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Clinical application of whole-genome sequencing in the management of extensively drug-resistant tuberculosis: a case report

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Abstract

Background Whole-genome sequencing (WGS)-based prediction of drug resistance in *Mycobacterium tuberculosis* has the potential to guide clinical decisions in the design of optimal treatment regimens.

Methods We utilized WGS to investigate drug resistance mutations in a 32-year-old Tanzanian male admitted to Kibong'oto Infectious Diseases Hospital with a history of interrupted multidrug-resistant tuberculosis treatment for more than three years. Before admission, he received various all-oral bedaquiline-based multidrug-resistant tuberculosis treatment regimens with unfavourable outcomes.

Results Drug susceptibility testing of serial *M. tuberculosis* isolates using Mycobacterium Growth Incubator Tubes culture and WGS revealed resistance to first-line anti-TB drugs, bedaquiline, and fluoroquinolones but susceptibility to linezolid, clofazimine, and delamanid. WGS of serial cultured isolates revealed that the Beijing (Lineage 2.2.2) strain was resistant to bedaquiline, with mutations in the *mmpR5* gene (Rv0678. This study also revealed the emergence of two distinct subpopulations of bedaquiline-resistant tuberculosis strains with Asp47f and Glu49fs frameshift mutations in the *mmpR5* gene, which might be the underlying cause of prolonged resistance. An individualized regimen comprising bedaquiline, delamanid, pyrazinamide, ethionamide, and para-aminosalicylic acid was designed. The patient was discharged home at month 8 and is currently in the ninth month of treatment. He reported no cough, chest pain, fever, or chest tightness but still experienced numbness in his lower limbs.

Conclusion We propose the incorporation of WGS in the diagnostic framework for the optimal management of patients with drug-resistant and extensively drug-resistant tuberculosis.

Keywords Whole-genome sequencing, XDR-TB, Genomic Diagnostics, Case Report, Clinical application

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Introduction

Tuberculosis (TB) is a highly contagious bacterial disease caused by *Mycobacterium tuberculosis*. TB has been a significant global health concern for centuries, and it continues to be a major cause of illness and death worldwide [1]. The discovery of streptomycin in the mid-20th century marked a turning point in TB treatment, making it one of the first diseases to be treated with antibiotics [2, 3]. However, over time, strains of TB have developed resistance to various antibiotics, leading to the emergence of drug-resistant (DR) forms of the disease [4–8].

Multidrug-resistant TB (MDR-TB) is defined as TB that is resistant to rifampicin and isoniazid [9]. In addition, pre-extensively drug-resistant TB (Pre-XDR-TB) is a TB that is resistant to rifampicin and any fluoroquinolone (a class of second-line anti-TB drugs) [9]. Extensively drug-resistant TB (XDR-TB) is the most severe form of drug-resistant TB and is defined as TB resistant to rifampicin plus any fluoroquinolone and to at least one of either bedaquiline or linezolid [9]. Recently, limited treatment options [10], increased treatment complexity and cost [11], increased rates of treatment failure and mortality [12], and the potential for further transmission of resistant strains [13] of drug-resistant TB (DR-TB) have been of great concern because they can undermine TB control efforts globally. Healthcare systems need to strengthen TB surveillance, improve diagnostic capabilities, ensure access to effective treatment regimens, and implement infection control measures to prevent the spread of DR-TB strains [14, 15].

The emergence of XDR-TB is primarily attributed to the improper use of antibiotics in TB treatment, inadequate supervision of treatment regimens, and a lack of comprehensive healthcare systems. The prevalence of XDR-TB varies across different regions, with higher rates found in parts of Eastern Europe, Asia, and Africa [9]. In Tanzania, the trend of MDR/Pre-XDR-TB notifications has been increasing in the past 7 years, with a slight decrease between 2019 and 2021 [16]. According to the Tanzania National Tuberculosis and Leprosy Programme (NTLP) 2021 report, 6 Pre-XDR-TB patients were detected among 442 RR/MDR/Pre-XDR-TB patients identified in 2021 [16].

