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Whole Genome Sequencing for Detecting Drug-Resistant Mutations in *Mycobacterium tuberculosis* in Zimbabwe

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ABSTRACT

Background: Drug-resistant tuberculosis (DR-TB) is a major public health issue in Zimbabwe. Whole-genome sequencing (WGS) is recommended for studying drug-resistant mutations, lineages, prevalence, and transmission patterns. However, there are limited data on drug-resistant conferring mutations and WGS use in Zimbabwe.

Aim: This study aimed to investigate the drug-resistant mutations, prevalence, and genetic diversity of DR-TB in Zimbabwe using WGS.

Methods: We conducted a retrospective study using WGS to analyze DNA extracted from 68 DR-TB isolates identified via phenotypic drug susceptibility testing (pDST) from studies conducted in Zimbabwe from 2011 to 2023. WGS was performed using an Illumina MiSeq machine (Illumina Inc., San Diego, California, USA).

Results: WGS identified 16 (23.5%) RR, 35 (51.5%) MDR, 5 (7.4%) Pre-XDR, 3 (4.4%) XDR, and 9 (13.2%) sensitive (non-resistant variants) isolates. The most frequent mutations were rpoB_Ser450Leu, Ser315Thr, embB_Met306Vol, rpsL_Lys43Arg, rrs_514A>C, and gid_102delG. The *mmpR5* p. Arg134 mutation conferred cross-resistance to bedaquiline and clofazimine. Concordant rates between WGS-based and pDST predictions of drug resistance were high for isoniazid (91.3%), rifampicin (86.3%), ethambutol (86.4%), fluoroquinolones (100%), linezolid (100%), and second-line injectables: amikacin (94.4%), kanamycin (91.7%), and capreomycin (100%). The study isolates belonged to lineages 1 to 4, with lineages 4 (n =38, 55.9%) and 2 (n =19, 27.9%) being the most predominant.

Conclusion: WGS provides valuable insights into the prevalence, drug-resistant mutations, and genetic diversity of DR-TB in Zimbabwe, thereby aiding the development of effective prevention, treatment, and control strategies.

Keywords: *Mycobacterium tuberculosis*, drug-resistant mutations, lineages.

Introduction

Tuberculosis (TB), a communicable disease caused by *Mycobacterium tuberculosis* (MTB), is a major cause of ill health and a leading cause of death worldwide (Global Tuberculosis Report 2024, n.d.; Yu *et al.*, 2022) Globally, in 2023, an estimated 10.8 million people were ill with TB, and there were an estimated 1.25 million deaths (Global Tuberculosis Report 2024, n.d.). Drug-resistant TB—in particular, rifampicin-resistant TB (RR-TB) and multidrug-resistant TB [(MDR-TB)—defined as resistance to both rifampicin and isoniazid]; extensively drug-resistant TB [(XDR-TB) defined as MDR-TB plus additional resistance to a fluoroquinolone and bedaquiline or linezolid or both OR formerly MDR-TB plus resistance to a fluoroquinolone and a second-line injectable] continues to challenge the control of TB (Viney *et al.*, 2021). Globally, in 2023, approximately 400,000 people developed MDR/RR-TB (Global Tuberculosis Report 2024, n.d.). Zimbabwe, a country on the World Health Organization (WHO) TB watch-list, has a TB prevalence of 275/100,000 population and an overall MDR-TB prevalence of 4.0% among new patients and 14.2% among retreatment TB patients, respectively (Timire *et al.*, 2019; Chipinduro *et al.*, 2022).

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The detection and treatment of MDR and XDR-TB remain global concerns. Although new treatment options and strategies for DR-TB are being proposed and recommended, access to the correct drug combinations is challenged by the limited availability of rapid and accurate drug-susceptibility testing platforms (Kadura *et al.*, 2020; World Health Organization 2023). Phenotypic drug susceptibility testing (pDST) remains the gold standard for detecting drug resistance (DR), but it is time-consuming and laborious. Rapid molecular assays such as GeneXpert/Ultra assays (Cepheid, Sunnyvale, CA, USA), the Hain GenoType MTBDRplus and MTBDRsl line probe assays (Hain Lifescience GmbH, Nehren, Germany), and INNO-LiPA Rif. TB (LiPA) are available, but they screen for DR mutations on a selected few *loci* of the TB genome, leaving many clinically relevant DR-associated *loci* unevaluated (Zijenah, 2018; Xiao *et al.*, 2023).

Whole-genome sequencing (WGS) detects the presence or absence of DR-conferring mutations, gives the identity of a mutation, and distinguishes between silent (mutation not associated with DR) mutations and those causing in vitro resistance (Curry International Tuberculosis Center, n.d.). The WGS has high concordance with pDST, offers detailed genomic data, identifies novel mutations, and detects very low levels of resistant variants missed by phenotypic growth methods (Colman *et al.*, 2015; 2016) It helps tailor individualized treatment for drug-resistant tuberculosis (DR-TB), improving patient outcomes (Mahomed *et al.*, 2017). The WGS also provides insights into strain types and transmission patterns, thereby aiding research and TB control programs (Brown *et al.*, 2015; Dippenaar *et al.*, n.d.)

