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Impact of drug-resistance diagnosis based on whole-genome sequencing on the treatment adequacy of patients with drug-resistant pulmonary tuberculosis in the state of São Paulo, Brazil: a protocol for a non-randomised controlled trial (Gen-TB PróCura)

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ABSTRACT

Introduction Since 2018, WHO has endorsed the use of whole-genome sequencing (WGS) of *Mycobacterium tuberculosis* complex isolates to detect drug-resistant tuberculosis (DR-TB). This endorsement was based on the assumption that a faster and more detailed description of the resistance profile would improve treatment prescription for DR-TB by healthcare providers, and hence the treatment outcomes of patients. Nonetheless, this assumption has not been tested in routine clinical practice and different scenarios. In Brazil, WGS is not routinely used for the diagnosis of DR-TB, having been carried out in only a few centres for research purposes. With this trial, we will evaluate whether a WGS-based drug-resistance report improves treatment adequacy in patients with pulmonary DR-TB, compared with the current standard-of-care diagnostic methods used in the state of São Paulo, Brazil.

Methods and analysis We will conduct a non-randomised controlled clinical trial with two arms to compare the intervention group (ie, individuals receiving a WGS-based report) with a historical control group (i.e., individuals who received resistance diagnostics based on the standard of care of conventional genotyping and phenotyping techniques). The primary outcome will be the proportion of patients whose treatment scheme was adequate based on complete resistance profile determined by WGS and/or phenotypic drug-susceptibility testing (pDST). Other secondary outcomes will also be considered. The target sample size is 88 eligible patients per group. The intervention group will be prospectively recruited over 18 months and the control group will be composed of patients diagnosed with pulmonary DR-TB up to 2 years before the start of the trial. To ensure comparability, isolates from the control group will undergo WGS retrospectively, and pDST will be performed retrospectively in both groups. This clinical trial will take place in six medical centres for the treatment of DR-TB in the state of São Paulo. This study is intended to support the implementation of the WGS in the routine diagnosis of DR-TB in the state of São Paulo.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The multicentre design with recruitment from diverse regions of the state of São Paulo will enhance representativeness.
⇒ The inclusion of both patients and healthcare professionals will allow the study to capture multiple perspectives on whole-genome sequencing (WGS) implementation.
⇒ The use of a Community Research Oversight Committee will ensure integration of community input into study conduct.
⇒ A limitation is the non-randomised design, adopted mainly for ethical reasons to ensure equitable access to WGS, with a retrospective cohort as control.
⇒ Another limitation is the limited sensitivity of WGS for newer drugs due to incomplete mutation catalogues.

Ethics and dissemination Ethical approval was obtained from the Human Research Committee of the Institute of Biomedical Sciences, University of São Paulo, Brazil (CAAE: 79497924.1.1001.5467). Study results will be published in peer-reviewed journals and disseminated to policymakers and stakeholders.

Trial registration number U1111-1308-4669.

INTRODUCTION

Background and rationale

Drug-resistant tuberculosis (DR-TB) is a serious public health concern. In 2023, it was estimated that 400 000 (95% CI (confidence interval): 360 000 to 440 000) TB cases resistant to rifampicin (RR-TB) or to rifampicin and isoniazid (MDR-TB) occurred worldwide. In Brazil, 1060 cases of DR-TB were reported in the same year,[1] but under-reporting is a concern.[2] The treatment success rate for RR/MDR-TB in the country is only 61.1%,[1] which is lower than the 90% treatment success rate for TB recommended by the Stop TB Partnership.[3] Recently, our research group reported the first four cases of extensively drug-resistant TB (XDR-TB) in Brazil,[4] that is, cases of MDR-TB that are also resistant to any fluoroquinolone and at least one other group A drug. Group A drugs include the fluoroquinolones (levofloxacin and moxifloxacin), bedaquiline and linezolid. The detected XDR-TB cases were resistant to bedaquiline, linezolid and/or delamanid,[4] the newest drugs in the fight against DR-TB. These data suggest that it is urgent to invest in the diagnosis of drug resistance, using fast and high-precision technologies that allow personalised treatment of patients with TB.

The laboratory diagnosis of DR-TB presents important challenges. In Brazil, different methods are used, including phenotypic drug susceptibility tests (pDST), such as the proportion and the BD BACTEC MGIT960 methods, and genotypic tests, such as the Xpert MTB/RIF Ultra and molecular Line Probe Assays (LPAs). The introduction of Xpert MTB/RIF in Brazil in 2014 improved the detection of RR-TB.[1] However, fewer than 50% of cases with bacteriological confirmation undergo susceptibility testing for rifampicin and only 12.6% of RR/MDR-TB cases are tested for second-line drugs.[5 6] One reason for this low coverage is that phenotypic methods are time-consuming, labour-intensive and unavailable for some drugs. On the other hand, genotypic tests do not cover all resistance-associated mutations, even for first-line drugs, and are also not available for all antimicrobials. Thus, since 2018, the World Health Organization (WHO) has endorsed next-generation sequencing (NGS), including whole-genome sequencing (WGS) and targeted sequencing, for the diagnosis of DR-TB.[7] By analysing the entire genome of the bacterium, WGS is capable of identifying variants in all genes associated with drug resistance in a single assay. To aid in this process, in 2021, the WHO published a catalogue of mutations associated with drug resistance or susceptibility, as well as mutations with unknown profiles.[8] A second edition of this catalogue was launched in 2023.[9]

This WHO endorsement was based on the assumption that detailed and faster NGS-based drug-susceptibility testing would improve the treatment prescription for patients with DR-TB by healthcare providers and, consequently, the treatment outcome of these individuals. However, these assumptions have not been tested in different routine clinical conditions globally. A recent systematic review did not find any interventional study assessing treatment outcomes of patients with DR-TB diagnoses based on WGS compared with conventional testing.[10] Van Rie *et al*[11] registered a randomised controlled trial in South Africa to assess a research question similar to the present study, but the results of this trial have not been published yet. Because WHO has many structured diagnostic recommendations for DR-TB detection, each country or region may use combinations of various strategies and diagnostic tests for this purpose. Therefore, there is a need to evaluate how the utilisation of WGS to detect drug resistance may impact the healthcare of patients in different scenarios of diagnostic workflow.

