed to sub-optimal exposures. While specific PK/exposure targets exist for some antibiotics (e.g., trimethoprim-sulfamethoxazole), for many agents commonly used in our setting, such as ampicillin and ceftriaxone, there is limited local data on whether these targets are consistently achieved in people living with HIV. This is an important area for future research, especially given the potential for altered drug metabolism in this population.

Reviewer: Since the central theme of this project is the prevention/mitigation of antimicrobial resistance, it would be helpful for the introduction to include some empirical data on the drivers of resistance to the specific antimicrobial agents of interest in this study. To what extent is the problem known to be driven by PK/PD mismatch compared to other mechanisms of AMR?

Response We thank the reviewer for the comment. Brusse-Keizer et al. demonstrated that suboptimal exposure to amoxicillin was associated with poor outcomes in patients with COPD (3). Resistance to fluoroquinolones has also been linked to subinhibitory drug concentrations (4), as has treatment outcomes of anti-TB drugs (5). That said, it is also true that other mechanisms like genetic mutations and horizontal gene transfer also play a significant role in antimicrobial resistance. We have expanded the introduction to reflect these points, starting from line 37 in the revised manuscript. **Reviewer:** The known and potential drug-drug interactions between common antimicrobials and ART should be presented.

Response: While ceftriaxone and penicillins like amoxicillin may not have significant interactions with ART, certain ARVs cause enzyme inhibition with resultant increase in antibiotic drug levels and risk of toxicity. For example, Lopinavir/ritonavir is predicted to cause inhibition of P-gp and MRP2 and could potentially increase azithromycin exposure with risk of QT interval prolongation and torsades des points (6). No clinical PK studies have been done, and no dose adjustments are currently recommended. We have added some more to this background on lines 40 to 45 in the revised manuscript.

Protocol and methods

The overall study design and methods reflect high levels of scientific rigour and appropriateness.

Reviewer: Study objective 2 should include “impact on clinical outcomes” – this could be sputum culture conversion or WHO-defined end-of-treatment outcomes.

Response: This study is aimed at describing PK data for antibiotics such as amoxicillin. We acknowledge that it is not powered to assess PD outcomes in individuals receiving these antibiotics. However, exploratory PK-PD data will be available for those on tuberculosis treatment, as they will have biweekly cultures from which colony counts can be utilized.

Reviewer: A brief justification for using trough concentrations as the PK parameter endpoint for each antimicrobial drug is necessary.

Response: Using through concentrations will help determine if the organisms are consistently exposed to concentrations above historical MIC. In addition to the sparsely sampled participants, we will intensively sample a subgroup of participants. By combining these data using population pharmacokinetics (PopPK) modeling techniques, we will be able to estimate the overall drug exposure for all participants, including those who were sparsely

sampled. This approach leverages the benefits of PopPK analysis while reducing the need for extensive sampling and resource-intensive drug concentration assays. Combining both datasets will provide a more comprehensive understanding of the PK of these drugs in HIV patients within our setting.

Reviewer: Using clinical responses to the commonly used antimicrobial agents is pragmatic but may be heavily confounded by the indications for the treatment and the appropriateness of the prescription. The authors should consider whether focusing their attention and effort on the most common indication/s for these drugs would be more useful.

Response: We agree with the reviewer, however the aim of this study is to describe the PK of these drugs in HIV and evaluate if we can utilize these models in routine practice when antibiotics are given. However, there is another parallel study taking place as part of this consortium where appropriateness and indications of the antibiotic use will be described and can inform these results. (<https://doi.org/10.12688/wellcomeopenres.23532.1>)

Reviewer: While the study makes provision for people receiving intravenous medication, it may be best to limit this study to ambulatory patients receiving oral treatment for all drugs except ceftriaxone.

Response: We thank the reviewer for this comment, however at this point in time, we have completed enrollment of patients receiving ceftriaxone. Since this is the most frequently prescribed intravenous antibiotic in Uganda, we believe the PK data will still be informative.

Reviewer: Studying the pharmacokinetics, drug-drug interactions, and PK/PD in the context of an acute diarrheal illness may be very challenging. If this is an exclusion criterion, it should be included in the exclusion criteria. If it is not an exclusion criterion, a brief section on how this will be handled in interpreting the data is necessary.

Response: Knowledge of acute diarrheal disease is important and we will review it as a covariate if this was present while interpreting the PK data especially for those who have unexpectedly low concentrations.

Reviewer: The sample size section should include a final statement on the proposed sample size. The total sample size appears to be 200 participants (50 for amoxicillin, 50 for ciprofloxacin, 50 for azithromycin, 25 for ceftriaxone, and 25 for TB treatment).

Response: This is true, the total sample size is 200 and this has been updated on line 155.

Reviewer: Co-treatment with more than one of these agents will likely occur (e.g. ceftriaxone plus azithromycin, or amoxicillin-clavulanate plus azithromycin). Would this be permissible? **Response:** This is very likely and we are enrolling those on multiple drugs of interest and recording this data so that it can be interpreted accordingly.

Reviewer: At present, the antiretroviral therapy regimen is not specified. Allowing *any* ART regimen is not practical and will limit the ability to study drug-drug interactions, in particular. Would it be possible to restrict this study to one (or at most, two) ART regimen/s to improve the methodological rigour?

Response: We agree with the reviewer. Most patients in Uganda are on dolutegravir based regimens with a few receiving efavirenz. This is given mostly with tenofovir and lamivudine.

We will therefore have enrolled participants on all these ART and will analyze the data with this as a potential covariate.

Reviewer: Have the investigators considered a control group of people who are HIV-negative and, therefore, not receiving ART? Without this group, it will be challenging to determine how any antimicrobial PK data relates to co-administration with ART (i.e., drug-drug interaction). **Response:** This is very useful feedback, and we believe having HIV-negative patients would have been very informative. Although this is not possible at this point, we plan to compare the antibiotic exposures in our study with previously published data (including those not on ART).

Therapeutic drug monitoring with dose-adjustment

The proposed study of therapeutic drug monitoring for optimising the treatment of tuberculosis is an attractive application of TDM for clinical impact and the mitigation of AMR.

Reviewer: In people with HIV, rifampicin C_{max} has been shown to occur later than expected (C_{4hr} or C_{6hr}) in a significant proportion of people. It would be helpful if a justification for the C_{2hr} timepoint is provided for this particular setting. What proportion of people with HIV from this setting has been shown to have T_{max} at 2 hours vs 4 hours vs 6 hours? What is the risk of increasing the dose based on a low C_{2hr} in a patient with C_{max} at 4 or 6 hours, for example? It may be worthwhile to mention some of the rapidly accumulating data on the safety of higher doses of rifampicin. The use of a DMC and stopping rules, as proposed, does strengthen this study,

Response: The reviewer is correct. We acknowledge that some patients may not have peak concentrations at C_{2hr} due to delayed absorption. Therefore, increasing rifampicin doses in this population may lead to adverse drug reactions. The protocol was amended such that blood draws will also be taken at C_{4hr} and C_{6hr} for all patients on TB treatment as previously published (7). We will therefore be able to estimate the C_{max} and overall exposure (area under the curve) more accurately to determine who requires dose adjustment.

Competing Interests: There is no conflict of interest to declare