In Zimbabwe, there is a paucity of data regarding DR mutation profiles of the prevailing DR-TB strains. A study conducted by Racheal *et al.* utilizing the Hain Line Probe Assay and GeneXpert to genotype MDR-TB isolates identified mutations conferring DR to Rif and INH only (Racheal *et al.*, 2015). Sagonda *et al.* (2014) identified three predominant lineages namely, LAM (50%), T-family (13.6%), and Beijing family (11.9%) among XDR strains using spoligotyping methods (Sagonda *et al.*, 2014). With WGS, a more accurate technique for describing the mutation profiles was developed. We therefore conducted a study using WGS to detect mutations conferring DR in the whole TB genome using RR, MDR, and XDR-TB isolates from previous studies conducted in Zimbabwe (Makamure *et al.*, 2023).

Materials and Method

Study setting

This study was conducted at the Biomedical Research and Training Institute (BRTI) laboratory in Harare, Zimbabwe. The BRTI laboratory is accredited for ISO 15189 standards of operations for TB diagnosis and is a center for Trials of Excellence in Southern Africa (TESA).

Study design and population

We retrospectively conducted WGS on DNA extracted from RR, MDR, and XDR-TB isolates identified by pDST from studies conducted in Zimbabwe from 2011 to 2023 and a known positive control. The studies included the Trap MDR-TB (TRP) study (2011–2015), the Zimbabwe National TB Prevalence (TBP) Survey (2014), the Zimbabwe MDR-TB (DRS) Survey (2015), TB diagnosis at BRTI [(NL) (2017)], the National Microbiology Reference Laboratory [(NMRL)-SP-(2019–2022)], and the National Tuberculosis Reference Laboratory [(NTBRL, SPC, 2020–2023)]. Participants were from both urban and rural settings. Phenotypic DSTs were performed following published guidelines (Canetti *et al.*, 1963; Makamure *et al.*, 2013)

Laboratory methods

From March 2021 to September 2023, 81 stored Mtb-Complex confirmed culture isolates and an H37Rv reference strain were re-cultured on the Löwenstein–Jensen slants with subsequent DNA extraction using the N-Cetyl-N,N,N-trimethylammonium-bromide (CTAB) method (Makamure *et al.*, 2023) The quantity and quality of the extracted DNA were measured using the qubit (Life Technologies, USA) and Nanodrop 2000 microfluidic (ThermoFisher Scientific, USA) instruments, respectively. DNA was shipped to the London School of Hygiene and Tropical Medicine (LSHTM), United Kingdom for WGS. Illumina library preparation kits (QIAseq and GeneRead Commercial Library Prep. Kits) were used to prepare DNA libraries following the manufacturer's instructions, and sequencing was performed using an Illumina MiSeq machine (Illumina Inc., San Diego, CA) following the manufacturer's protocol producing 2×151 base pair reads.

Bioinformatics analysis

The Public Health England bioinformatics pipeline at Genomics England processed the reads. The reads were trimmed using trimmomatic (v0.39) to remove low-quality data and adapter sequences (Danecek *et al.*, 2021; Ez, 2024). Trimmed reads were mapped to the H37Rv genome (NC_000962.3), which is phenotypically susceptible to all TB drugs, using the Burrows–Wheeler Aligner (BWA) (v0.7.17) and converted into bam alignment format using SAMtools (v1.10). Bcftools (v1.10) was used to call small variants {SNPs and insertion or deletions (indels)} and perform filtering based on allelic depth. Large deletions were detected using Delly (v0.8.1) and filtered using Bcftools. Variants were cross-referenced against the TBProfiler database tbdb (Phelan, 2025) to identify DR variants and ascertain the strain types. Mtb-Complex strains were stratified into lineages and subgroups using the classification schemes proposed by Napier *et al.* (2020).

To facilitate the phylogenetic cluster analysis, SNP positions within repetitive regions and DR–associated genes were excluded. An SNP-based phylogenetic tree was constructed using BCFtools (v1.10.2), SNP-sites (v2.5.1), and IQTree (v2.2.0) (Nguyen *et al.*, 2015) with the ModelFinder option and ascertainment bias correction. The ultrafast bootstrap (UFBoot) approximation was employed with 1000 replicates combined with a further optimization step to reduce the risk of overestimating the branch support. Phylogenetic trees were midpoint rooted using FigTree v1.4.4 (FigTree, n.d.), and nodes were arranged in increasing order. The tree was annotated using the EvolView online tool. A cluster threshold of 20 SNP difference or less was employed to cover a time span of 12 years of difference in sample collection periods.