Here, we will evaluate whether treatment guided by a WGS-based diagnostic report leads to more adequate antimicrobial therapy for patients with pulmonary DR-TB compared with the routine diagnostic practice provided by the Brazilian Universal Healthcare System (Sistema Único de Saúde (SUS)) in six medical centres for DR-TB treatment in the state of São Paulo, Brazil. We hypothesise that the implementation of the WGS of *Mycobacterium tuberculosis* complex isolates as a diagnostic tool for detecting antimicrobial resistance in TB will improve treatment adequacy for patients with pulmonary DR-TB compared with the current diagnostic routine for DR-TB in the state of São Paulo. The protocol is described according to the 'Standard Protocol Items: Recommendations for Interventional Trials statement'.[12]

METHODS AND ANALYSIS

Trial design

A non-randomised, controlled, non-blinded, multicentre superiority trial with two groups will be conducted.[12] The intervention group will consist of prospectively recruited patients with pulmonary DR-TB who will receive the intervention (ie, a WGS-based drug-resistance report sent to the patient and the responsible physician). The control group will be composed of patients with pulmonary DR-TB who were treated at any time during the 2 years before the start of the clinical trial and who received standard diagnostic results. This is the first version of this clinical trial protocol, dated 4 January 2025.

Participants, interventions and outcomes

Study setting

This clinical trial will take place in six medical centres for the treatment of DR-TB located in three different cities in the state of São Paulo, Brazil (table 1). These are tertiary medical centres under the SUS umbrella. In addition, the Tuberculosis and Mycobacteriosis Laboratory from

Table 1 Medical centres that will participate in this clinical trial and their locations

Medical centre	City
Hospital das Clínicas, School of Medicine of Ribeirão Preto, University of São Paulo	Ribeirão Preto
Hospital das Clínicas, University of Campinas	Campinas
Reference and Training Center of Sexually Transmitted Infections and AIDS, State Secretariat of Health	São Paulo
Emilio Ribas Institute of Infectious Diseases	São Paulo
University Hospital, Federal University of São Paulo	São Paulo
Hospital das Clínicas, School of Medicine, University of São Paulo	São Paulo

the Adolfo Lutz Institute (NTM-IAL), São Paulo, which is the state reference laboratory for the diagnostics of TB, will also be involved in this study. Treatment schemes and monitoring provided to patients with DR-TB, patient management and strategies to prevent loss of follow-up are performed under routine conditions established by the State and National Programmes of Tuberculosis Control (*Plano Estadual pelo Fim da Tuberculose como Problema de Saúde Pública* and *Programa Nacional de Controle da Tuberculose*).[13] Notably, the state of São Paulo is the most populous state in Brazil, with 46 363 573 million inhabitants.[14] It also reports the highest absolute number of TB cases per year in the country (~16 000 cases/year).[15]

Eligibility criteria

The following inclusion criteria must be met for each patient in both groups: (i) be a patient (any age or sex) at one of the six medical centres listed in table 1 during the data collection period; (ii) have a diagnosis of active pulmonary DR-TB; (iii) have *M. tuberculosis* isolate(s) obtained from clinical sample(s) during the study period; (iv) belong to one of the priority populations listed in box 1 and (v) meet at least one of the following diagnostic workflow conditions: (a) have resistance to rifampicin detected by Xpert MTB/RIF, or to any drug detected by LPA-1 or LPA-2, in accordance with the priority flow chart based on the diagnostic workflow of NTM-IAL (figure 1); (b) be experiencing treatment failure, with clinical suspicion of drug resistance, and have no available drug-susceptibility testing results and/or a negative rifampicin resistance result on Xpert MTB/RIF or (c) have experienced relapse following a prior diagnosis of DR-TB.

Box 1 List of priority populations for drug-susceptibility testing in the state of São Paulo, Brazil

1. Re-treatment cases.
2. Population deprived of liberty.
3. Population experiencing homelessness.
4. Patients with HIV co-infection.
5. Patients with other immunosuppressive conditions.
6. Healthcare professionals, correction workers and other professionals working in enclosed institutions (eg, shelters, mental health hospitals).
7. Contact individuals of patients diagnosed with drug resistance.
8. Patients with drug and/or alcohol addiction.
9. Immigrants.
10. Patients from enclosed institutions.
11. Patients with an Xpert MTB/RIF Ultra-positive test for rifampicin resistance.
12. Patients with positive culture or smear microscopy after the second month of treatment.

The following exclusion criteria will apply for both groups: (i) extrapulmonary TB diagnosis at the time of enrolment or during the study; (ii) an unviable or contaminated bacterial culture; (iii) co-infection with non-tuberculous mycobacteria; (iv) transfer to another treatment centre not listed in table 1 during the study and/or (v) withdrawal from or refusal to participate in the study.

Interventions

Explanation for the choice of comparators

Because we decided to offer the intervention (i.e., the WGS-based diagnostic report) to all prospectively recruited participants, we selected a retrospective group as the comparison/control group. More specifically, the control group will involve patients diagnosed with pulmonary DR-TB in the six medical centres up to 2 years before the start of the intervention (i.e., 2023–2025) who met eligibility criteria. These patients received the standard of diagnostics and care determined by the State and National Programmes of Tuberculosis Control.

As with many high-burden TB countries worldwide, drug-susceptibility testing is not universal in Brazil. A list of priority populations (box 1) and a priority algorithm (figure 1) is used to decide which sample/isolate will be tested for resistance to first-line and second-line drugs using techniques such as Xpert MTB/RIF Ultra assay, first-line and second-line LPA and selected phenotypic testing (figure 1). The control group was subjected to these conditions, without the possibility of WGS, and received standard of care. Noteworthy, Xpert MTB/RIF Ultra is not universally available in the state; however, it is used for all cases of pulmonary TB in the six medical centres involved in this trial, except when the sputum sample is <2 mL, in which case smear microscopy is preferred.

Intervention description

The intervention group will be prospectively recruited for 1.5 years (18 months). The intervention will consist of a WGS-based report of drug resistance that will be

