

Molecular determinants of multidrug-resistant tuberculosis in Sierra Leone

Harriet N. A. Blankson,^{1,2,3} Rashidatu Fouad Kamara,⁴ Ivan Barilar,^{1,2} Sönke Andres,⁵ Ousman S. Conteh,⁴ Tobias Dallenga,^{2,6} Lynda Foray,⁴ Florian Maurer,^{2,5,7} Katharina Kranzer,⁸ Christian Utpatel,^{1,2} Stefan Niemann^{1,2,5}

AUTHOR AFFILIATIONS See affiliation list on p. 11.

ABSTRACT Multidrug-resistant tuberculosis (MDR-TB) management has become a serious global health challenge. Understanding its epidemic determinants on the regional level is crucial for developing effective control measures. We used whole genome sequencing data of 238 of *Mycobacterium tuberculosis* complex (MTBC) strains to determine drug resistance profiles, phylogeny, and transmission dynamics of MDR/rifampicin-resistant (RR) MTBC strains from Sierra Leone. Forty-two strains were classified as RR, 196 as MDR, 5 were resistant to bedaquiline (BDQ) and clofazimine (CFZ), but none was found to be resistant to fluoroquinolones. Sixty-one (26%) strains were resistant to all first-line drugs, three of which had additional resistance to BDQ/CFZ. The strains were classified into six major MTBC lineages (L), with strains of L4 being the most prevalent, 62% ($n = 147$), followed by L6 (*Mycobacterium africanum*) strains, (21%, $n = 50$). The overall clustering rate (using $\leq d12$ single-nucleotide polymorphism threshold) was 44%, stratified into 31 clusters ranging from 2 to 16 strains. The largest cluster ($n = 16$) was formed by sublineage 2.2.1 Beijing Ancestral 3 strains, which developed MDR several times. Meanwhile, 10 of the L6 strains had a primary MDR transmission. We observed a high diversity of drug resistance mutations, including borderline resistance mutations to isoniazid and rifampicin, and mutations were not detected by commercial assays. In conclusion, one in five strains investigated was resistant to all first-line drugs, three of which had evidence of BDQ/CFZ resistance. Implementation of interventions such as rapid diagnostics that prevent further resistance development and stop MDR-TB transmission chains in the country is urgently needed.

IMPORTANCE A substantial proportion of MDR-TB strains in Sierra Leone were resistant against all first line drugs; however this makes the all-oral-six-month BPaLM regimen or other 6-9 months all oral regimens still viable, mainly because there was no FQ resistance. Resistance to BDQ was detected, as well as RR, due to mutations outside of the hotspot region. While the prevalence of those resistances was low, it is still cause for concern and needs to be closely monitored.

KEYWORDS multidrug resistance, *Mycobacterium tuberculosis*, Sierra Leone, tuberculosis

Tuberculosis (TB) is among the leading causes of death from a single infectious agent, accounting for 1.6 million deaths in 2021 (1). TB is caused by pathogens of the *Mycobacterium tuberculosis* complex (MTBC), consisting of *Mycobacterium tuberculosis sensu stricto* (*Mtb*), *Mycobacterium africanum* (*Maf*) (2), *Mycobacterium bovis*, and strains of other animal-adapted species (3–5). *Mtb* strains cause most human diseases globally, while *Maf* strains are responsible for 20%–40% of diseases in West Africa (Benin—37%, Ghana—20%, Gineau Bissau—47%, and Sierra Leone—23%) (6–11). MTBC strains can be further classified into nine main lineages (L) and several sublineages using

Editor Silvia T. Cardona, University of Manitoba, Winnipeg, Manitoba, Canada

Address correspondence to Stefan Niemann, sniemann@fz-borstel.de.

Katharina Kranzer, Christian Utpatel, and Stefan Niemann contributed equally to this article.

The authors declare no conflict of interest.

See the funding table on p. 12.

This project's results were presented at the Keystone eSymposia dubbed Tuberculosis: Science Aimed at Ending the Epidemic.

Received 28 June 2023

Accepted 28 October 2023

Published 30 January 2024

Copyright © 2024 Blankson et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

single-nucleotide polymorphism (SNP)-based barcoding classification (12). *Mtb* strains belong to L1–L4, L7, and L8, while *Maf* belongs to L5, L6, and L9 (11).

Drug-susceptible TB is treatable with 6 months of a standard drug regimen composed of isoniazid (INH), rifampicin (RIF), pyrazinamide (PZA), and ethambutol (EMB) (13). However, increasing drug resistance, especially rifampicin-resistant (RR)-TB, multidrug-resistant (MDR, resistance to both RIF and INH)-TB, pre-extensively drug resistance [pre-XDR, MDR plus resistance to one fluoroquinolone (FQ)], and XDR-TB [pre-XDR plus additional resistance to one other World Health Organization (WHO) group A drug], threaten TB control (14). In 2021, an estimated 450,000 people developed MDR/RR-TB globally, ~167,000 were diagnosed, and only ~162,000 were initiated on MDR/RR treatment (1). Global treatment success for MDR/RR-TB is only ~60% (1). In MDR/RR-TB, additional resistance to other first-line drugs, such as PZA and EMB, and second-line drugs, including FQ or bedaquiline (BDQ), is problematic as it reduces effective treatment options (15).

In May 2022, the WHO recommended a novel all-oral 6-month regimen of BDQ, preteonamid (Pa), and linezolid (L) plus moxifloxacin (M) (in the absence of FQ resistance) for the treatment of MDR-TB (BPaLM) (16, 17). This new regimen was found to be non-inferior to standard 9- and 12-month MDR-TB regimens and was found to be favorable because of a reduction in adverse events (16, 18). While this new regimen holds great promise, the emergence of FQ and/or BDQ resistance described recently may threaten the longevity of the regimen (19, 20). Thus, understanding the underlying prevalence of FQ/BDQ resistance before introducing the new regimen is crucial (21, 22). Equally important is the ongoing monitoring of resistance development, for example, fostered by the transmission of MTBC strains with particular resistance profiles that can negatively impact diagnostic or treatment strategies in a given geographical region, as shown for the I491F outbreak clone in Eswatini (19, 23, 24). Furthermore, strains of particular MTBC lineages may have minimum inhibitory concentration (MIC) differences to particular drugs, for example, L1 *Mtb* strains have been found to have intrinsically higher Pa MIC compared to L2–L4, and L7 strains (25). Whether or not this is clinically important has not yet been established.

Sierra Leone, a country in West Africa with an estimated TB incidence of 289 per 100,000 population, had a prevalence of MDR/RR-TB of 2.5% and 12% among newly diagnosed and retreatment TB in 2021 (1). Xpert MTB/RIF is the primary molecular TB diagnostic in most hospitals, but smear microscopy continues to be used in primary care clinics. Molecular and phenotypic drug susceptibility testing for all other TB drugs is not performed in the country (26). As such, detailed first- and second-line drug resistance data are unavailable, and the information on phylogeny and transmission dynamics of MDR/RR MTBC strains in the country is very limited.