Ethical approval

Approvals to conduct the study were obtained from the BRTI Institutional Review Board (AP155/2020), the Parirenyatwa Joint Research Ethics Committee (JREC/183/2021), and the Medical Research Council of Zimbabwe (MRCZ/A/2662). Participants provided consent to participate in the surveys. To maintain confidentiality, isolates were stripped of patient identifiers. Approval to ship samples was obtained from the MRCZ and the Research Council of Zimbabwe.

Results

Distribution of sequenced samples according to study name

In total, 68 isolates were sequenced. Among the isolates with known demographic data, 34/58 (58.6%) isolates were from males, 30/55 (54.5%) of participants were >34 years old, and 32/47 (68.0%) were HIV-positive. The distribution and demographics of the sequenced samples by study are shown in Supplementary Table 1. Of these 68 isolates, 38 (55.9%) were from lineage 4 (Euro American/Haarlem), 19 (27.9%) from lineage 2 (East Asian/Beijing), 8 (11.8%) from lineage 1 (Beijing), and 3 (4.4%) from lineage 3 (CAS/Delhi/CAS1). From lineage 4 sub-lineage 4.3.4.2.1, 13 (34.2%) was the most predominant, followed by sub-lineage 4.1.2, 7 (18.4%), with 5 (71.4%) isolates being MDR-TB. From lineage 2 sub-lineage 2.2.1, 17 (89.5%) with 10 (58.8%) isolates being MDR-TB were the most predominant. DR profiles were predicted from sequence data using TB-Profiler, which reported 16 (23.5%) RR-TB, 35 (51.5%) MDR-TB, 5 (7.4%) Pre-XDR-TB, 3 (4.4%) XDR-TB, and 9 (13.2%) sensitive results (Table 1). Of the nine sensitive samples detected, one was a known sensitive positive control, two had missing pDST results, and six had been falsely reported as RR-TB by the pDST from the same study.

Table 1. The distribution of drug resistance types according to lineage.

		TB Type					
Main lineage	**Sub lineage**	**MDR**	**Pre-XDR**	**RR-TB**	**Sensitive**	**XDR**	**Total**
lineage1	lineage1.1.3.2	1		2			3
	lineage1.2.2.1	5					5
lineage2	lineage2.2.1	10	2	4	1		17
	lineage2.2.2					2	2
lineage3	lineage3				2		2
	lineage3.1.1				1		1
lineage4	lineage4.1.1.3			2	1		3
	lineage4.1.2	5			2		7
	lineage4.1.2.1		1				1
	lineage4.1.4				1		1
	lineage4.3.4.1			2			2
	lineage4.3.4.2	1	1				2
	lineage4.3.4.2.1	6	1	4	1	1	13
	lineage4.4.1.1	3					3
	lineage4.8	1					1
	lineage4.8.1	2		1			3
	lineage4.9.1	1		1			2
Total		35	5	16	9	3	68

TB = Tuberculosis; MDR = Multidrug-resistant TB; Pre XDR-Extensively drug resistant; RR-TB = Rifampicin-resistant TB; XDR-Extensively drug resistant.

Drug resistance

First line

Resistance to rifampicin was most common, with 59 (86.8%) isolates containing at least one resistance mutation. The majority of 31/59 (52.5%) patients had the canonical S450L mutation in *rpoB*, with other mutations in the RR-DR having a lower frequency (Table 2). Isoniazid resistance was reported in 43

Table 2. Table showing the most common resistance mutations and confidence levels according to WHO grading.

Antibiotic	Gene	Change	Frequency	Confidence
Rifampicin	*rpoB*	p. Ser450Leu	31	High
		p. His445Asp	5	High
		p. Leu452Pro, p. His445Tyr, c.1297_1305delTTCATGGAC	4 each	Moderate
		p. Asp435Tyr, c.1312_1314delAAC,	3 each	Moderate
		p. His445Asn, p. Gln432Lys, p. Gln432Pro, p. His445Leu, p. Leu430Pro, p. His445Cys	1 each	Low
Isoniazid	*katG*	p. Ser315Thr	38	High
Isoniazid/ Ethionamide	*inhA*	c.-777C>T	5	High
		c.-770T>A	2	High
		c.-154G>A	1	Moderate
Ethionamide	*ethA*	p. Gln459*, c.65delA	1 each	Low
Ethambutol	*embB*	p. Met306Ile	17	High
		p. Gln497Arg, p. Met306Val	4 each	Moderate
		p. Gly406Ala	3	Low
		p. Gly406Ser	1	Low
Streptomycin	*gid*	c.102delG	4	Low
		p. Val105Glu, c.-674_248del, c.210_477del, c.386delG, p. Ala134Glu	1each	Low
	rpsL	p. Lys43Arg	12	High
Streptomycin, amikacin, and capreomycin	*rrs*	n.514A>C	5	Low
		n.1401A>G	1	High
Kanamycin				
Pyrazinamide	*pncA*	p. Thr160Pro, c.374delT	3 each	Low
		p. Cys14Arg	2	Low
		p. Gly78Ser, p. His71Tyr, p. Gly97Asp, c.64delA, c.515delT, c.459_466delCAGGGTGC, c.-11A>G, c.171_172insA	1 each	Low
(Fluoroquinolone)	*gyrA*	p. Asp94Gly	4	High
Moxifloxacin	*gyrA*	p. Asp94His, p. Ala90Val	2 each	High
Levofloxacin				
Bedaquiline	*mmpR5*	p. Arg134*	2	Low
		p. Glu138*, c.198dupG, c.141_142dupTC,	1each	Low
Clofazimine	*mmpR5*	p. Arg134*	2	Low
		c.198dupG, c.141_142dupTC	1each	Low