Efforts to address pre-XDR-TB include improving diagnostic capabilities, enhancing surveillance systems, developing new drugs and treatment regimens, and strengthening healthcare infrastructure to ensure proper treatment and prevent further spread [17, 18]. Combating pre-XDR-TB and XDR-TB requires a multifaceted approach involving international collaboration, research, healthcare system strengthening, and addressing socioeconomic factors that contribute to its persistence and spread.

The World Health Organization (WHO) has established guidelines for the disease diagnosis and management of TB. Clinical diagnosis of DR-TB in Tanzania, as reported in other resource-limited countries, relies on microscopy, culture, and molecular diagnostic assays such as the Xpert MTB/RIF and line probe assays. Molecular assays such as the Xpert MTB/RIF and line probe assays have more variable sensitivities for TB detection [19, 20]. In developed countries with robust healthcare systems, WGS plays a pivotal role in augmenting diagnostic accuracy and treatment precision for DR-TB patients [21–24]. However, in low-income settings with high TB incidences, the clinical application of WGS for the diagnosis and management of DR-TB patients can be challenging due to obstacles such as limited infrastructure, cost constraints, and technical expertise [23–25].

This case study presents the case of a patient with TB who first presented with MDR-TB but, due to inadequate adherence to treatment, evolved to XDR-TB. To understand the microevolution of the XDR-TB infection, this study investigated the evolutionary changes in *M. tuberculosis* in eight isolates obtained over two consecutive years (Table 1) by using WGS. This study reports the genotypic mutations and microevolution of two distinct XDR subpopulations from the same strain in serial isolates and highlights the potential of WGS in shaping the management and control of XDR-TB.

Case presentation

A 32-year-old man from Tanzania was transferred to Kibong'oto Infectious Diseases Hospital after his second-line treatment for drug-resistant TB had failed for 16 months. Upon admission, he exhibited symptoms including a productive cough, chest pain, chest tightness, fever, and weight loss. Microscopy and LJ cultures yielded positive results. He had no prior history of HIV, diabetes, or hypertension and did not smoke cigarettes or drink alcohol.

During the physical examination, the patient exhibited normal vital signs, including a normal temperature (36.7 °C), pulse rate (73 bpm), blood pressure (117/71 mmHg), oxygen saturation of 97% on room air, and respiration rate (17 breaths/minute). His weight was 65 kg, and his height was 1.69 m, equivalent to a body mass

Table 1 Serial sputum samples collection dates

Sputum Sample ID	Collection Date
1	May 2021
2	June 2021
3	April 2022
24	June 2022
4	August 2022
5	October 2022
6	November 2022
7	March 2023

index of 23 kg/m^2. Respiratory examination revealed decreased air entry into the left chest. His electrocardiogram, liver and kidney function tests and electrolyte levels were all within the normal range. However, he presented with leukocytosis, neutrophilia (8.6), and lymphopenia (11.4%). Chest radiography revealed cavitary disease and prominent fibrotic changes in the right lung (Fig. 1).

Since his initial MDR-TB episode in 2019, the patient has undergone various treatment regimens for either pre-XDR-TB or XDR-TB, with inconsistent treatment durations and outcomes documented in Table 2. Monthly monitoring utilizing clinical assessments, smear microscopy, and culture in MGIT indicated improvement with the revised medications. Particularly in his current XDR-TB regimen, which was tailored based on WGS results, the patient experienced severe neuropathy associated with a high dose (1200 mg) of linezolid at month 4 of therapy, which was then reduced to 600 mg for an additional 2 months. By month 6 of XDR-TB treatment, the patient developed mild hearing loss attributed to the injection of 1 gm of amikacin. e treating clinicians adjusted the frequency of amikacin daily to three times per week until month 8 of therapy, when it was completely discontinued. e patient was discharged home at month 8 and is currently in the ninth month of treatment. He reported no cough, chest pain, fever, or chest tightness but still experienced numbness in his lower limbs.