To close these knowledge gaps, we performed a whole genome sequencing (WGS) study of 238 RR/MDR MTBC strains collected in Sierra Leone between 2016 and 2020. WGS data of the MTBC strains were investigated for phylogenetic classification, resistance prediction, and cluster analysis.

MATERIALS AND METHODS

Study design and population

Sputum samples from the National TB Reference Laboratory Freetown, Sierra Leone, of individuals with RR-TB as determined by Xpert MTB/Rif and those with suspected RR-TB who had failed first-line treatment in Sierra Leone between November 2016 and March 2020 were sent to the Supranational Reference Laboratory in Borstel for mycobacterial culture. All RR isolates based on genotypic drug susceptibility tests were included in this analysis. Each strain included in the study came from a different patient. A schematic representation of the study design is shown in Fig. 1.

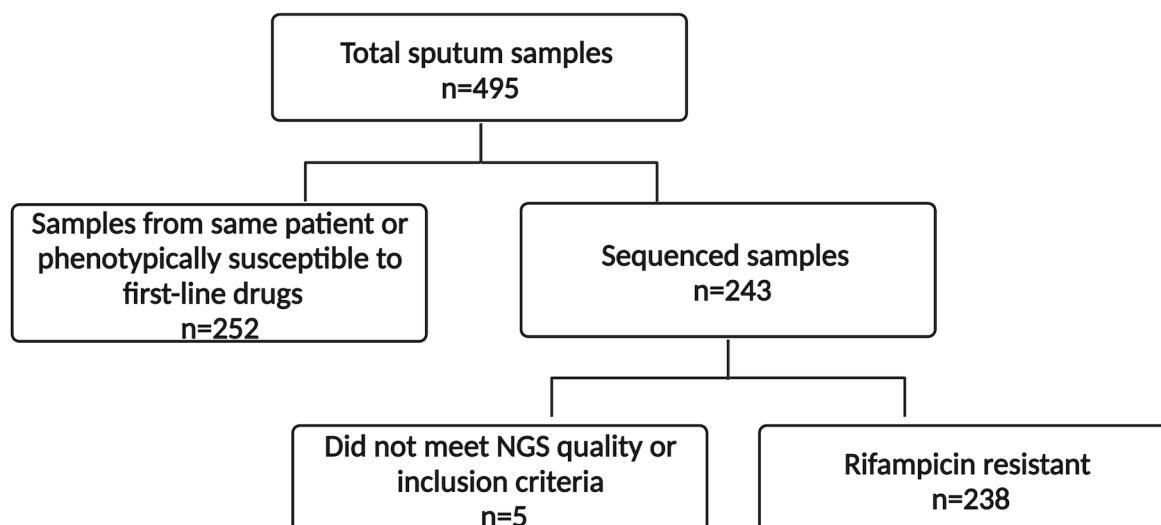


FIG 1 Study design. Inclusion criteria were patient sputum samples positive for *Mycobacterium tuberculosis* complex strains, which were genotypically rifampicin resistant. Only one cultured sample per patient was included in the study and sequenced samples that did not meet NGS quality standards were excluded. *n*, number of samples; NGS, next-generation sequencing. Created with BioRender.

Whole genome sequencing and analysis

The DNA of MTBC strains was extracted as previously described (27). The genomes were sequenced and analyzed with bioinformatics pipelines as described by Grobbel et al. (28) and Merker et al. (29). Briefly, genomic DNA was sequenced using Illumina Technology (NextSeq 500 or MiSeq) using the Baym protocol (30) and Nextera library preparation kits following the manufacturer's instructions (Illumina, San Diego, CA). The FASTQ data were analyzed with the MTBseq pipeline (31). Briefly, reads were mapped to the H37Rv genome [GenBank ID: NC_000962.3 (32)] with BWA (33), processed with SAMtools (34), and the mappings were refined with the GATK 3.8 (35).

For variant detection, SAMtools (34)-derived mpileup files were filtered for minimum thresholds of at least two reads indicating a variant in both forward and reversed orientation, two reads calling the allele with at least a phred score of 4, and 5% allele frequency for resistance determination, and for phylogeny, four reads mapped in each forward and reverse direction, respectively, with 75% allele frequency and at least four calls with a phred score of at least 20.

Genomic SNP positions with a reliable base call in at least 95% of the strains and covered in all strains were concatenated to a sequence alignment. SNPs within a window of 12 bp from each other and those located in repetitive regions or resistance-associated genes were excluded to avoid calling SNPs related to insertions and deletions artifacts (29). Strains were phylogenetically classified, and transmission clusters were inferred with single linkage clustering using distance cutoffs of ≤ 5 and ≤ 12 SNPs. Raw FASTQs were uploaded to the European Nucleotide Archive (Table S1).

Resistance analysis

Genotypic resistance predictions were based on a curated Research Center Borstel mutation catalog, as described by Grobbel et al. (28), and the WHO's catalog (36). Mutations linked to phenotypic drug resistance were marked as resistant, and unclear ones were classified as not resistant. When no mutation was detected, strains were defined as susceptible. Drug-resistant types were classified based on the WHO's classification (14).

Phylogenomic analysis

A maximum parsimony tree (MPT) was built with the software BioNumerics version 7.6.3 (BioNumerics) from the aligned sequences of concatenated SNPs. Numbers on branches indicate the number of distinct SNP positions between isolates. Using distinct SNP sites, maximum likelihood trees (MLTs) were calculated with IQ-TREE 2, an efficient method for phylogenetic inference (37), using ModelFinder (38), an automated model selection, including ascertainment bias correction, and ultrafast bootstrap approximation (39) with resampling of 1,000. The MLT was midpoint rooted with the FigTree software version 1.4.4. The trees were visualized with Interactive Tree of Life (iTOL) 5.7 (40).

Statistical analysis

Descriptive statistics were performed (distribution frequency) and graphs were drawn with R software version 4.2.1.

RESULTS

Study population

A total of 238 MTBC strains were included in the study (Table 1), 63% ($n = 151$), and they were from retreatment patients. The majority of strains were obtained from men (43%, $n = 102$), and the median age was 34 years (interquartile range 30–39). Among patients with known HIV status, 15% (25/162) were HIV positive.

MTBC population structure

WGS data analysis revealed 15,089 informative SNPs differentiating any of the 238 MTBC strains. These SNPs were used to calculate a maximum likelihood phylogeny based on a concatenated SNP alignment (Fig. 2). Based on canonical SNP signatures (12), the 238 strains were classified into six main MTBC lineages (L1–L6) (Fig. 2; Fig. S1; Table 2). Strains of L4 were most frequent ($n = 147$, 62%), followed by strains of L6 (*Maf*, $n = 50$, 21%), L2 ($n = 22$, 9%), L1 ($n = 10$, 4%), L5 (*Maf*, $n = 7$, 3%), and L3 ($n = 2$, 0.8%). The strains were further categorized into sub-lineages as follows: 4.1.2.1 Haarlem ($n = 45$, 19%), 4.1 Euro-American ($n = 38$, 16%), 6.2.2 West Africa 2 and 4.8 mainly T ($n = 23$, 9.7%), and 2.2.1 Beijing Ancestral 3 ($n = 22$, 9.2%) (Fig. 2; Tables S1 and S2.1).