Table 3. Genotypic versus phenotypic resistance comparison among study isolates.

Drug	Numbers of isolates with phenotypic results	Concordant results	% Match
Rifampicin	51	44	86.3
Isoniazid	46	42	91.3
Ethambutol	44	38	86.4
Pyrazinamide	0	0	N/A
Moxifloxacin	2	2	100.0
Levofloxacin	2	2	100.0
Bedaquiline	2	2	100.0
Delamanid	0	0	N/A
Linezolid	1	1	100.0
Streptomycin	45	35	77.8
Amikacin	17	17	94.4
Kanamycin	24	22	91.7
Capreomycin	24	24	100.0
Clofazimine	2	2	100.0
Ethionamide	7	4	57.1

(63.2%) isolates (meaning 36.8% were RR but Isoniazid sensitive), with the majority 38/43 (88.4%) caused by *katG* S315T. Other mutations were also found in the *inhA* promoter region. Resistance to ethambutol and pyrazinamide was less frequent in 27 (39.7%) and 16 (23.5%) isolates, respectively.

Second line

Resistance to second-line treatment was also observed in 25 isolates carrying streptomycin resistance mutations in *rpsL, rrs*, and *gid* (Table 2). Resistance to the fluoroquinolones moxifloxacin and levofloxacin was found in eight isolates and was conferred through mutations in *gyrA*. Resistance to bedaquiline was found in three XDR isolates based on the *mmpR5* (*Rv0678*) gene. Two of the isolates carried the p. Arg134* mutation, and the remaining isolate carried the c.198dupG mutation, both conferring resistance to bedaquiline and clofazimine. A single mutation *(rrs* n.1401A>G) conferring cross-resistance to the SLIDs amikacin, kanamycin, and capreomycin was found in one sample. Finally, ethionamide resistance was found in nine samples and was conferred by mutations in the *ethA* and the *inhA* promoter region. Interestingly, a novel variant (p. Val75Gly) was detected in rplC, a candidate gene for linezolid resistance. Additional mutation (*ethA* p. Ala381Pro), which is currently annotated with "Uncertain significance" in the WHO catalog with respect to ethionamide resistance, was also detected; however, evidence from literature suggests an association (Klopper *et al.*, 2020; World Health Organization, 2023).

Genotype versus phenotype

Phenotypic resistance results were compared with genotypic resistance to characterize the agreement between the two methods. Concordance ranged from 57.1% to 100%, and isolates with phenotypic results varied by drug, ranging from 51 for rifampicin to 1 for linezolid (Table 3). Some samples did not have phenotypic results because either the results could not be obtained from the study documents or some samples had limited numbers of antibiotics tested.

Sample relatedness

Highly related genome sequences were characterized using an SNP distance of 20 and are presented in Figures 1 and 2. Eleven distinct clusters ranging between 2 and 4 isolates in size from 68 isolates were identified, giving a clustering rate of 25%. As expected, samples were clustered by lineages, with the largest clusters formed by lineages 2 and 4, each comprising four members. Additionally, several clades with short terminal branch lengths revealed high relatedness indicating that the isolates were linked by a recent transmission event, one of which was the largest lineage 4 cluster.

Discussion

This is the first study to demonstrate the use of WGS to detect mutations conferring DR in DR-TB in Zimbabwe and to determine the lineage and transmission patterns of DR-TB.

Patient demographics

The burden of DR-TB was higher in males than in females and in those aged >34 years. This finding is similar to that of the Zimbabwe TB prevalence Survey conducted in 2014 (Chipinduro *et al.*, 2022). This consistent pattern means that targeted interventions targeting males are crucial for effective TB prevention, care, and management. The gender disparity may be influenced by biological, behavioral, and socio-economic factors, (Marçôa *et al.*, 2018), whereas the age-related prevalence highlights the need for age-specific strategies to address TB. These findings underscore the importance of tailored public health policies and resource allocation to mitigate the impact of DR-TB in Zimbabwe."