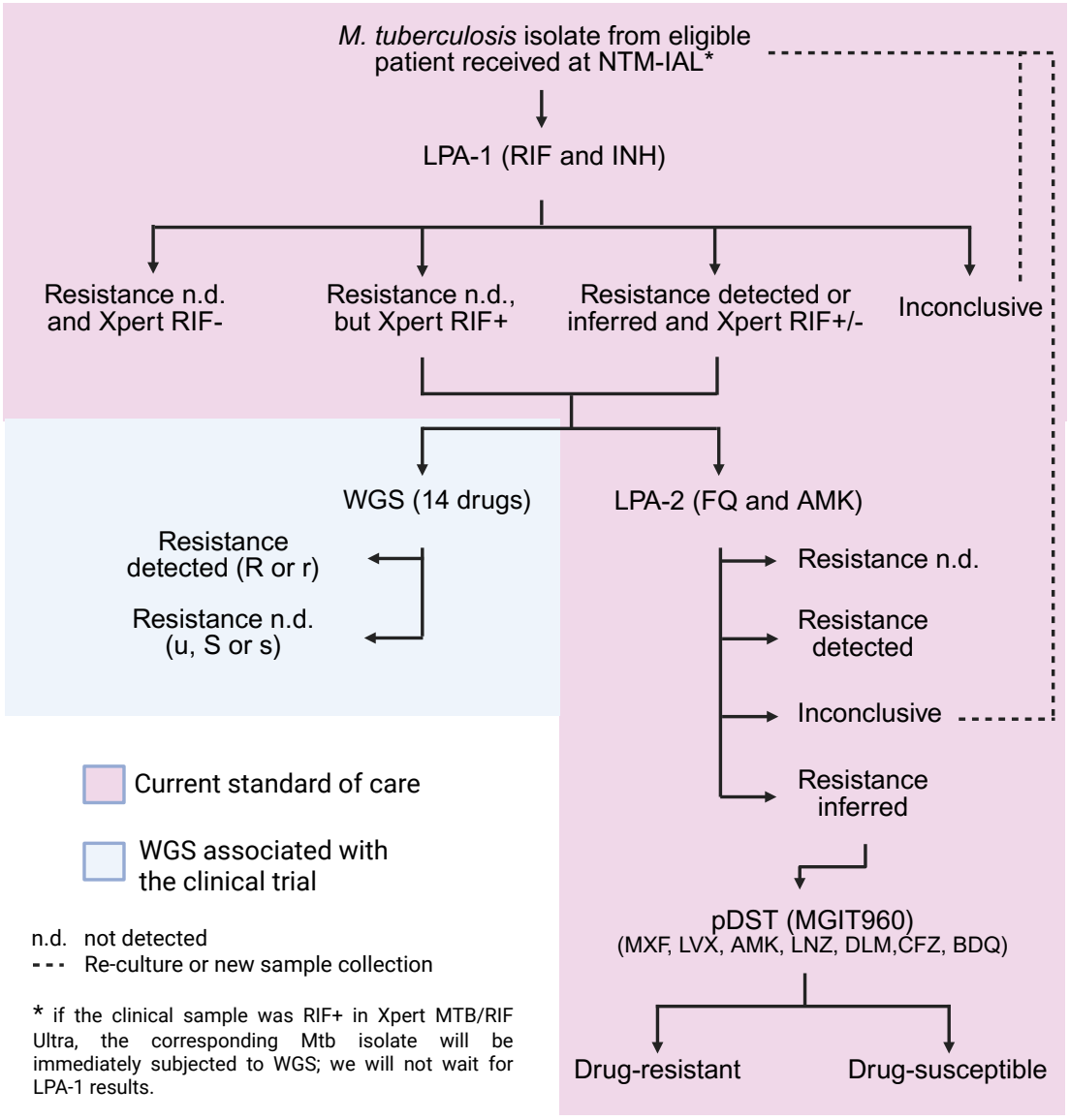

Figure 1 Diagnostic workflow of antimicrobial resistance for patients with tuberculosis at the Tuberculosis and Mycobacteriosis Laboratory from the Adolfo Lutz Institute (NTM-IAL)**.** Standard-of-care workflow (in pink) and added workflow associated with the clinical trial (in blue) are shown. Clinical samples are collected at the medical centres and subjected to Xpert MTB/RIF Ultra at the site. If patients meet the priority populations (box 1), clinical samples are subjected to culture at the laboratories of the medical centres. These laboratories are part of the NTM-IAL laboratory network. Once a mycobacterial isolate is obtained, it is sent to NTM-IAL headquarters for the diagnostics of antimicrobial resistance using Line Probe Assay (LPA) and phenotypic testing. Whole-genome sequencing (WGS) of the isolate, as part of the clinical trial, will be performed if the clinical sample shows positive results for resistance to rifampicin according to Xpert MTB/RIF Ultra or the isolate is resistant (or inferred) according to LPA-1. The WHO's catalogue of mutations associated with resistance in *Mycobacterium tuberculosis* (Mtb) classifies the mutations as: R (associated with resistance), r (associated with resistance—interim), u (uncertain significance), S (not associated with resistance), s (not associated with resistance—interim). AMK, amikacin; BDQ, bedaquiline; CFZ, clofazimine; DLM, delamanid; LNZ, linezolid; FQ, fluoroquinolones; INH, isoniazid; LVX, levofloxacin; MXF, moxifloxacin; RIF, rifampicin; pDST, phenotypic drug-susceptibility testing. Figura was created in BioRender, under Pires dos Santos, A's account, current in 2025 (https://app.biorender.com/citation/6779a5919acd7f1fa5e719d8).

available to the responsible physicians at GAL (Laboratory Environment Management System), the web-based system used by the SUS to manage and deliver diagnostic results generated by the public laboratory network. The structure of the WGS-based report has been provisionally validated by the reference laboratory NTM-IAL and the GAL team (online supplemental file 1). Each patient's physician is responsible for informing the results to their patients.

Before being delivered to the responsible physician and patient, the report will be evaluated by two validators. The first validator is a licensed biomedical professional from NTM-IAL, which will guarantee that all quality control standards of the WGS have been met. The second

validator is a highly trained reference physician. A similar model established for the clinical interpretation of HIV genotyping used by the Brazilian SUS will be applied.[16] In this model, this highly trained reference physician will read the report and provide a clinical interpretation of its findings along with treatment guidance to the responsible physician of the medical centre. The reference physician will be based at the Division of Tuberculosis of the Center for Epidemiological Surveillance of the State of São Paulo (CVE-SP).

WGS will be performed in DNA extracted[17] from *M. tuberculosis* cultures in Mycobacterium Growth Indicator Tube (MGIT) or Löwenstein-Jensen medium. DNA libraries will be constructed using DNA Prep (Illumina) and sequenced using MiSeq Reagent Kit V.2 or p1 NextSeq 1000 (300 cycles, 2X151 bp paired-end, Illumina) in MiSeq or NextSeq (Illumina) platforms, respectively, according to the manufacturer's instructions. Generated FASTQ will be analysed by a bioinformatic pipeline developed and validated previously by the researchers from the NTM-IAL and the Institute of Biomedical Sciences (ICB), University of São Paulo (USP) (manuscript in preparation).

To guarantee the safety of the intervention group, standard-of-care diagnostics will be made available to all participants. In other words, the WGS report will serve only as an addition to the current standard of care and will not interfere with the current diagnostic workflow. Therefore, the standard diagnostic results can be used in case the WGS fails for different reasons (poor sequencing, negative culture, etc).

Under the current diagnostic workflow, phenotypic testing is not performed for all second-line drugs (figure 1). However, at the end of the trial, phenotypic testing will be conducted for all drugs in a research capacity, for both groups, to serve as the gold standard for the evaluation of the primary outcome of the WGS report and any discrepancy that may emerge between the current diagnostic workflow and the WGS. WGS will also be retrospectively performed in the control group by retrieving their isolates from the NTM-IAL biobank.