TABLE 1 Main demographic characteristics of study participants^a

		<i>n</i> (RR/MDR)	(%)
Total		238	
Sex	Female	41	17
	Male	102	43
	Unknown	95	40
Age range (years)	≤19	13	6
	20–29	50	21
	30–39	48	20
	40–49	37	16
	50–59	20	8
	≥60	8	3
Type	Unknown	62	26
	New	10	4
	Retreatment	151	63
HIV status	Unknown	77	32
	Negative	137	58
	Positive	25	11
	Unknown	76	32

^aHIV, human immunodeficiency virus; MDR, multidrug-resistant; RR, rifampicin-resistant.

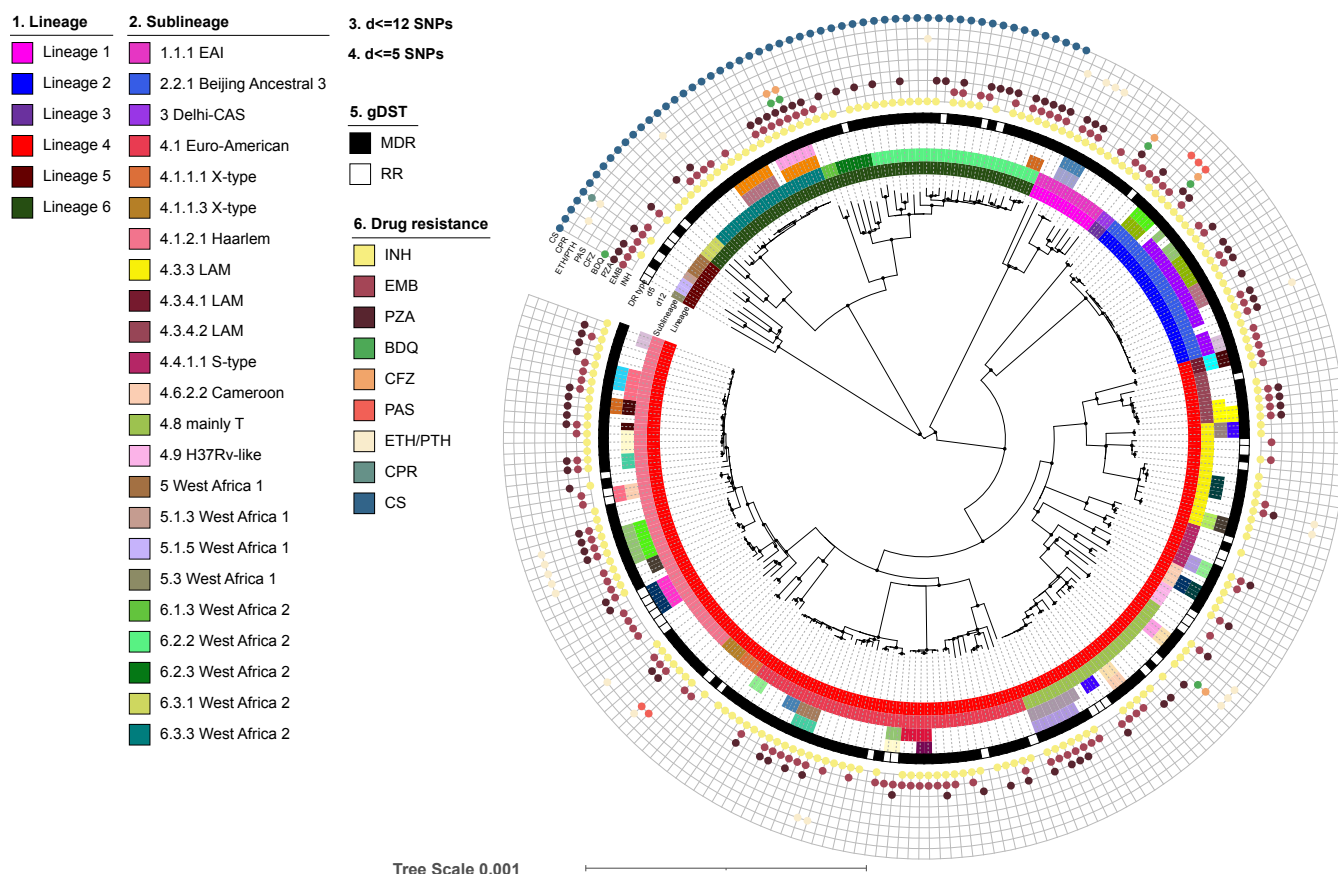


FIG 2 Phylogeny, lineage classification, and drug resistance patterns of the *Mycobacterium tuberculosis* complex strains from Sierra Leone. The maximum likelihood tree was based on a concatenated single-nucleotide polymorphism (SNP) alignment of 328 *M. tuberculosis* complex (MTBC) strains. The alignment was based on 15,089 informative SNP sites. Ultrafast bootstrap values (+0.95) are indicated on the tree branches with black dots and the circles from the inner ring to the outer show: 1. The seven main MTBC lineages; 2. the MTBC sublineages; 3. and 4. show the genome clusters based on strain SNP distances of ≤ 12 SNPs and ≤ 5 SNPs, respectively (strains sharing the same color belong to the same cluster), 5. genotypic drug susceptibility types (gDST); and 6. the colored dots indicate genotypic resistance to the respective drug. INH, isoniazid; EMB, ethambutol; PZA, pyrazinamide; PAS, para-aminosalicylic acid; CS, cycloserine; ETH, ethionamide; PTH, proteonamid; CFZ, clofazimine; BDQ, bedaquiline; CPR, capreomycin; MDR, multidrug-resistant (resistance to at least isoniazid and rifampicin); RR, rifampicin-resistant.

Drug resistance

We then performed a genotypic resistance prediction based on high-confidence resistance mutations. Overall, resistance prevalence was 82% ($n = 196$), 53% ($n = 126$), and 39% ($n = 92$) for INH, EMB, and PZA, respectively (Fig. 3; Fig. S2–S6; Table S2.2). No resistance to FQ and linezolid was detected; however, five strains were resistant to BDQ/clofazimine (CFZ) based on mutations in *Rv0678* (Fig. S4). A total of 61 (26%) strains were found to be resistant to all first-line drugs; three had additional resistance to BDQ/CFZ (Table S2.3). Resistance patterns were similar across strains from all lineages, except for cycloserine (CS), as all L5 and L6 strains were inherently resistant to CS (Fig. 3). Out of the five BDQ/CFZ resistant strains, one belonged to L1.1.1, one to L4.8, one to L5, and two to L6 (Table S2.4).