Lineages

There was high genetic diversity from lineages 1 to 4, with the most predominant lineages being lineage 4 (55.9%) and lineage 2 (27.9%). While the dominance of lineages 2 and 4 in Southern Africa is known, this study provides specific evidence of their prevalence in Zimbabwe's DR-TB population over 12 years. These data are important for understanding the dynamics and potential transmission pathways within the country and contribute to confirming lineage trends at the national level, which is crucial for tailoring local TB control strategies. The predominant lineages found in Southern African countries, specifically Zambia, South Africa, and Mozambique are lineages 4, 2, and

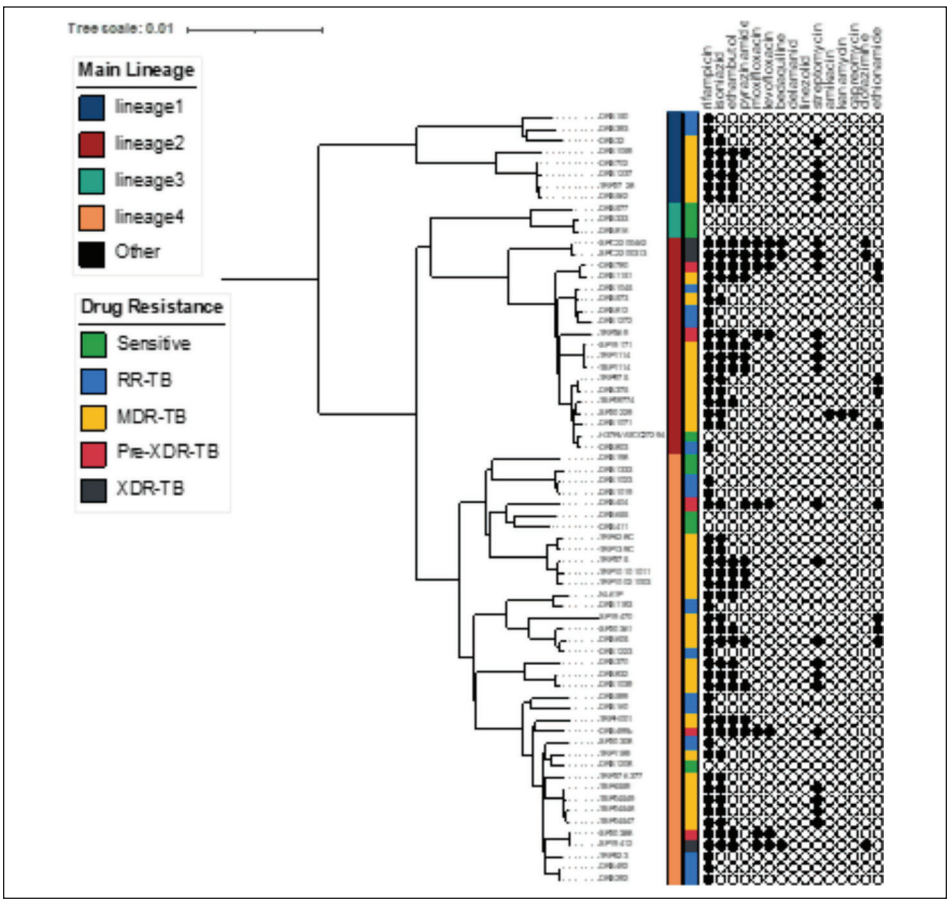

Fig. 1. Phylogenetic tree annotated with *Mycobacterium tuberculosis* complex lineages and drug resistance information.

1, respectively (Kone *et al.*, 2020) and also in other studies conducted ("Drug-Resistant Tuberculosis: A Survival Guide for Clinicians, 3rd edition/2022 Updates | Curry International Tuberculosis Center," n.d.), Liang *et al.*, 2023). The high migratory activity of Zimbabweans to and from these neighboring countries could account for the presence of these lineages in Zimbabwe, particularly the highly virulent lineage 4, which is most prevalent in Harare and Beitbridge compared with the other lineages (Supplementary Fig. 1). This could be because the international airport is in Harare, and Beitbridge is the Zimbabwe South African border post used by many vendors. We recommend TB control strategies that focus on border regions based on the well-documented role of cross-border movement in influencing TB transmission dynamics in Southern Africa, particularly in Zimbabwe, which shares porous borders with multiple high-burden countries. Although lineages 2 and 4 circulation may have historical origins, the persistence and potential amplification of these lineages in border areas warrant targeted interventions. However, this recommendation also requires further investigation.

Drug resistance

Among the 68 samples sequenced, MDR-TB had the highest prevalence (51.5%), followed by RR-TB (23.5%), Pre-XDR-TB (7.4%), and XDR-TB (4.4%). This study did not assess the overall burden of MDR-TB due to the non-representative sample. However, the results highlight resistance patterns among the isolates collected from the studied sample rather than national MDR-TB prevalence. Future studies with representative sampling are necessary to evaluate the burden accurately. The majority of MDR-TB cases were in lineage 4, 19/35 (54.3%), followed by lineage 2, 10/35 (28.6%), and the least in lineage 1, 6/35 (17.1%). Lineages 4 and 2 are also predominant in SA (Kone *et al.*, 2020), which has a known large burden of DR-TB. This could mean that there is significant cross-border transmission between the two countries. Lineage 1 has inherent resistance to nitroimidazoles, but it is rare in SA, which probably explains why it is the least prevalent.