Criteria for discontinuing or modifying allocated interventions

No serious adverse events of the intervention are expected, thus, there are no criteria for discontinuing or modifying the allocated interventions.

Strategies to improve adherence to interventions

In many countries, healthcare professionals responsible for the treatment of patients with DR-TB have limited knowledge of how to use drug resistance reports based on WGS to define a personalised treatment. Therefore, to improve adherence and the correct use of the intervention by physicians, besides having the reference physician validator, capacity building with the healthcare professionals of the six medical centres will be performed before the initiation of the trial. Physicians who attend to patients with DR-TB at the six medical centres will be trained on the principles of WGS-based diagnostics, its strengths and limitations, the clinical interpretation of the mutations and the choice of treatment based on the findings of the drug-susceptibility testing based on WGS. No strategies to improve adherence to the treatment by the patient per se will be made beyond what is currently done in routine care (e.g., close follow-up, directly observed treatment, among others) defined by the Federal and State Programmes of Tuberculosis Control.

Relevant concomitant care permitted or prohibited during the trial

Participants from the intervention group in this trial will continue their usual care except for receiving the detailed WGS-based diagnostic report. During the trial, these participants may receive concurrent care interventions as needed.

Outcomes

The primary outcome of interest is treatment adequacy (table 2), defined as the proportion of patients in each group who were prescribed an antimicrobial treatment regimen that is fully appropriate based on the complete resistance profile of their isolate. The index treatment regimen used for this comparison will be defined as the regimen prescribed within 30 days after all relevant diagnostic test results became available to the treating physician. For the control group, this includes results from Xpert MTB/RIF Ultra, LPA-1, LPA-2 (if performed) and pDST (if performed). For the intervention group, this includes all of the above plus the WGS report. A complete resistance profile will be established using WGS, pDST or a combination of both, for all patients in both groups. Accordingly, three separate analyses of treatment adequacy will be conducted: one using WGS alone, one using pDST alone and one using the combination of WGS and pDST as the reference standard. To enable these analyses, WGS will also be retrospectively performed on stored isolates from the control group, available at the NTM-IAL biobank. Similarly, pDST using MGIT960 for 13 drugs will be conducted for both groups for the sole purpose of outcome evaluation, not for clinical decision-making (with the exception of any pDST conducted as part of routine care). A treatment regimen will be considered adequate if: all drugs in the treatment regimen are active against the strain, based on the WGS and/or pDST profile (i.e., no resistance to any drug in the regimen), and the regimen is consistent with national and/or WHO guidelines for treatment of drug-resistant TB, including RR/MDR/XDR-TB where applicable.

We will also collect data on secondary outcomes (table 2). Although this is not a therapeutic intervention trial, we will compare treatment outcomes between groups to provide contextual information on the overall clinical trajectory of patients and to support the characterisation of the cohort, including potential stratification of treatment adequacy by eventual outcome. The SITE-TB system categorises treatment outcomes into the following:

Table 2 Outcomes, source, individual and aggregate metrics and specific measurement time points

ID	Outcome	Source	Individual metric	Aggregate metric	Specific measurement time point
1	Treatment adequacy (primary outcome)	SITE-TB	Yes or no	Number of individuals	After all test results become available
2	Cure from TB	SITE-TB	Yes or no	Number of individuals	At the end of the treatment
3	Treatment completed	SITE-TB	Yes or no	Number of individuals	At the end of the treatment
4	Death from TB	SITE-TB, SIM	Yes or no	Number of individuals	At the end of the treatment
5	Death from other causes	SITE-TB, SIM	Yes or no	Number of individuals	At the end of the treatment
6	Treatment failure	SITE-TB	Yes or no	Number of individuals	At the end of the treatment
7	Loss to follow-up	SITE-TB	Yes or no	Number of individuals	At the end of the treatment
8	Change in diagnostics	SITE-TB	Yes or no	Number of individuals	At the end of the treatment
9	Change in treatment regimen	SITE-TB	Yes or no	Number of individuals	At the end of the treatment
10	Change in resistance pattern	SITE-TB	Yes or no	Number of individuals	At the end of the treatment
11	Still undergoing treatment	SITE-TB	Yes or no	Number of individuals	At the end of the trial
12	Transfer to another country or medical centre	SITE-TB	Yes or no	Number of individuals	At the end of the trial
13	Change in treatment based on WGS results*	Medical records, GAL, SITE-TB	Yes or no	Number of individuals	At the end of the treatment
14	Sputum culture conversion	Medical records, GAL, SITE-TB	Number of days, yes or no	Mean/Median number of days, number of individuals	Every month following treatment initiation
15	Time elapsed between DR-TB diagnosis based on Xpert MTB/RIF and the delivery of LPA-1, LPA-2, pDST and WGS results to the physician	GAL, SITE-TB	Number of days	Mean/Median number of days	At the start of treatment
16	Time patients were treated with ineffective drugs	SITE-TB	Number of days	Median number of days	At the end of treatment
17	Number and length of hospital stay, if applicable	Medical records, SITE-TB	Number of times and days	Number of individuals requiring hospital stay and mean/median number of days	At the end of treatment
18	Total treatment time until last treatment outcome recorded	SITE-TB	Number of days	Mean/Median number of days	At the end of treatment(s)
19	Use of the WGS report by healthcare professionals*	Questionnaire to physicians	Score (1–10), categorical, narrative	Median score per question, category frequency, narrative analyses	At the WGS report receival
20	Physician uptake of WGS-guided recommendations	Questionnaire to physicians and SITE-TB	Yes or no	Number of individuals	After all test results become available

*Descriptive analysis performed on intervention group only.
DR-TB, drug-resistant tuberculosis; GAL, Laboratory Environment Management System; LPA, Line Probe Assay; pDST, phenotypic drug-susceptibility testing; SIM, Mortality Information System; SITE-TB, Tuberculosis Special Treatment Information System; TB, tuberculosis; WGS, whole-genome sequencing.

cure, treatment completed, death from TB, death from other causes, treatment failure, loss to follow-up, change in diagnostics, change in treatment regimen, change in resistance pattern, still undergoing treatment, transfer to another country or medical centre and others. All outcome categories will be initially retained and reported for transparency. However, depending on the distribution of cases across categories, we may group them into

broader outcome categories (e.g., successful vs unsuccessful or favourable vs unfavourable) to facilitate statistical analysis. The decision to regroup outcomes will be made after data review, based on sample size considerations and the feasibility of meaningful comparisons.