All the RIF resistance-conferring mutations were found in the *rpoB* gene. The most prevalent mutation was *rpoB* S450L (41%, $n = 98$), which is part of the WHO group_1 mutations (2001) associated with resistance (36) (Table S3.1). The *rpoB* S450L mutation was found in strains of all lineages except L2 (Table S4). Despite this, there was a diversity of *rpoB* mutations, with 32 (13%) of the strains having one of the so-called RIF-borderline mutations (*rpoB* D435Y, L452P, H445L/N, and L430P) (41, 42). Such strains may test phenotypically susceptible to RIF. Also, three strains had the *rpoB* I491V and V170F

TABLE 2 MTBC strain proportions, clustering, and cluster rate within the lineages

Main lineages	Σ no. of strains (%)	# Clustered	Cluster rate
Lineage 1	10 (4.2)	3	30
Lineage 2	22 (9.2)	19	86
Lineage 3	2 (0.8)	0	0
Lineage 4	147 (61.8)	70	48
Lineage 5	7 (2.9)	0	0
Lineage 6	50 (21)	12	24
Total	238	104	44

resistance mutations, which are found outside of the RIF-resistance-determining region (RRDR) (23, 24, 36, 43) (Tables S1, S3.1, and S4), and thus, not detected by commercial molecular RR tests such as Xpert MTB/RIF assay.

Mutations linked with resistance to INH were found in the *katG* gene and the *fabG1-inhA* regulatory region (Tables S1, S3.2, and S5) (44). The *katG* S315T resistance mutation was most prevalent ($n = 133, 56\%$) and found in strains of all lineages except L3 and L5 (Tables S1, S3.2, and S5). Besides, 53 strains had mutations in *fabG1-inhA/inhA*,

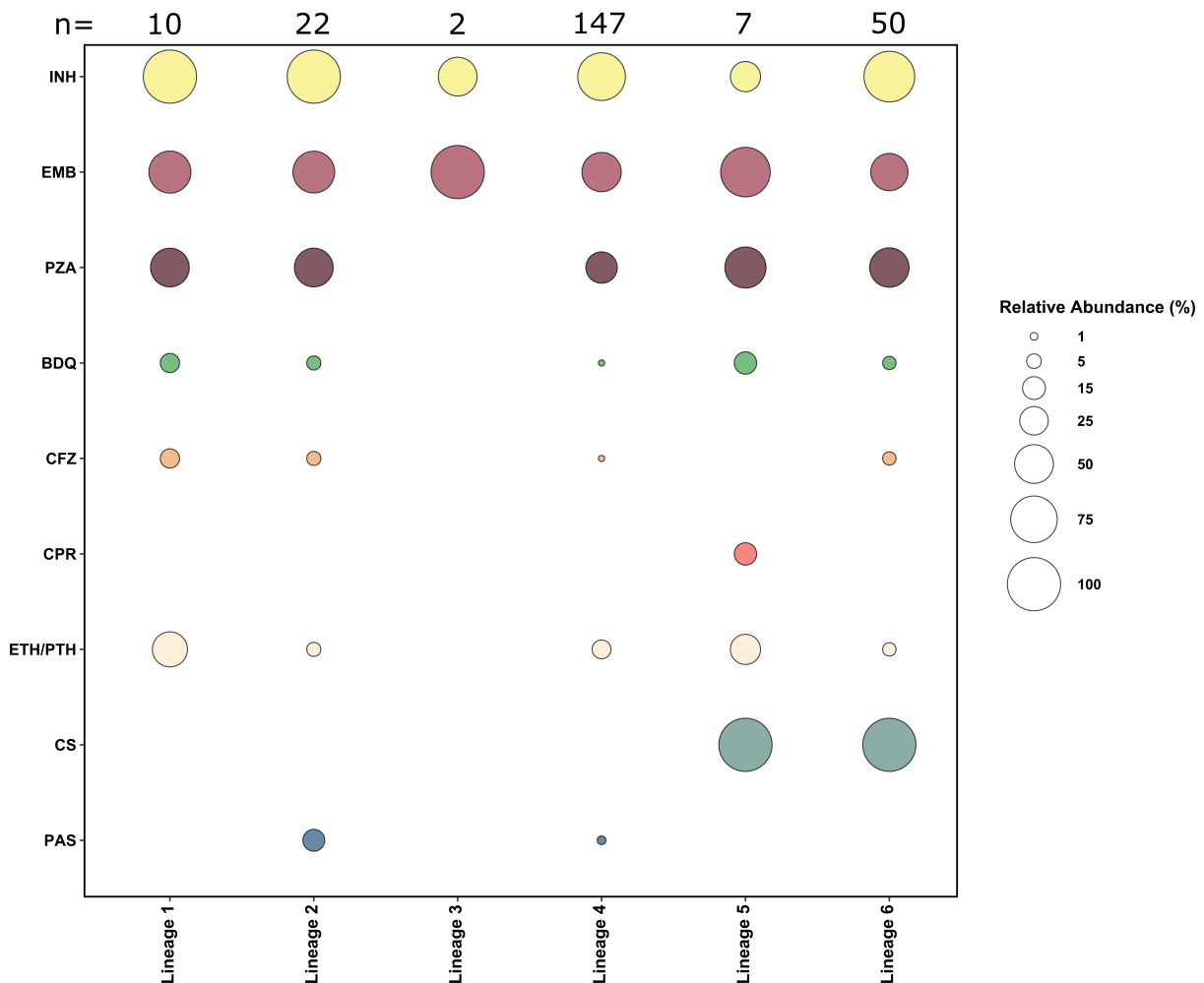


FIG 3 Drug resistance to first- and second-line anti-tuberculosis drugs per lineage of *Mycobacterium tuberculosis* complex strains investigated. The bubble plot shows the relative abundance of *M. tuberculosis* complex (MTBC) strains for each lineage that developed resistance to the corresponding drug. On the left y-axis are the abbreviated names of drugs: INH, isoniazid; EMB, ethambutol; PZA, pyrazinamide; CS, cycloserine; ETH, ethionamide; PTH, prothionamide; PAS, para-aminosalicylic acid; CFZ, clofazimine; BDQ, bedaquiline; and CPR, capreomycin. On the x-axis at the bottom are the MTBC strain lineages and indicated at the top are the total number of MTBC strains per lineage. n = number of isolates

out of which 37 acquired a second mutation in *katG* (Tables S3.2 and S5). Of those, the most prevalent mutation was *fabG1* 15t>c (18 strains), which is classified as WHO group_2 borderline mutation; 12 did not have any additional INH mutations, and 4 had an additional *katG* S315T mutation (Tables S1 and S3.2). Also, of note were the 12 strains with *fabG1* L203L mutations; however, 10 of these strains had also developed the higher level *katG* S315T resistance mutation (Tables S1, S3.2, and S5).