The most frequently encountered mutations were those of RIF (*rpoB* p. Ser450Leu, 31/59 (52.5%); INH (*katG* p. Ser315Thr) in 38/43 (88.4%), EMB

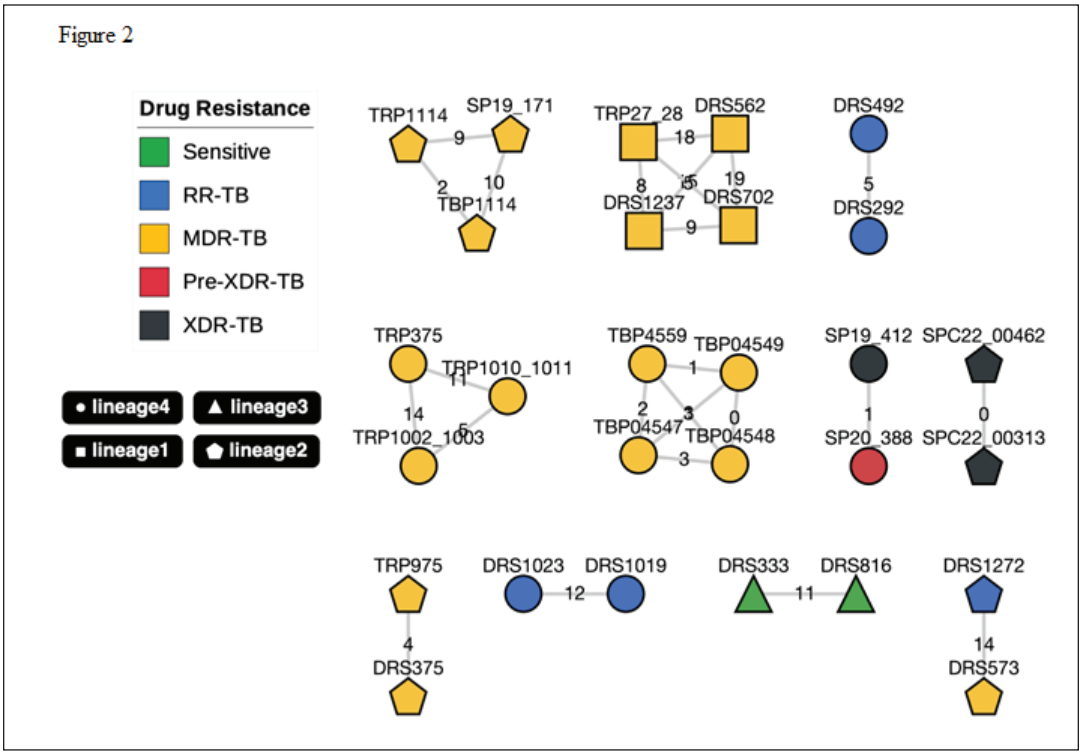

Fig. 2. Transmission clusters revealing potential recent transmission events. Isolates sequences included in the cluster analysis were from the following studies: Trap MDR-TB (TRP), the Zimbabwe National TB Prevalence (TBP) Survey, the Zimbabwe MDR-TB (DRS) Survey, the National Microbiology Reference Laboratory (SP), and the National Tuberculosis Reference Laboratory (SPC).

(*embB*p. Met306Vol) in 17/27 (63.0%), SM (*rpsL*p. Lys43Arg) 12/25 (48.0%), (*rrs*n.514A>C) 5/25 (20%), and (*gid*p.102delG) 4/25 (16.0%), as in the WHO guidelines and similar to other study findings (Racheal *et al.*, 2015; McNerney *et al.*, 2017; Curry International Tuberculosis Center, n.d.; World Health Organization, 2023; Xiao *et al.*, 2023; Liang *et al.*, 2023). However, there were other frequently encountered mutations in more drugs: fluoroquinolones (moxifloxacin and levofloxacin) *gyrA* p. Asp94Gly, 4/8 (50.0%), p. Asp94His 2/8 (25.0%), p. Ala90Val 2/8 (25.0%), and ethionamide *inhA* p. C777C>T 5/9 (55.5%), which were not listed in these studies. While *rpoB* S450L, *katG* S315T, *gyrA* D94, and i*nhA* C-15T mutations are well-documented globally, their high frequency in Zimbabwe underscores the importance of incorporating molecular diagnostic tools targeting these mutations into local TB diagnostic algorithms. The study identified the presence of "disputed" mutations in the *rpoB* gene (p. Leu430Pro, p. Asp435Tyr, p. His445Cys/Leu/Asn, and p. Leu452Pro) (Miotto *et al.*, 2018). These findings confirm the presence of these mutations in Zimbabwe, which can be falsely reported as sensitive using phenotypic methods. This result also supports the use of genotypic testing to accurately detect these mutations.