Methods

Culture and phenotypic testing

Eight spot and early morning sputum samples from the patient were collected over two consecutive years (Table 1) and processed by Petro 's method according to the manufacturer's instructions [26]. e culture, identification, and DST of the XDR-TB isolates were performed at the Central Tuberculosis Reference Laboratory, Muhimbili National Hospital, Dar es Salaam, Tanzania, in **M**ycobacterium **G**rowth **I**ncubator **T**ubes (BBL™ MGIT™ Becton–Dickinson, Sparks MD, USA) on Middlebrook 7H11 agar and selective 7H11 agar following the manufacturer's instructions. Drug susceptibility testing (DST) was conducted in MGIT™ at the recommended critical concentrations [27

Table 2 The patient's previous history of mycobacterial diagnosis, multiple treatment regimens, and inconsistent outcomes

Years	Form of TB	Presentations	Diagnostic tool	Treatment Regimens	Progress and actions	Final outcome
October 2019	MDR-TB	TB symptoms and signs	Xpert® MTB/RIF	BDQ + LZD + LFX + PZA + CS + CFZ	• Lost to follow up after 7 months in April 2020	Lost to follow up
May 2021	MDR-TB	TB symptoms and signs reverted	Xpert® MTB/RIF, smear microscopy and culture for monitoring	BDQ + LZD + LFX + CFZ + PTO + PZA	• Converted to culture negative at month 4 but reverted to positive at month 8 through month 14. • Phenotypic DST in LJ culture revealed resistant to isoniazid, fluoroquinolones and ethambutol but susceptible to injectable aminoglycosides (amikacin, kanamycin & capreomycin) and ethionamide	MDR-TB Treatment failure
September 2022	Pre-XDR-TB	TB symptoms and signs	DST in LJ Culture Xpert® MTB/XDR	BDQ + LZD + LFX + CFZ + PTO + PZA + PAS + DLM	• Culture remained positive for 7 months from until September 2022 to April 2023 • WGS of cultured isolates performed in April 2023 revealed resistant to BDQ, CFZ, fluoroquinolones (LFX), SM, EMB, ETH, INH, and RIF.	Pre-XDR TB treatment failure
April 2023	XDR-TB	TB symptoms, signs and culture positivity	Whole genome sequencing of cultured isolates	Amikacin 1 g, LZD (1200 mg), PAS 4 mg daily, DLM 100 mg twice per day & CS 750 mg daily.	Achieved culture conversion at month 2. He has been discharged home and now at month 9. TB symptoms has resolved and culture remained negative	Continuation phase of optimize treatment regimen

Anti-TB medicines: BDQ, bedaquiline; CFZ, Clofazimine; CS, Cycloserine; DLM, delamanid; EMB, ethambutol; ETH, Ethionamide; INH, isoniazid; LFX, Levofloxacin; LZD, Linezolid; PAS, para-amainosalicylic acid; PTO, prothionamide; PZA pyrazinamide; RIF, rifampicin and SM, streptomycin

DNA extraction and quantification

The bacteria were grown on Löwenstein–Jensen slants at 37 °C until colonies were visible. Colonies were then scraped from the surface of the solid medium using a sterile loop, suspended in 400 mL Tris-EDTA (TE) buffer, and heat-killed at 90 °C for 30 min. Genomic DNA was extracted at the Genomic Laboratory at Muhimbili University of Health and Allied Sciences by using a modified protocol adopted from the Centre for Clinical Microbiology (CCM), University College London (UCL) [28]. Briefly, lysozyme (50 µL, 10 mg/mL), 5 µL of proteinase K (20 mg/mL), 70 µL of SDS (10% w/v) and 100 µL of prewarmed CTAB/NaCl (10% CTAB in 0.7 M NaCl) were added and then incubated at 60 °C for 10 min, 750 µL of chloroform/isoamyl alcohol (24:1 v/v) was added, and the debris was pelleted by centrifugation for 5 min at 10,000×g. Ice-cold isopropanol (450 µL) was added to the aqueous supernatants, which were subsequently centrifuged at 10,000×g for 5 min before the pellets were washed with 1 mL of 70% ethanol. DNA pellets were rehydrated in 100 µL of molecular grade water overnight at 4 °C. DNA was quantified using NanoDrop™ spectrophotometry and the Qubit™ dsDNA BR Assay Kit (Thermo Fisher). The size and integrity of the DNA fragments were confirmed using genomic DNA reagents and ScreenTape from the TapeStation 4150 (Agilent Technologies Inc.). DNA extractions were shipped to CCM and UCL for sequencing.