Transmission (genomic clustering)

Using a maximum distance of 12 SNPs between two strains to define a cluster, 104 (44%) strains were grouped into 31 clusters ranging in size from 2 to 16 strains (Fig. 4; Fig. S7; Table 2). We linked the cluster data with resistance mutation profiles to better understand the transmission dynamics of RR/MDR strains. Indeed, while all strains of the largest cluster group_3 shared the *katG* S315T mutation (Fig. 5A) (16 isolates of 2.2.1 Beijing sublineage), they had different *rpoB*, *embB*, and *pncA* resistance mutations, subdividing the clustered strains into sub-clusters as indicated in the MPT (Fig. S8). The

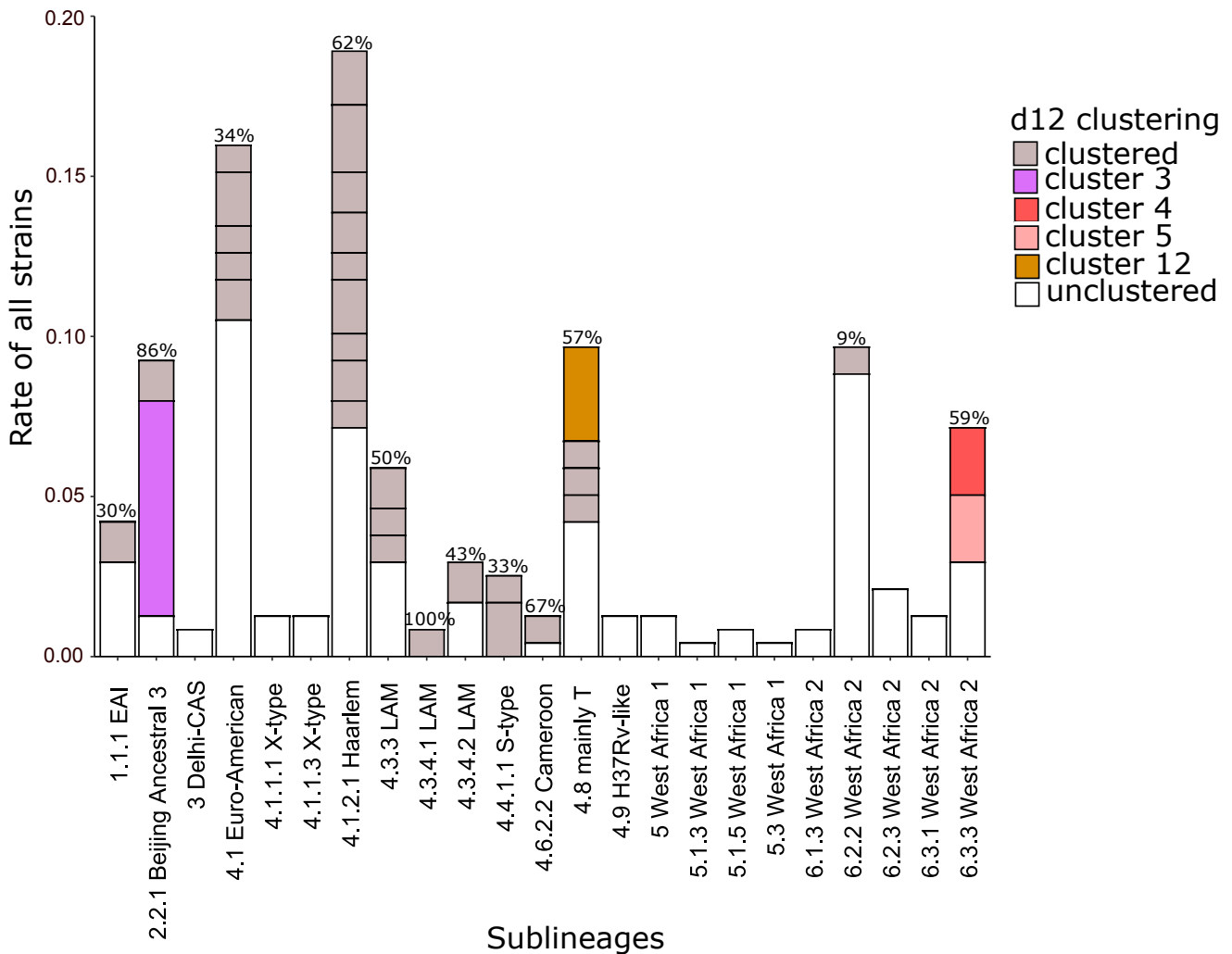


FIG 4 Clustering proportions of *Mycobacterium tuberculosis* complex strains by sublineage. The bar plot shows the distribution of *M. tuberculosis* complex strains that clustered versus unclustered based on sublineages. The total cluster rate per sublineage is indicated on the top of the bars and distinct clusters are separated by lines. Large clusters with five or more strains are presented in specific colors, while gray represents clusters with less than five strains and white represents the proportion of unclustered strains.