The significant proportion (36.8%) of isolates showing rifampicin resistance while remaining susceptible to isoniazid is surprising and notable resistance with isoniazid susceptibility is surprising and notable and challenges the current treatment algorithms that assume isoniazid resistance when RR is detected, especially with the use of Xpert MTB/Rif, the first-line TB diagnostic test in Zimbabwe. There is a need to explore the implications for diagnostic algorithms and treatment protocols and validate this finding in a larger, representative sample.

The significant proportion of streptomycin resistance mutations may reflect the drug-resistant regimens (aminoglycosides injectables) used during the study period. In this study, the observed bedaquiline-resistant mutations in the mmpR5 (Rv0678) gene also accounted for cross-resistance between clofazimine and bedaquiline. This study identified frequently encountered mutations in a wide range of antibiotics compared with findings from other studies suggesting a high burden of DR in Zimbabwe (Timire *et al.*, 2019). The novel variant detected in an XDR isolate in the linezolid resistance gene *rplC* requires further investigation to characterize the relationship with linezolid. In addition, the finding of *ethA* p. Ala381Pro, which was labeled with "Uncertain significance"

in the WHO catalog, but found to be associated with ethionamide resistance, highlights the careful consideration of all variants present, including those not associated with the catalog.

Phenotype versus genotype

In this study, there were high concordance rates between WGS-based prediction of DR with pDST for isoniazid (91.3%), rifampicin (86.3%), ethambutol (86.4%), fluoroquinolones (100%), linezolid (100%), and SLIDs amikacin (94.4%), kanamycin (91.7%), and capreomycin (100%). However, there were lower concordance rates for streptomycin (77.8) and ethionamide (57.1%). The high concordance between genotype and phenotype observed in this study validates the reliability of molecular diagnostics in the Zimbabwean context and supports their scale-up for rapid diagnosis and treatment.

The discordant results where WGS found six sensitive samples that had been reported as RR by the phenotypic method could be due to a technical error in the pDST method, as all the discordant results were from one study and from one antibiotic only.

Sample relatedness

To identify related isolates in our study, we calculated pairwise SNP distance. Usually, the threshold used to define recent transmission clusters is 12 or 5. In our study, the pairwise SNP distance used was 20, to identify highly related isolates, which were collected across a wide timeframe (2011 to 2023) (Makamure *et al.*, 2023). The cluster analysis results should be interpreted cautiously, considering the non-representative nature of the sample. The cluster analysis only provides insights into genetic relatedness, although the primary focus of this study is on genotypic drug susceptibility testing and the use of genetic methods.

Study limitations

We could not obtain the demographic and DST data for the isolates that were sequenced, which limited our analysis. The sample size was limited to only 68 due to constrained availability of archived DR-TB isolates, some samples failing to meet optimal concentrations or purity levels, and limited resources for WGS. The limited number of isolates and sampling bias significantly limited the generalizability of the results. A larger, stratified sample would improve the epidemiological conclusions. However, our study demonstrated the use of WGS to identify resistance mutations and potential transmission clusters. Although these findings are not representative of the national prevalence, they provide evidence for applying WGS in Zimbabwe's TB surveillance and control programs.

The WGS data were limited to isolates of RR-TB, MDR-TB, Pre-XDR-TB, and XDR-TB. This excluded isoniazid-resistant, rifampicin-susceptible TB (Hr-TB), which is another form of DR-TB. This form of DR is missed during routine program settings because the first-line Xpert MTB/Rif diagnostic test does not detect isoniazid resistance profiles. There was a lack of sufficient numbers of second-line pDSTs for newer repurposed drugs in the current DR-TB regimen bedaquiline, pretomanid, linezolid, and moxifloxacin (BPaLM); therefore, we could not assess the performance of WGS on repurposed drugs in the DR-TB regimen comprehensively.

Conclusion

Using WGS, we provided comprehensive genomic data on DR mutations for both first- and second-line antibiotics used in treating TB, along with insights into the lineages, strain types, and transmission patterns of DR-TB in Zimbabwe. Our findings revealed high diversity of the four major lineages (L1–L4) and a significant prevalence of multidrug-resistant TB (MDR-TB) in Zimbabwe.

We recommend incorporating WGS as an additional layer in future DR-TB surveys, research, monitoring of TB treatment failures, and applications in TB control programs to ultimately end TB.

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Authors' contributions

Beauty Makamure designed the study, collected the data, retrieved samples from the archives, resuscitated them, performed WGS, analyzed the data, and wrote the first draft of the manuscript. Isabel Mashita collected some of the samples from the archives. Forget Makoga extracted the DNA. Dan Ward performed the WGS. Jody Phelan, Tsitsi Bandason, and Martha Chipinduro analyzed the data. Pasipanodya Nziramasanga, Junior Mutsvangwa, John Z Metcalfe, Jody Phelan, and Justen Manasa supervised the study. Martha Chipinduro, Collins Timire, Stephen Stephen, and Shungu Munyati contributed to the writing of the manuscript.