Library preparation and sequencing

Libraries were prepared from the MTB DNA extracts using the PathHiT protocol, which is a modification of CoronaHiT [29], for subsequent sequencing on Illumina sequencers. Sequencing of the prepared libraries was carried out in the UCL Genomics unit using a MiSeq, NextSeq 500/550 or NextSeq 2000 sequencer (Illumina) and the most appropriate sequencing kit (with a minimum of 150 cycles, i.e., generating 75 bp paired-end reads) depending on the number of samples and aiming for 100X mean read depth. Sequences were analysed at CCM and UCL and deposited in the European Nucleotide Archive (ENA) (project accession number: PRJEB74918).

Bioinformatics analysis

Sequence reads were mapped to the H37Rv reference genome (RefSeq accession: NC_000962.3) using bwa mem v.0.7.17 [30], and alignments were sorted using samtools v.1.12 [31]. Site statistics were generated using BCFtools mpileup v.1.12, and gene annotations were generated using snpEff (v.4.3.1t) software [32]. Sequence purity was determined using Kraken2 [33] to exclude potential contamination with nontuberculous mycobacterial sequences. Variants in the genes conferring resistance to pretomanid and bedaquiline and

upstream regions, as described in the second edition of the WHO catalogue of mutations [34], were investigated.

The genotype of the drug resistance-conferring variants was determined using TB-profiler v.5.0.1/database tbdb_e25540b_Oct 26, 2023 and Mykrobe v.0.13.0 [35].

The percentage of variants of interest in the genes conferring resistance to bedaquiline was verified by viewing the BAM files against the H37Rv reference genome (RefSeq accession: NC_000962.3) using the genome viewer IGV v.2.15.2. The percentages of variants were calculated from the proportion of sequence reads showing variants of interest in relation to the sequence depth at the corresponding genomic position. Maximum likelihood phylogenetic tree was constructed using IQ-TREE (v2.0.3) with a general time reversible model of nucleotide substitution (model selection restricted to those supported by RAxML); branch support values were determined using 1000 bootstrap replicates) [36]. Phylogenetic comparisons for the tree construction filter out all mixed sites and INDELS.

Results

WGS analysis revealed that all eight (8) serial isolates were pure *M. tuberculosis*; lineage 2 (Beijing); sublineage 2.2, with good coverage > 99.5% and a mean depth of sequencing ≥ 100 x. All isolates, apart from isolate number 24, were closely related to very small genetic distances in the phylogenetic tree (Fig. 2). According to the sequence data for isolate number 24, 7% of the reads were classified as lineage 4 (Latin American Mediterranean, LAM). The results also indicated that the MTB evolved from MDR to Pre-XDR to XDR (Fig. 2). Subsequent resistance mutations in the gyrA gene (p.Asp94Gly) against moxifloxacin gave rise to pre-XDR strains, and further

mutations in gyrA (p.Asp94Gly) against the levofloxacin gene facilitated the emergence of XDR strains.

WGS revealed the presence of mutations conferring resistance to the following anti-tuberculosis drugs: rifampicin, isoniazid, pyrazinamide, ethambutol, streptomycin, fluoroquinolones, ethionamide, cycloserine, clofazimine, and bedaquiline in all the serially tested isolates. Mutations were detected in the *rpoB*, *katG*, *pncA*, *embB*, *gid*, *gyrA*, *ethA*, *ald* and *mmpR5* genes. The specific variants involved in drug resistance are listed in Table 3. A subpopulation (17%) of the 6th (PR0943-5) and 7th (PR0943-6) isolates developed a frameshift mutation in the *ald* gene due to the duplication of 2 "GC" bases in coding positions 436–437 of the gene, which is associated with resistance to cycloserine.