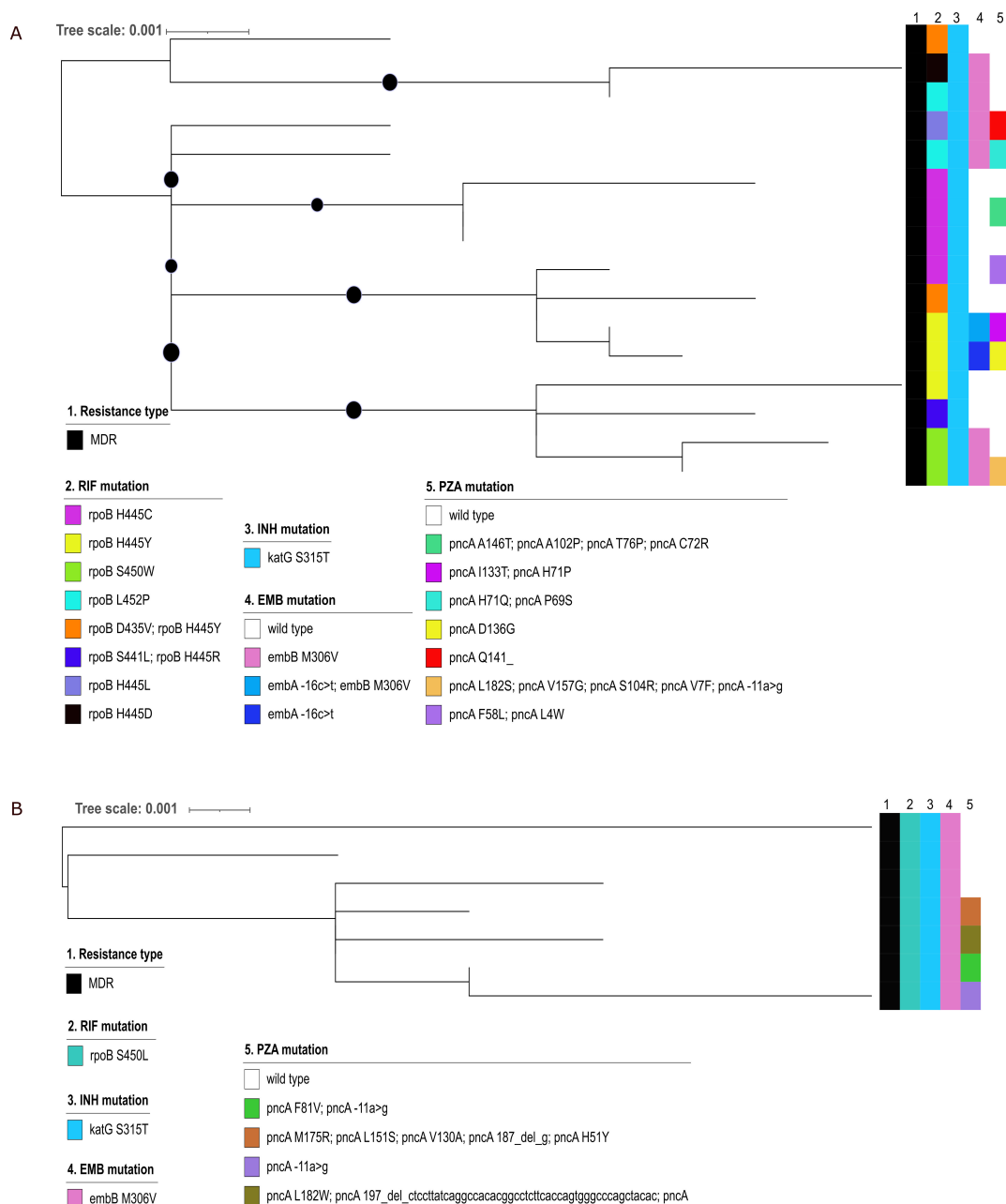


FIG 5 Phylogeny of the strains of the two largest clusters of multidrug-resistant *Mycobacterium tuberculosis* strains. Maximum likelihood trees were calculated for the *M. tuberculosis* strains of the two largest multidrug-resistant clusters identified in the study. (A) The midpoint-rooted phylogeny of the 16 clustered *M. tuberculosis* strains of "cluster 3" belonging to the sublineage 2.2.1 Beijing Ancestral 3. The alignment was based on 1,174 informative SNP sites. This tree shows the transmission of an isoniazid-resistant clone with multiple independent developments to multidrug resistance (MDR—resistance to at least isoniazid and rifampicin), shown by the different rifampicin resistance mutations. The strains also had developed different ethambutol and pyrazinamide resistance mutations. (B) The midpoint-rooted phylogeny of the seven clustered *M. tuberculosis* strains of "cluster 12" belonging to the sub-lineage 4.8 mainly T. The alignment was based on 466 informative SNP sites. In this tree, a single MDR clone was transmitted, which was also resistant to ethambutol, shown by the same resistance mutation for all the seven strains. However, the strains developed different pyrazinamide resistance mutations. 1. The genotypic drug resistance type; 2–5. the color-coded resistance mutations of the first-line drugs identified for the respective drugs; and the black dots on the tree branches indicate the ultrafast bootstrap values (+0.95). RIF, rifampicin; INH, isoniazid; EMB, ethambutol; PZA, pyrazinamide; MDR, multidrug-resistant.

second-largest cluster group₁₃ consisted of seven 4.8 mainly T sublineage MDR strains (Fig. 5B); all strains shared the same RIF *rpoB* S450L, INH *katG* S315T, and EMB *embB*

M306V resistance mutations indicating the ongoing spread of this MDR strain in the country (Fig. 5B).

Clustering also indicated smaller transmission events of RR/MDR L6 strains (Fig. 6). Out of the 51 L6 strains, 12 (24%) clustered (Tables S1 and S2.5). Of the 6.3.3 West Africa 2 sublineage strains, 10 out of 17 (59%) were clustered, forming two clusters (group_4 and group_5) of five strains each (Fig. 6). Interestingly, while the cluster analysis separated the strains into two clusters, they were closely related in the phylogeny and share the RIF *rpoB* D435Y mutation and INH *fabG1-17g>t/katG* S315T double mutation, indicating the emergence from a common MDR ancestor (Fig. 6). However, only the strains of group_5 developed further resistance to EMB and PZA, rendering them fully first-line resistant (Fig. 6). Also, two of the strains developed BDQ/CFZ resistance due to mutations in *Rv0678* (Table S1).

DISCUSSION

In this study, we employed WGS to characterize RR/MDR MTBC strains from Sierra Leone. We found that one in five strains investigated was resistant to all first-line drugs. While no resistance to FQ was observed, five strains had resistance to BDQ/CFZ due to *Rv0678* mutations. The cluster rate exceeded 40%, indicating ongoing transmission of RR/MDR TB, which contributes significantly to the disease burden in the country. Strikingly, *Maf* L6 strains constituted 21% of the MDR MTBC strains analyzed and formed a longitudinal outbreak with two branches, each constituting five strains. Within one of these branches, all strains were resistant to all first-line drugs, and two strains had developed additional BDQ/CFZ resistance. There was a high diversity of drug resistance mutations with a significant number of so-called borderline INH and RIF resistance mutations, potentially allowing the use of these first-line drugs albeit with higher dosing.

None of the investigated MTBC strains had FQ resistance, which contrasts with data from other West African countries. For instance, in Nigeria, FQ resistance was 13%