Other important secondary outcomes will be collected and analysed solely from the intervention group: the extent to which WGS data inform therapeutic decision-making (change of treatment based on WGS results), physicians' perceptions (provided by the questionnaire: utility, clarity and interpretability of WGS results, confidence in decision making, perceived impact on workflow, barriers and facilitators, satisfaction) and physician uptake of WGS-guided recommendations. Since this is a pragmatic trial, the ultimate goal is to model a workflow for this diagnostic test in the state of São Paulo, including evaluating and making necessary adjustments to sample ordering and collection, laboratory processing, result communication, clinical decision-making and follow-up, and treatment adjustments, even though these aspects are not explicitly listed as secondary outcomes. All outcomes will be collected from medical records and notifications systems (Tuberculosis Patient Control System (TBWeb), Tuberculosis Special Treatment Information System (SITE-TB), Mortality Information System (SIM) and Laboratory Environment Management System (GAL)). These online systems were or will be filled by the healthcare professionals responsible for the treatment of the study's participants.

Participant timeline

The schedule of screening, enrolment, interventions and assessments is shown in table 3.

Sample size

An unpublished study from our group retrospectively analysed 123 MDR-TB cases in the state of São Paulo using WGS. Of these, approximately nine patients (about 7%) were likely receiving inadequate treatment based on WGS results, which were not available to physicians at the time (i.e., patients had only standard diagnostic care). Based on this, we conservatively estimate that 85% of patients in the control group received adequate treatment. Assuming an improvement to 97% in the intervention group, the number of participants, in each group, needed to identify this difference if it exists is 88, considering an α=0.05 and a power=0.80. The intervention group will be prospectively recruited for 18 months. Considering the number of eligible patients with TB the medical centres treat annually and the proportion of people with pulmonary DR-TB in São Paulo,[1] we estimate that during the data collection period, we will achieve 120 eligible participants with positive culture. The control group will be composed of people diagnosed with pulmonary DR-TB during the 2 years before the start of the trial (2023–2025). We estimate

Table 3 Schedule of screening, enrolment, interventions and assessments of the participants

	Study period							
	Enrolment	**Allocation**	**Postallocation**					**Close-out**
Time point	$-t_1$	**0**	M1	M2	M3	M4	Etc*	End of treatment
Enrolment								
Eligibility screen	X							
Informed consent (intervention group)		X						
Initiation of standard treatment (intervention group)		X						
Allocation		X						
Interventions								
WGS-based report to intervention group			X					
Assessments								
Baseline sociodemographic and clinical characteristics		X						
Clinical sample for culture	X	X						
Smear microscopy or culture control			X	X	X	X	X	X
Medical records and other information (intervention group)			X					X
Medical records and other information (control group)		X						X
Questionnaire to physicians			X					

M1=first month, M2=second month and so on.
*Postallocation time has a variable time, according to the duration of treatment of each patient.
WGS, whole-genome sequencing.

that it will involve 160 eligible participants for this group. Thus, the study will be adequately powered, even in the case of some proportion of loss to follow-up (eg, 10%).

Recruitment

Participants in the intervention group will be prospectively recruited, for 18 months, during their routine care. Recruitment will occur at one of the following moments: (i) immediately after the participant's clinical sample presents a positive result for rifampicin resistance in Xpert MTB/RIF Ultra; (ii) immediately after the participant's clinical isolate presents a positive result for antimicrobial resistance in LPA-1; (iii) immediately after a culture-positive isolate is obtained from a participant who is experiencing treatment failure and has no available drug-susceptibility testing results and/or Xpert MTB/RIF shows a negative result for rifampicin resistance or (iv) immediately after a culture-positive isolate is obtained from a participant who experienced relapse following a prior diagnosis of DR-TB. This period of recruitment should provide enough participants to identify sufficiently precise differences between the intervention and control groups on the outcomes of interest.[18] Participants in the control group were already enrolled in treatment for pulmonary DR-TB and their data will be accessed through the TB surveillance systems. No monetary compensation will be provided for recruitment.

Assignment of interventions: allocation

Sequence generation and concealment mechanism

There is no need for sequence generation or concealing the allocation because all prospectively recruited participants eligible for the study will be allocated to the intervention group.

Implementation

The healthcare professionals of each medical centre are responsible for the enrolment of prospectively recruited participants. Intervention is delivered by the reference laboratory through the GAL system. The responsible physician of each patient will make appropriate use of the intervention and decide on the treatment plan based on prior training and recommendations from validators. The leading researchers and responsible physicians are responsible for the enrolment of participants from the control group.

Assignment of interventions: blinding

This study is not blinded.

Data collection and management

Plans for assessment and collection of outcomes and baseline

Results and dates of Xpert MTB/RIF Ultra and LPA, clinical sample culture positivity, follow-up smear microscopy and cultures, WGS-based drug resistance report will be extracted from each centre's medical records, NTM-IAL records, SITE-TB and/or the GAL. Sociodemographic, clinical and laboratory patient data, including treatment history and time, hospitalization, adverse events and outcomes, will be extracted from each centre's medical records and the TB surveillance systems (TBWeb, SITE-TB, SIM and GAL). Raw sequencing data will be retrieved from the Illumina sequencing platforms and analysed using a bioinformatic pipeline of antimicrobial resistance detection at NTM-IAL or ICB-USP. If sequencing or bioinformatic analysis fails, this information will be entered into the data collection system designed for this study. The decision to repeat the procedure will be evaluated on a case-by-case basis. For the assessment of the responsible physician's experience using the WGS report, a structured questionnaire will be applied (online supplemental file 2).

Plans to promote participant retention and complete follow-up

Participants in the intervention group will follow their usual routine care. No additional strategy will be applied to improve participants' retention, as this is a pragmatic trial.

Data management

Datasets will be registered under a data collection system designed for this study, secured by passwords to protect the identity of participants. Whenever possible, information will be de-identified of any personal data at the source of its generation and provided a serial number. Informed consent will be stored in physical form according to the guidelines of each medical centre, and scanned into PDF documents for safe electronic storage.

The datasets with information about the outcomes will be screened to identify and correct inconsistencies and (possible) duplicated registration of the same participant.[19 20] Whenever necessary and possible, healthcare professionals will be consulted to clarify inconsistent or missing information. If missing data for a variable are considered substantial (ie, may change the conclusions), sensitivity analyses will be performed.[21]

Plans for collection, laboratory evaluation and storage of biological specimens for genetic or molecular analysis in this trial/future use

Clinical samples (sputum, bronchoalveolar lavage, pleural fluid, gastric lavage, among others) will be collected from patients in each medical centre and processed for culture in BD BACTEC MGIT960 or Löwenstein-Jensen medium at their laboratory. The NTM-IAL will receive the cultured isolates of *M. tuberculosis*. Clinical samples will be discarded according to the guidelines of each medical centre, but the bacterial isolate will be saved for this trial and future use at NTM-IAL in a controlled biorepository. This biorepository has been approved by the Research Ethics Committee and follows specific conservation and care guidelines from NTM-IAL.