Longitudinal analysis of the eight serial isolates over two years revealed a dynamic picture of the evolution of resistance to bedaquiline, fluoroquinolones and cycloserine in the *M. tuberculosis* population. The evolution of bedaquiline resistance started in the third isolate (PR0943-3) through the emergence of a subpopulation with a frameshift mutation (Asp47f) in the *mmpR5* gene (Rv 0678) due to the insertion of 2 bases, "GA", at positions 139–140 in the coding region of the gene. Another distinct subpopulation emerged in the fifth isolate (PR0943-4) with a frameshift mutation (Glu49fs) in the *mmpR5* gene (Rv 0678) due to the insertion of a cytosine at position 144 in the coding region of the gene. No single read in the six serial isolates carried both alleles on the same sequencing read (Fig. 3), which indicates that these were two distinct subpopulations. Over time, the first subpopulation diminished, with the frequency of the reads carrying this allele gradually declining in the subsequent isolates, while the second subpopulation increased gradually and predominated the population in the eighth
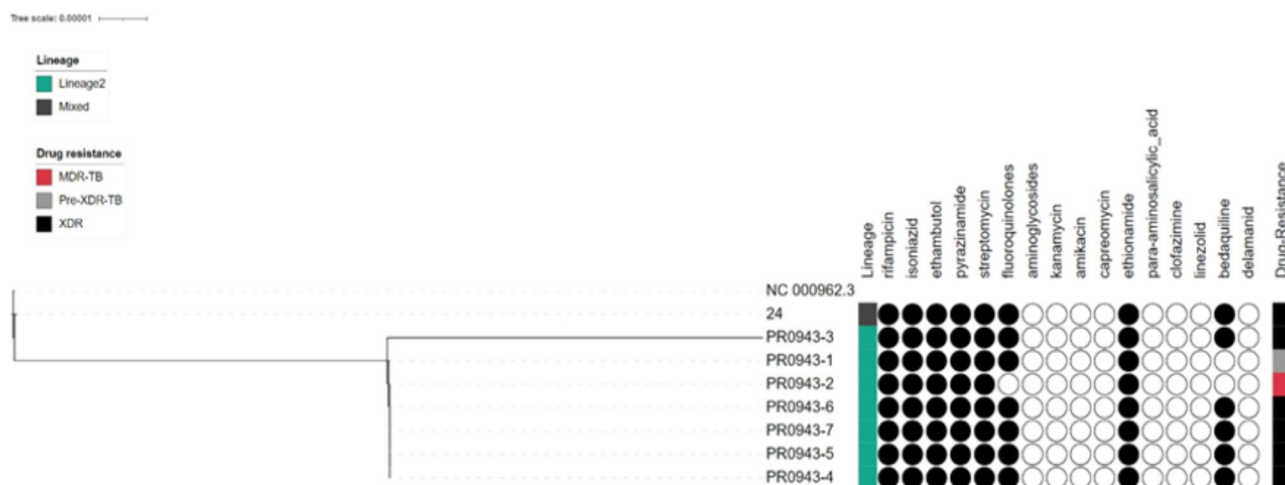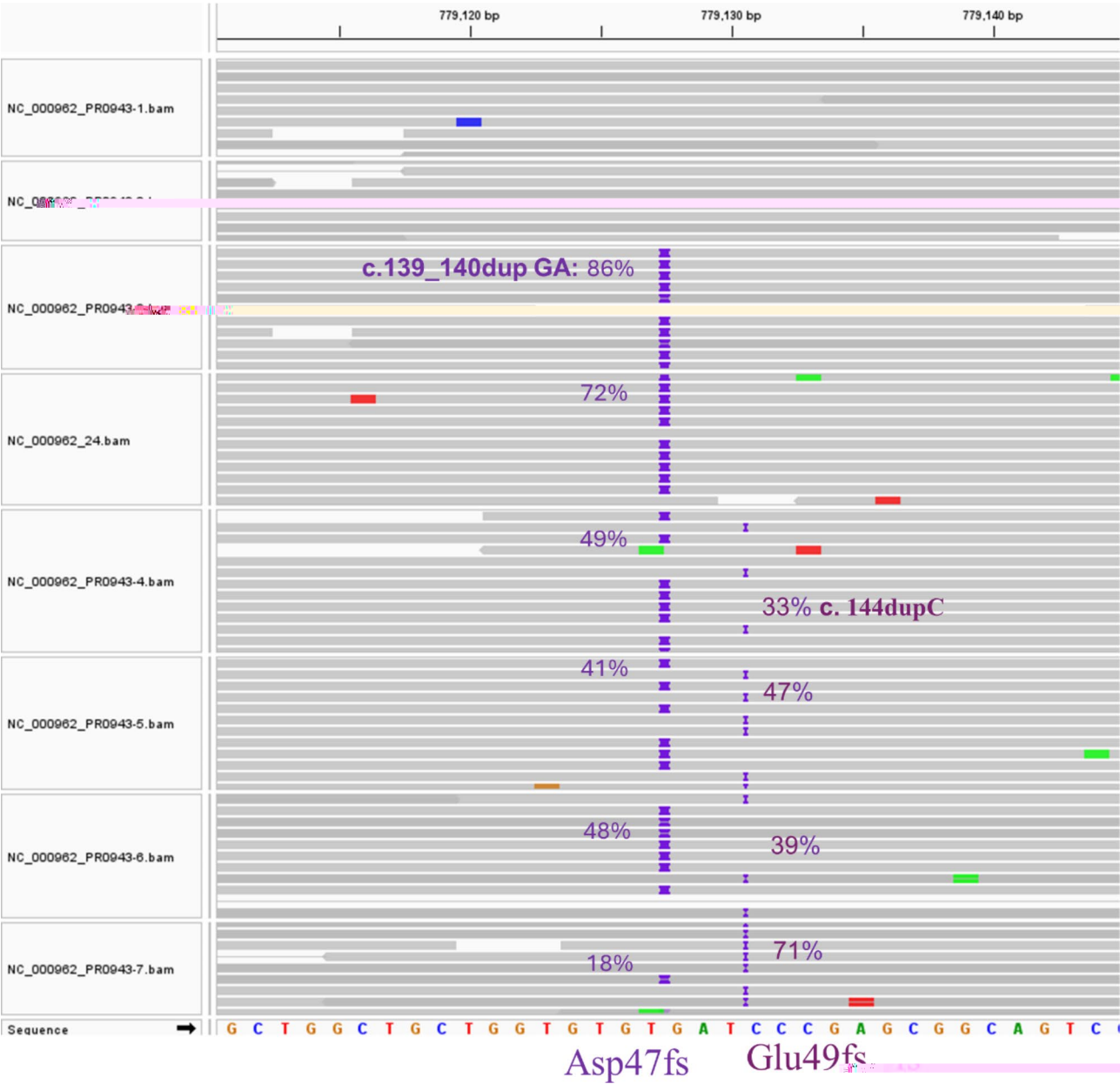