Conflict of interest

All authors declare that they have no conflicts of interest related to this study. None of the authors have any financial, personal, or other relationships with individuals that could influence, or be perceived to influence their work.

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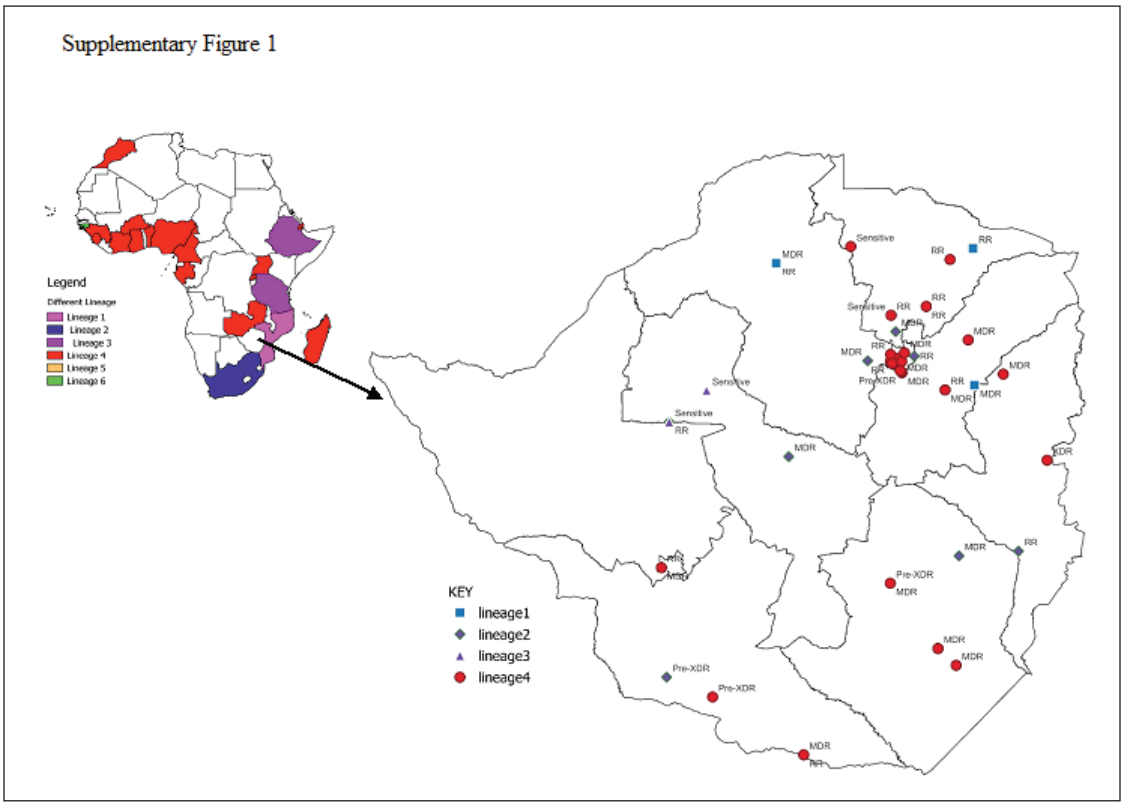
Supplementary Material

Supplementary Table 1. Table showing the distribution of sequenced samples according to study name, gender, HIV status, and age.

		Study Name						
		TRP	**TBP**	**DRS**	**NL**	**SP**	**H**	**Total**
Samples with DNA extracted		22	7	40	1	11	1	82
Samples with optimal concentrations or purity levels of WGS		13	6	38	1	9	1	68
Sex	Male	6	3	21	1	4	-	34
	Female	7	3	12	0	2	-	24
	Unknown	0	0	5	0	3	-	9
HIV status	Positive	7	0	22	0	3	-	32
	Negative	6	1	7	0	1	-	15
	Unknown	0	5	9	1	5	-	20
Age (years)	<25	1	0	5	0	0	-	6
	25-34	3	1	13	0	2	-	19
	>34	9	5	12	0	4	-	30
	Unknown	0	0	8	1	3	-	12

TRP: Trap MDR-TB Survey; TBP: Zimbabwe National TB Prevalence Survey; DRS: Zimbabwe MDR-TB Survey; NL: Routine workout at BRTI; SP: Routine workouts at NMRL and NTBRL; H: Positive control H37RVATCC27294; HIV: Human immunosuppressing virus; DNA: Deoxyribonucleic acid; WGS: Whole Genome Sequencing.



Supplementary Fig. 1. The distribution of *Mycobacterium tuberculosis* complex lineages in Zimbabwe compared with the main lineages found in other African countries.