Statistical methods

Statistical methods for primary and secondary outcomes

To facilitate the interpretation of results, some continuous outcomes can be categorised. Risk ratios (RRs) will be calculated for dichotomous outcomes.[22] Additionally,

hazard ratios (HRs) and a Kaplan-Meier curve will be produced for the culture conversion variable.[23–25] As for continuous variables, differences in means will be compared using the Welch t-test[26] and medians with the Mann-Whitney U test.[27] When applicable, analyses will be carried out controlling for possible confounding variables, as long as these differ substantially between groups at baseline and are prognostic of the outcome.[28–30] The analyses will be based on the intention-to-treat principle.[31] Data interpretation will be carried out based on estimates and their 95% CIs and p values will be reported.[18 32] All analyses will be performed using IBM SPSS software or in R.

Interim analyses

An interim analysis of treatment adequacy will be performed when reaching 50% of the recruited patients.

Methods for additional analyses (e.g., subgroup analyses)

When analysing secondary outcomes such as cure, death or other unsuccessful outcomes, we will adjust for differences in treatment regimens and year of treatment initiation between groups using multivariable logistic regression, with regimen type and treatment year included as covariates. The introduction of the bedaquiline, pretomanid and linezolid (BPaL) regimen in national treatment guidelines is expected to occur during the historical period, in January 2025. To assess its potential impact, we will conduct subgroup analyses stratified by regimen type and perform sensitivity analyses excluding patients treated with BPaL or, alternatively, excluding those not treated with BPaL. If data allow, we will also examine process indicators, such as time to treatment initiation, time to DST results, frequency of culture conversion testing and the proportion of patients receiving standardised versus individualised regimens, to explore whether changes in care quality occurred over time between groups.

We also anticipate conducting analyses that control for differences in the proportion of participants experiencing homelessness, deprived of liberty, living with HIV and/or presenting different drug-resistance profile (RR, MDR, pre-XDR and XDR), as these variables may be prognostic of the treatment adequacy and outcomes and may differ between groups.[1 15 33] If possible, we may also run exploratory subgroup analyses to investigate differences in the outcomes according to the participants' drug resistance profiles.

Methods to handle protocol non-adherence and missing data

Because the intervention involves the delivery of a WGS report to physicians and participants in a pragmatic trial, specific strategies to decrease non-adherence to treatment will not be implemented. However, there may be non-adherence regarding the use of the WGS-based report by the responsible physician, that is, the physician does not use the information provided by the WGS to decide on the treatment plan. In the questionnaire that will be applied to the physicians, they will have to answer if they used the information to confirm or change the treatment plan or not. If data allow, we will perform two separate analyses: an intention-to-treat analysis, including all participants in the groups they were assigned to, regardless of whether the WGS report was used in treatment decisions and a per-protocol analysis, restricted to participants in the intervention group whose physicians used the WGS results to guide treatment. We will also check, through sensitivity analysis, the potential influence of any missing data.[21]

Plans to give access to the full protocol, participant-level data and statistical code

Only the healthcare professionals responsible for the care of the research participants and the research team will have access to participants' data. These data cannot be shared with other people. De-identified individual participant data underlying the results reported in this study can be made available to researchers who provide a methodologically sound proposal, whose proposed use of the data has been approved by an independent review committee, and who agree to a data use agreement. To gain access, data requestors will need to sign a data access agreement. The purpose of data sharing is to enable researchers to achieve the aims outlined in their study proposal. The study protocol, statistical analysis plan and analytic code can also be shared on request under the same conditions. Additionally, genomic data generated during the study will be deposited in public databases, such as the National Center for Biotechnology Information and/or the European Nucleotide Archive, with all patient identifiers removed to ensure confidentiality and privacy. Data will be made available beginning 6 months after publication and will remain accessible for 5 years thereafter.

Oversight and monitoring

Composition of the coordinating centre and trial steering committee

The coordinating committee will be composed of two researchers from the NTM-IAL and two researchers from ICB-USP, who will meet on a daily or weekly basis. A trial steering committee will be composed of two principal investigators, one from NTM-IAL and one from ICB-USP, one research coordinator from each medical centre and seven members of the Community Research Oversight Committee (Comitê Comunitário de Acompanhamento de Pesquisa (CCAP)). The CCAP will be composed of members of the community, including TB activists and survivors, with at least two individuals from each city involved in the trial, totalling seven individuals. In addition to the research coordinator and physicians, each medical centre will have a healthcare worker specifically hired to aid in data collection and management, as well as the overall trial organisation within each centre.

Composition of the data monitoring committee, its role and reporting structure

While no serious adverse events are expected from the intervention, data monitoring will be conducted by an independent statistician. Results will be presented to the coordinating and steering committees every 4 months. Interim analysis on treatment adequacy, sputum conversion, time from trial enrolment to the delivery of the WGS-based diagnostic report, the physician questionnaire and any reports of distress or other alterations provided by the responsible physicians will be evaluated by this independent statistician.

Adverse event reporting and harms

If any harm comes to the patient from the knowledge of the WGS results, such as distress for knowing that the resistance profile is more severe than previously thought, this will be reported in the data collection system designed for this study. In addition, an open-ended question about how the knowledge of the WGS result impacted the well-being of the patient will be part of the physician's questionnaire. All adverse events related to treatment are managed by the responsible physician of each patient and reported under SITE-TB.

Frequency and plans for auditing trial conduct

Data entered into the data collection system designed for this study will be checked weekly by the research team of the coordination centre. Monthly meetings will be performed with the representatives of each medical centre, NTM-IAL and the coordination centre. The trial will not undergo auditing by an independent team separate from the investigators. The sponsor will have no role in auditing the clinical trial.

Trial status

The trial has not started. The expected start date is April 2025, with an end date of October 2026. This trial is registered in Registro Brasileiro de Ensaios Clínicos under Universal Trial Number U1111-1308-4669.

Patient and public involvement

Members of the CCAP will be part of the trial steering committee, as described in the 'Methods and analysis' section. While not involved in the initial design of the study, they will follow all other stages of the research, which include overseeing and recommending potential changes in protocol if needed. They will meet monthly and visit each trial site once a year, and generate communication materials to the general public and research community about the research and antimicrobial resistance in TB (eg, Instagram account, LinkedIn account, podcast). Healthcare professionals will follow-up with the clinical trial participants regarding any burden of the intervention.