Fig. 2 Phylogenetic relationship between the eight serial *M. tuberculosis* isolates collected over two years from the presented case and genotypic drug resistance pattern: black circle: drug resistant variants were detected, white circles: susceptible

Table 3 Comparison of genotypic and phenotypic drug resistance profiles of *M. tuberculosis* strains using whole genome sequencing

Drugs	Genotype		Genotypic mutations in drug targets								Phenotypic DST							
			PR0943-1	PR0943-2	PR0943-3	PR0943-24	PR0943-4	PR0943-5	PR0943-6	PR0943-7	1	2	3	24	4	5	6	7
Isoniazid	*katG*		p.Ser315Thr	p.Ser315Thr	p.Ser315Thr	p.Ser315Thr	p.Ser315Thr	p.Ser315Thr	p.Ser315Thr	p.Ser315Thr	R	R	R	R	R	R	R	R
Rifampicin	*rpoB*		p.Ser450Leu	*p.Ser450Leu*	p.Ser450Leu	p.Ser450Leu	p.Ser450Leu	p.Ser450Leu	p.Ser450Leu	p.Ser450Leu	R	R	R	R	R	R	R	R
Ethambutol	*embB*		p.Gln497Lys p.Met306Ile	Gln497Lys Met306Ile	p.Gln497Lys p.Met306Ile	p.Gln497Lys p.Met306Ile	p.Gln497Lys p.Met306Ile	p.Gln497Lys p.Met306Ile	p.Gln497Lys p.Met306Ile	p.Gln497Lys p.Met306Ile	R	R	R	R	R	R	R	R
Pyrazinamide	*pncA*		p.Cys14Arg	Cys14Arg	p.Cys14Arg	p.Cys14Arg	p.Cys14Arg	p.Cys14Arg	p.Cys14Arg	p.Cys14Arg	R	R	R	R	R	R	R	R
Streptomycin	*gid* *rrs*		p.Leu79Ser n.514 A>C	Leu79Ser n.514 A>C	p.Leu79Ser n.514 A>C	p.Leu79Ser n.514 A>C	p.Leu79Ser n.514 A>C	p.Leu79Ser n.514 A>C	p.Leu79Ser n.514 A>C	p.Leu79Ser n.514 A>C	R	R	R	R	R	R	R	R
Flouroquino- lones	*gyrA*		p.Ala90Val (10%) *	-	p.Asp94Gly	p.Asp94Gly	p.Asp94Gly	p.Asp94Gly	p.Asp94Gly	p.Asp94Gly	R	R	R	R	R	R	R	S
Bedaquiline	*MmpR5 (Rv0678)*		-	-	c.139_140dup GA: p.Asp47f	c.139_140dupGA: p.Asp47fs	c.139_140dupGA: p. Asp47fs	c.139_140dupGA: p.Asp47fs	c.139_140dupGA: p.Asp47fs	c.139_140dupGA: p.Asp47fs	S	S	S	S	S	S	R	S
							c.144dupC; p.Glu49fs	c.144dupC; p.Glu49fs	c.144dupC; p.Glu49fs	c.144dupC; p.Glu49fs								
Clofazimine			-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S
Delamanid			-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S
Levo oxacin	*gyrA*		p.Ala90Val (10%) *	-	p.Asp94Gly	p.Asp94Gly	p.Asp94Gly	p.Asp94Gly	p.Asp94Gly	p.Asp94Gly	S	S	S	S	R	S	R	R
Linezolid			-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S

S = culture sensitive

R = culture resistant

*% variant

Fig. 3 The evolution Bedaquiline resistance in the presented case through the emergence of two distinct subpopulations: the first developed Asp47fs mutation through insertion of 2 bases "GA" at genomic position 779,127 and the second with Glu49fs through insertion of 1 base "C" at genomic position 779,130. Across the subsequent *M. tuberculosis* isolates the first subpopulation was diminishing while the second was rising; the percentages of both variants are displayed on each sequence pane next to the corresponding mutation position

isolate (PR0943-7), with an allele frequency reaching 71% (Fig. 3).

The genotypic mutations detected in the *gyrA* gene are associated with resistance to fluoroquinolones. In the first isolate (PR0943-1), 10% of the reads showed a variant of *gyrA* with the missense mutation Ala90Val, and this subpopulation was eliminated in the second isolate, PR0943-2. Starting from the third isolate (PR0943-3), another variant of the *gyrA* gene, Asp94Gly, associated

with fluoroquinolone resistance, evolved and continued to persist across the subsequent isolates.

For cycloserine resistance, a subpopulation (17%) emerged in the 6th (PR0943-5) and 7th (PR0943-6) isolates with a frameshift mutation in the *ald* gene due to the duplication of the 2-base "GC" in coding position 436–437 of the gene but was subsequently eliminated in the eighth isolate (PR0943-7).

Discussion

In this study, we analysed a series of eight isolates of the same strain that were collected consecutively from one patient who first presented with MDR-TB and developed XDR-TB over the course of treatment due to a history of intermittent treatment and noncompliance.

This study also investigated the genotypes and phylogenetic relationships of the isolates and revealed that all the XDR-TB isolates belonged to the Beijing (lineage 2.2) strain (Fig. 2), which has been associated with the spread of MDR-TB and unfavourable treatment outcomes [37].

The Beijing TB genotype is not common in Tanzania [38, 39], but documented TB isolates in Tanzania indicate that the Beijing genotype has been established among TB patients in Tanzania.

The present study revealed that the phenotypic DST and WGS results of *M. tuberculosis* clinical isolates were concordant for isoniazid, rifampicin, ethambutol, streptomycin, pyrazinamide, and fluoroquinolones among all eight isolates. However, there was a discrepancy between the phenotypic DST and genotypic drug resistance patterns for bedaquiline in isolates PR0943-3, PR0943-24, PR0943-5 and PR0943-7 and for levofloxacin in isolates 3rd PR0943-3, 4th PR0943-24, and 6th PR0943-5 (Table 3). This could be explained by the emergence of the subpopulation with the variants conferring resistance to these classes of antibiotics and the relatively low proportions in the early isolates. The latter isolates were found to be phenotypically resistant by DST.