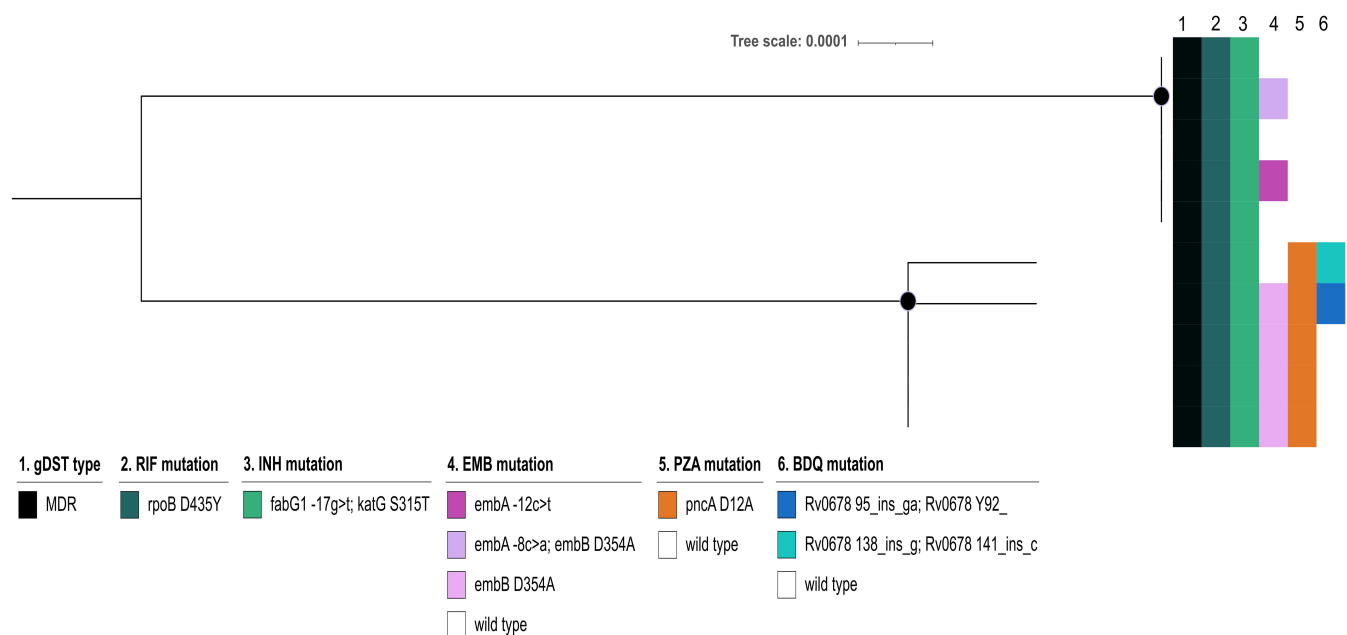


FIG 6 Phylogeny of the largest cluster of endemic multidrug-resistant *Mycobacterium africanum* strains. The mid-point-rooted maximum likelihood tree was calculated for the 10 multidrug-resistant (MDR, resistance to at least isoniazid and rifampicin) *M. africanum* strains belonging to sublineage 6.3.3 West Africa 2. The alignment was based on 1,924 informative SNP sites. The tree shows a single sublineage 6.3.3 West Africa 2 MDR clone transmitting, which formed two distinct clusters (clusters 4 and 5). 1. The genotypic drug susceptibility type (gDST); 2–6. are the color-coded resistance mutations of the first-line drugs identified for the respective drugs; and the black dots on the tree branches indicate the ultrafast bootstrap values (+0.95). RIF, rifampicin; INH, isoniazid; EMB, ethambutol; PZA, pyrazinamide; BDQ, bedaquiline; MDR, multidrug-resistant.

(17/132 of RR-TB) in a study conducted between 2018 and 2020 (45), while in Ghana, 36% (11/31) of MDR-TB strains were FQ resistant (46).

We found five MTBC strains (2%) with resistance to BDQ and CFZ due to mutations in *Rv0678*, a transcriptional repressor of the MmpS5–MmpL5 efflux pump (47, 48). Mutations in *Rv0678* are the primary drivers of BDQ/CFZ resistance in clinical strains in South Africa (20) and Moldova (21). Also, these mutations are linked to treatment failure and can potentially cause an increase in BDQ/CFZ resistance over a short timeframe (20).

Considering the low prevalence of BDQ resistance (2%) and the absence of FQ resistance in Sierra Leone, both the 9–12-month all-oral regimens and the new 6-month BPaLM are likely to be effective treatment regimens for most patients (16, 17). BDQ/CFZ resistance may emerge without direct drug exposure (23, 24, 43, 49), and since the exact mechanism is yet to be identified, it is crucial to prioritize prospective surveillance for BDQ/CFZ resistance and mechanisms involved in selecting MTBC strains with *Rv0678* mutations. This is especially significant as BDQ is an essential drug in the new BPaLM regimen and the rapid development of resistance may reduce the efficacy of the regimen (50).

Our investigation of the MTBC strain diversity identified strains of six main MTBC lineages circulating and causing RR/MDR-TB in Sierra Leone. Overall, the widely distributed and diverse L4 strains were the most prevalent (51). L4 strains are highly successful globally due to their genotypic and phenotypic diversity (51–53). The high proportion of L4 strains in this study linked to a cluster rate of 62% is in line with previous studies (2, 51). Lineage 2 strains were the third most prevalent strains; surprisingly, they all belonged to the 2.2.1 Beijing Ancestral 3 sublineage, also showing a high cluster rate of 86%, indicating effective MDR-TB transmission. Recent studies, for example, from Eastern European countries, have found associations between modern L2 strains such as L2.2.3 (54) and L2.2.1 (55) with high rates of clustering and transmission (56–58). Studies from India and South Africa indicate that strains of ancestral Beijing lineages may also develop high drug resistance rates linked with ongoing transmission of few MDR/pre-XDR/XDR strains in a given setting (19, 59). The emergence of ancestral Beijing strains in Sierra Leone warrants close monitoring and further investigations towards cross-border spread in Africa, introduction by migration followed by local spread, and the overall importance of this strain type for the MDR epidemic.

In contrast to other geographical regions, 24% of strains belonged to *Maf*, the majority were L6 (classified as West African 2), confirming the findings from another study from West Africa (60). All *Maf* strains were resistant to CS, a drug classified as group B by the WHO because of 1 bp frameshift deletion in *ald*, as described previously (61).

In line with findings from other African countries such as Namibia, we found a high diversity of INH/RIF resistance mutations with a substantial proportion of strains having so-called borderline resistance mutation in *rpoB* and/or in the *inhA-fabG1* promoter region, which are difficult to detect by phenotypic assays (41, 42, 62). In patients infected with MTBC strains with lower-level resistance mutations, high-dose INH and/or RIF may overcome resistance, presenting a viable treatment option, especially for patients with MDR/pre-XDR/XDR TB with advanced resistance patterns (63, 64). However, host genetic factors that lead to enhanced drug metabolisms and/or reduced bioavailability need to be investigated as they are likely to contribute to the higher frequency of strains with borderline resistance mutations in the region (65–67). High-dose regimens can only be applied when both pathogen and host genetics/phenotypes are available.

Still, the high prevalence of MTBC strains in Sierra Leone and Namibia with RR/MDR-TB patients underlines their significance for the drug resistance TB epidemiology. Moreover, since strains with borderline INH resistance mutations can also develop higher-level resistance by a second mutation (42), it is, therefore, essential to have molecular methods for resistance detection to keep track of low-level resistance mutations to prevent misclassification of resistant strains and reduce treatment failure.

We detected three strains with resistance mutations outside the rifampicin RRDR (*rpoB* I491V and V170F), which are not detected by commercial molecular RR tests such

as Xpert MTB/RIF assay (23, 24, 43). Consequently, patients are usually treated with drug-susceptible regimens resulting in higher rates of treatment failures and possibly enhanced transmission. Such strains have also proven to be challenging for severe MDR-TB control in other African countries such as Eswatini (23).

Conclusion

Our data indicate that resistance to group A, B, and C MDR-TB treatment drugs is limited in Sierra Leone. Hence, the short all-oral-6-month BPaLM or the recently proposed 6–9-month or 9–12-month regimens offer great potential to treat most of the MDR-TB cases in the country successfully. Our data on the MTBC strains' population structure point toward the potential importance of ancestral Beijing strains for the MDR-TB epidemic in Africa. We also demonstrated that MDR *Maf* strains contribute significantly to the MDR-TB burden in the country.

Limitations

Our study was limited by the small sample size and lack of metadata to help make associations among the isolates. However, the thorough genotypic testing and in-depth analysis revealed noteworthy findings.

ACKNOWLEDGMENTS

We thank the patients for the samples; Doris Hilleman for expert information; and Fenja Boysen, Vanessa Mohr, Tanja Niemann, and Ilse Radzio for technical assistance.