Ethics and dissemination

Research ethics approval

Ethical approval was obtained from the Human Research Committee of the ICB-USP, Brazil (CAAE: 79497924.1.1001.5467).

Protocol amendments

Any changes to the protocol that could impact the trial's design, execution or the potential risks and benefits to participants will necessitate a formal amendment. These amendments will be reviewed and approved by the Steering Committee and submitted for Research Ethics Committee approval before being implemented. These changes will be communicated via email or meetings to the relevant parties, including the medical centres, the NTM-IAL, the CVE-SP, the Health Secretariat of the State of São Paulo, the CCAP and other relevant associated committees.

Consent

Who will take informed consent and how?

Written informed consent form (online supplemental file 3) will be obtained during the prospective data collection from each participant and/or responsible guardian by trained healthcare professionals (nurse technicians, nurses or physicians) from each of the six medical centres. Participants will be invited to participate voluntarily in the research, without any monetary compensation or cost. People aged ≥6 years but <18 years will be invited to fill in an age-appropriate version of the form; their responsible guardian must also consent to their participation. Responsible guardians will also provide consent for people under 6 years of age or those unable to understand the study. The informed consent form covers the possibility of ancillary studies. The consent from participants in the retrospective control group will not be feasible; therefore, the Research Ethics Committee authorised the analysis of their data without directly asking each participant or responsible for consent. No clinical samples will be collected from participants of the retrospective group; only previously stored *M. tuberculosis* isolates, and secondary clinical and epidemiological data collected from national and state surveillance registers will be used.

Additional consent provisions for the collection and use of participant data and biological specimens

Participants' additional consent will be requested for the storage of the *M. tuberculosis* isolates in a biorepository at the NTM-IAL for 10 years and to access their medical records and other information entered in four notification systems: TBWeb, SITE-TB, SIM and GAL.

Confidentiality

We will follow the principles of the Brazilian Law for the General Protection of Personal Data (Lei Geral de Proteção de Dados Pessoais (LGPD), n° 13.709/2018) to protect the personal identification of patients. Only the healthcare professionals responsible for the care of the research participants and selected members of the

research team will have access to participants' personal information. After completion of the study, participants' data will be securely stored on a password-protected computer.

Declaration of interests

The authors, including the principal investigators, declare that they have no competing interests.

Access to data

Only the study and site coordinators will have access to the final trial dataset. Access to patient-level data will follow the Brazilian General Data Protection Law (LGPD, n° 13.709/2018).

Ancillary and post-trial care

Healthcare professionals will check whether the results provided by the WGS report provoked any kind of discomfort in research participants and offer mental health assistance if necessary, under the Brazilian SUS. No cost to participants is anticipated due to the present study. The trial is not insured for compensation to individuals who may be harmed by trial participation. Ancillary and post-trial care are not anticipated.

Dissemination policy and authorship

Study results will be published in peer-reviewed journals and disseminated to policymakers and stakeholders. An executive summary will be provided to the Brazilian Ministry of Health as part of the agreement with the sponsor. The CCAP will also develop activities to share study information with the community. Participant-level data cannot be shared.

Authorship eligibility for any resulting publications from this trial will require substantial contributions to the conception or design of the work; or the acquisition, analysis or interpretation of data; drafting or revising the work critically and final approval of the version to be published. All individuals meeting these criteria will be offered authorship, and all authors will be accountable for the integrity of the work. No professional medical writers will be used in the development of this protocol or in the preparation of any manuscripts or reports unless explicitly stated and appropriately acknowledged.

DISCUSSION

This will be the first clinical trial in Brazil to evaluate the impact of WGS-based antimicrobial resistance detection on TB treatment adequacy. In 2023, the state of São Paulo reported 182 cases of DR-TB.[34] The six medical centres participating in this study manage approximately 50% of these DR-TB cases, reflecting a significant proportion of the routine diagnostics conducted at NTM-IAL. In this study, a pragmatic clinical trial design was chosen to evaluate the diagnostic intervention in real-world conditions, integrating major medical centres and existing diagnostic workflows. It was designed to provide a realistic assessment of the effectiveness of WGS-based antimicrobial resistance detection, ensuring that this intervention can work effectively across diverse populations, settings and variables, benefiting everyone. This approach ensures that the generated evidence informs the potential integration of this diagnostic tool into São Paulo's routine practices in the future. Additionally, this study will help identify barriers to implementation, such as logistical challenges, resource limitations, and the acceptance and compliance of patients and healthcare professionals. By demonstrating real-world feasibility and benefits, we aim to build trust among policymakers, healthcare workers and the public, facilitating the adoption of WGS-based diagnostics in São Paulo and potentially other regions in Brazil.

With approximately 16 000 cases of TB reported annually,[15] universal culturing and phenotypic or genotypic drug-susceptibility testing are not feasible within the current laboratory infrastructure of São Paulo. As a result, the State Programme of Tuberculosis Control, managed by the State Secretariat of Health, has developed a selection algorithm to determine which cases are eligible for culture and drug-susceptibility testing. At NTM-IAL headquarters, isolates are received from the laboratory network solely for the purpose of conducting drug-susceptibility testing. The selection algorithm prioritises certain populations (box 1) and includes a combination of Xpert MTB/RIF Ultra (when available), LPA-1, LPA-2 (if resistance is detected in LPA-1) and phenotypic testing for selected second-line drugs. In the future, if WGS becomes a routine tool for detecting DR-TB, a similar selection algorithm will likely be used. Therefore, in our pragmatic trial, only clinical samples positive for rifampicin resistance via Xpert MTB/RIF Ultra, or isolates resistant or inferred to be resistant to any antimicrobial in LPA-1 and/or LPA-2, will be selected for WGS. This approach may miss DR-TB cases with other resistance profiles not detected by these tests. To assess the scope of undetected antimicrobial resistance, we will prospectively sequence 20% of isolates that are assumed to be drug-susceptible based on standard care, as part of a parallel study not described here.

This trial aligns with the latest recommendations for involving communities in biomedical research and clinical trials.[35] A Community Research Oversight Committee will play a pivotal role in guiding the study to address community needs and ensuring effective dissemination of findings to the broader public. Members of CCAP will conduct periodic visits to the medical sites, collaborating with coordinators and researchers to oversee all aspects of the project, including independent ethical reviews. To enhance community engagement, the research project has been named Gen-TB PróCura (Portuguese for 'Gen-TB Seeking a Cure'), and dedicated social media accounts have been established. These platforms will share posts, videos and podcasts to communicate research findings and promote general awareness about DR-TB.