With respect to fluoroquinolones, both variants, gyrA_p. Ala90Val and gyrA_p. Asp94Gly is classified as a group 1 gene associated with resistance to levofloxacin and moxifloxacin; however, the former was reported to be associated with a low level of resistance to moxifloxacin, and the latter persisted with a high level of resistance to moxifloxacin.

The serial isolates from the present patient developed mutations in the *mmpR5* (Rv0678) gene encoding the *MmpR5* repressor protein over time, and variants of *mmpR5* are associated with resistance to bedaquiline and clofazimine. Recently, it was reported that not all frameshift mutations in *mmpR5* (Rv0678) cause loss of function of the protein and may not confer resistance to bedaquiline [40]. However, the two variants detected in our patient were associated with resistance, especially the latter. The former variant in *mmpR5*_p. Asp47f detected in the first subpopulation resistant to bedaquiline is classified as group 1 (the variants associated with resistance) for bedaquiline and group 2 (variants associated with resistance-intrem) for clofazimine in the second version of the WHO catalogue of drug resistance mutations in *M. tuberculosis* (2023). The latter variant *mmpR5*_p. Glu49fs is classified as group 1 and is associated with resistance to bedaquiline and clofazimine.

Genotypic mutations in the *mmpR5 (Rv0678)* gene in bedaquiline before exposure to clofazimine have been identified in settings with limited prior use of bedaquiline [41]. However, there is limited knowledge about the mechanisms of action, drug susceptibility, pharmacokinetics, resistance mechanisms, and cross-resistance of bedaquiline and clofazimine [42].

Neither the TB profiler nor Mykrobe predicted resistance to either bedaquiline or clofazimine. Nevertheless, the variants identified in our longitudinal subpopulation analysis are likely the mechanism for bedaquiline resistance (BDQ-R), especially because the patient did not respond to bedaquiline treatment. We detected 144dupC in some of the clinical trials in a BDQ-R isolate, which has been reported elsewhere [43–46]. Even using the most sensitive approach possible, where any mutation in any of the genes that have been identified as potentially associated with bedaquiline resistance is assumed to be resistance-conferring, only approximately half (49–69%) of bedaquiline resistance genes were identified [41]. In other studies, mutations in *mmpR5* were significantly associated with resistance according to minimum inhibitory concentration tests [41].

The history of the patient involved the transfer from a health centre, signifying the non-responsiveness of the patient to the prescribed individualized treatment regimen. The patient was reported to be culture positive after receiving TB treatment at KIDH and presented with cough, chest pain, chest tightness, fever, and weight loss, indicating failure of the prescribed current regimen. Unresponsiveness to the treatment regimen might be attributed to nonadherence to the regimen, as evidenced by reports including loss to follow-up. As observed in phenotypic DST, resistance to clofazimine was not observed in any of the isolates. However, resistance to bedaquiline was detected phenotypically in the later isolates, and WGS demonstrated the evolution of bedaquiline resistance in this patient, emphasizing the importance of WGS. As revealed by the WGS results, acquired resistance to bedaquiline, as evidenced by the emergence of subpopulations of MDR-TB strains, requires the inclusion of drugs with high resistance preventing capacity through high and early bactericidal activity [47]. The United Republic of Tanzania is among the 124 countries that have adopted bedaquiline as a treatment regimen for DR-TB. The management of XDRTB patients is challenging, and there are implications associated with the management of XDR-TB patients, including limited treatment options, potential for transmission, and the importance of infection control measures. Thus, the detection of bedaquiline resistance at the infant stage of its use in the management of TB in the country advocates for early confirmation of drug resistance in TB patients by obtaining specimens for culture and DST. It

Katale *et al. Annals of Clinical Microbiology and Antimicrobials*   (2024) 23:76

Page 9 of 10

is recommended that implementing rigorous infection control measures, including proper isolation and venti-

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