Treatment outcomes (cure, death and other unsuccessful treatment outcomes) were included as secondary

outcomes of the trial. Given that DR-TB treatment outcomes are influenced by a wide range of patient-, disease-, treatment-, healthcare- and social-related factors, the independent impact of a WGS report on treatment success is uncertain and likely limited. Nevertheless, we opted to analyse these data to address any unbalance between control and intervention groups and to provide a complete picture of the outcomes of patients with DR-TB. However, it is important to note that Brazil introduced pretomanid into DR-TB treatment regimens in January 2025, which may introduce bias into this non-randomised study regarding treatment outcomes and sputum conversion. Since our trial will start in April 2025, the historical control group will include only a small number of patients from the first half of 2025 who underwent BPaL treatment. In contrast, we expect a great proportion of the patients in the intervention group to undergo BPaL treatment. Only a smaller proportion of the intervention group will not be eligible for BPaL treatment due to certain contraindications, including previous exposure to any of the BPaL drugs for more than 30 days, resistance to drugs in the BPaL regimen, patients under 14 years of age, patients who have experienced adverse events related to BPaL, and other factors. A comparison of treatment schemes before and after the introduction of BPaL is provided in online supplemental file 4.

It is also important to note that Brazil is currently adopting the BPaL regimen, not BPaLM. Therefore, fluoroquinolone resistance, which would compromise the moxifloxacin ("M") component, may have a greater impact on the historical control group, in which moxifloxacin-containing regimens were more frequently used (online supplemental file 4). In an unpublished study conducted by our group, 22 (17.9%) out of 123 MDR-TB patients treated in the state of São Paulo between 2022 and 2023 showed phenotypic fluoroquinolone resistance. BPaLM will likely be adopted as a standard treatment regimen in the future, but a start date has not been made available by the Brazilian Ministry of Health.

Uptake of WGS results by physicians will also be analysed as a secondary outcome in the intervention group. This provides insight into the real-world implementation of the diagnostic tool and helps contextualise differences between the intention-to-treat and per-protocol effects. Non-use of WGS may reflect systemic, behavioural or logistical barriers, and analysing these patterns may guide future implementation strategies. In addition, the selection algorithm may introduce bias by favouring the enrolment of more complex TB cases. Patients with DR-TB who are not diagnosed through the current standard of care but later fail treatment may be classified as re-treatment cases and only then become eligible for enrolment. At that point, the patient could have developed additional drug resistance. This means the patient originally had a simpler resistance profile that went undetected and later progressed to a more complex one, and only then was enrolled in the trial. This reflects a real-world diagnostic delay, and we may need to address it through appropriate analytical methods and careful selection of historical controls to ensure comparability. This decision will be made after data review.

This study has limitations. While it would be ideal to include all patients with DR-TB from the state of São Paulo in the trial, Brazil's SUS is highly decentralised, encompassing numerous community medical centres, clinics and hospitals. Many patients are treated outside large tertiary centres, making it infeasible to recruit all eligible patients with DR-TB into a clinical trial, as each centre requires independent ethics approval. Therefore, we selected six large tertiary centres, where the general infrastructure, including medical staff and ethics committees, could support the conduct of a clinical trial of this nature. Another limitation is the non-randomised design, with a retrospective control group. We acknowledge that randomised or stepped-wedge trials are generally stronger designs, but they were not feasible or ethical in this setting. DR-TB remains relatively rare in São Paulo (~200 cases/year), meaning that prospective randomisation, or even a stepped-wedge design, would require an extended time period of recruitment within the six medical centres, far exceeding our available funding and delaying results. Importantly, randomising patients or delaying access to WGS across centres would have required physicians to knowingly withhold potentially beneficial diagnostic information, raising ethical concerns regarding equitable access within participating centres. Considering the successful application of this technology to therapeutic decisions in other countries such as South Africa and the UK, we felt an ethical obligation to provide every participant from the medical centres the intervention as early as possible and simultaneously. We believe it would be unethical to randomise sites to wait for the intervention as it would be to randomise half of the participants to the intervention and half to standard diagnostic care.

A stepped-wedge design was also problematic given the chronic nature of TB treatment: patients enrolled during the control phase would often still be under care when their centre transitioned to the intervention phase. In such cases, patients who initially did not receive the intervention but later experienced poor outcomes might become eligible for re-enrolment under the intervention arm. This scenario introduces two key issues. First, treatment failures, potentially resulting from the absence of early WGS-based resistance profiling, could disproportionately accumulate in the intervention group, introducing systematic bias against the intervention. Second, it would require duplicative recruitment efforts and could cause discomfort or distress for patients who may feel disadvantaged by not having had access to the intervention earlier. These concerns, both methodological and ethical, underscore the limitations of the stepped-wedge design in chronic disease contexts and further justify our choice of an alternative study design.

Overall, the use of historical controls allowed us to include all previously treated patients in a comparable timeframe, increasing sample size, enabling timely study

completion, reducing regulatory burden and preserving ethical integrity, while still enabling evaluation. While this approach has some limitations, it represents a pragmatic balance of ethical, logistical and methodological considerations, and it aligns with the primary aim of this trial, which encompasses both treatment outcomes and the systematic assessment of assessment of workflow, physicians' perceptions and the integration of WGS into routine care.

Finally, the sensitivity of WGS for detecting antimicrobial resistance may be low for certain drugs, particularly newer ones for which not all resistance-associated mutations are known. In comparison with the phenotypic drug-susceptibility testing using BD BACTEC MGIT960, the bioinformatics pipeline to be used in this trial demonstrated sensitivities ranging from 33.3% for delamanid to 100% for ethambutol and moxifloxacin across 13 drugs tested (manuscript in preparation). Additionally, discrepancies between the current diagnostic workflows and WGS results may arise. These discrepancies will be addressed on a case-by-case basis, considering emerging evidence, the specific drugs involved and updated recommendations from WHO and the State Programme of Tuberculosis Control. This approach ensures that the interpretation of WGS results remains aligned with the latest scientific and clinical developments.

In conclusion, this trial, alongside the SMARTT (Sequencing Mycobacteria and Algorithm-determined Resistant Tuberculosis Treatment) trial being conducted in South Africa,[11] represents a critical effort to evaluate WGS-based detection of drug-resistant TB under real-world conditions in the Global South. By generating robust evidence on the feasibility and impact of this technology, these studies aim to bridge the gap between cutting-edge diagnostics and routine healthcare practices, guiding the individualised treatment of patients with TB. Nonetheless, the broader impact of WGS will depend on the feasibility of widespread implementation. In Brazil, significant barriers remain, including limited laboratory infrastructure, reliance on culture-based workflows and the absence of routine genomic surveillance, which must be addressed to fully realise the benefits of WGS in routine care. We expect to contribute to the broader global effort to eliminate TB as a public health concern, particularly in resource-limited settings where the burden of the disease remains high.

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