H.N.A.B. conducted the experiment, analyzed results, and wrote the manuscript; R.F.K., O.S.C., L.F., and I.B. provided samples and data; S.A. and F.M. supported the laboratory work and provided the input for the manuscript; T.D. analyzed the results and reviewed the manuscript; K.K. supported the laboratory work and analysis and reviewed and commented on several versions of the manuscript; C.U. and S.N. conceived, designed concept, provided analysis tools, and wrote the manuscript, S.N. also provided reagents and materials. All authors reviewed and approved the final version of the manuscript.

AUTHOR AFFILIATIONS

¹Molecular and Experimental Mycobacteriology, Research Center Borstel Leibniz Lung Center, Borstel, Germany

²German Center for Infection Research, Partner Site Hamburg-Lübeck-Borstel-Reims, Borstel, Germany

³School of Biomedical and Allied Health Sciences, College of Health Sciences, University of Ghana, Korle-Bu, Accra, Ghana

⁴National Leprosy and Tuberculosis Control Programme Sierra Leone, Freetown, Sierra Leone

⁵National and WHO Supranational Reference Center for Mycobacteria, Research Center Borstel Leibniz Lung Center, Borstel, Germany

⁶Cellular Microbiology, Research Center Borstel Leibniz Lung Center, Borstel, Germany

⁷Institute of Medical Microbiology, Virology and Hygiene, University Medical Center Hamburg-Eppendorf, Hamburg, Germany

⁸Clinical Research Department, London School of Hygiene and Tropical Medicine, London, United Kingdom

AUTHOR ORCID*s*

Harriet N. A. Blankson  <http://orcid.org/0000-0003-2997-9112>

Katharina Kranzer  <http://orcid.org/0000-0001-5691-7270>

Christian Utpatel  <http://orcid.org/0000-0001-5416-8253>

Stefan Niemann  <http://orcid.org/0000-0002-6604-0684>

FUNDING

Funder	Grant(s)	Author(s)
The Ministry of Education Ghana, the German Academic Exchange Service (DAAD)	Ghanaian-German Postgraduate Training Programme (57344816)	Harriet N. A. Blankson
The German Center of Infection Research (DZIF)	FKZ 8013802812	Christian Utpatel Harriet N. A. Blankson Stefan Niemann Ivan Barilar
Precision Medicine in Chronic Inflammation (PMI)	EXC 2167. Precision Medicine in Chronic Inflammation (PMI)	Stefan Niemann Harriet N. A. Blankson
The World Health Organization (WHO)	WHO Agreement for performance of work, Purchase order 202945290	Katharina Kranzer Sönke Andres

ETHICS APPROVAL

The project was approved by the Sierra Leone Ethics and Scientific Research Committee on 6 February 2020 (email: efoday@health.gov.sl).

ADDITIONAL FILES

The following material is available [online](#).

Supplemental Material

Tables S4 and S5 (Spectrum02405-23-S0001.docx). Supplementary tables.

Figures S5, S6, S7, S8 (Spectrum02405-23-S0002.pdf). Supplementary figures.

Figures S1, S2, S3, S4 (Spectrum02405-23-S0003.pdf). Supplementary figures.

Table S1 (Spectrum02405-23-S0004.csv). Supplementary table.

Table S2 (Spectrum02405-23-S0005.docx). Supplementary table.

Table S3 (Spectrum02405-23-S0006.docx). Supplementary table.

REFERENCES

- World Health Organization. 2022. Global tuberculosis report 2022. World Health Organization, Geneva. Available from: <https://apps.who.int/iris/handle/10665/363752>. Retrieved 29 Nov 2022.
- Asante-Poku A, Otchere ID, Osei-Wusu S, Sarpong E, Baddoo A, Forson A, Laryea C, Borrell S, Bonsu F, Hattendorf J, Ahorlu C, Koram KA, Gagneux S, Yeboah-Manu D. 2016. Molecular epidemiology of *Mycobacterium africanum* in Ghana. *BMC Infect Dis* 16:385. <https://doi.org/10.1186/s12879-016-1725-6>
- Malone KM, Gordon SV. 2017. *Mycobacterium tuberculosis* complex members adapted to wild and domestic animals. *Adv Exp Med Biol* 1019:135–154. https://doi.org/10.1007/978-3-319-64371-7_7
- Brites D, Loiseau C, Menardo F, Borrell S, Boniotti MB, Warren R, Dippenaar A, Parsons SDC, Beisel C, Behr MA, Fyfe JA, Coscolla M, Gagneux S. 2018. A new phylogenetic framework for the animal-adapted *Mycobacterium tuberculosis* complex. *Front Microbiol* 9:2820. <https://doi.org/10.3389/fmicb.2018.02820>
- Clarke C, Van Helden P, Miller M, Parsons S. 2016. Animal-adapted members of the *Mycobacterium tuberculosis* complex endemic to the southern African subregion. *J S Afr Vet Assoc* 87:1322. <https://doi.org/10.4102/jsava.v87i1.1322>
- Yeboah-Manu D, Asare P, Asante-Poku A, Otchere ID, Osei-Wusu S, Danso E, Forson A, Koram KA, Gagneux S. 2016. Spatio-temporal distribution of *Mycobacterium tuberculosis* complex strains in Ghana. *PLoS One* 11:e0161892. <https://doi.org/10.1371/journal.pone.0161892>
- Asare P, Asante-Poku A, Prah DA, Borrell S, Osei-Wusu S, Otchere ID, Forson A, Adjapong G, Koram KA, Gagneux S, Yeboah-Manu D. 2018. Reduced transmission of *Mycobacterium africanum* compared to *Mycobacterium tuberculosis* in urban West Africa. *Int J Infect Dis* 73:30–42. <https://doi.org/10.1016/j.ijid.2018.05.014>
- Gehre F, Antonio M, Faïhun F, Odoun M, Uwizye C, de Rijk P, de Jong BC, Affolabi D. 2013. The first phylogeographic population structure and analysis of transmission dynamics of *M. africanum* West African 1—combining molecular data from Benin, Nigeria and Sierra Leone. *PLoS One* 8:e77000. <https://doi.org/10.1371/journal.pone.0077000>
- Groenheit R, Ghebremichael S, Svensson J, Rabna P, Colombatti R, Riccardi F, Couvin D, Hill V, Rastogi N, Koivula T, Källenius G. 2011. The Guinea-Bissau family of *Mycobacterium tuberculosis* complex revisited. *PLoS ONE* 6:e18601. <https://doi.org/10.1371/journal.pone.0018601>
- Homolka S, Post E, Oberhauser B, George AG, Westman L, Dafaë F, Rüsç-Gerdes S, Niemann S. 2008. High genetic diversity among *Mycobacterium tuberculosis* complex strains from Sierra Leone. *BMC Microbiol* 8:103. <https://doi.org/10.1186/1471-2180-8-103>
- Silva ML, Cá B, Osório NS, Rodrigues PNS, Maceiras AR, Saraiva M, Blumenthal A. 2022. Tuberculosis caused by *Mycobacterium africanum*: knowns and unknowns. *PLoS Pathog* 18:e1010490. <https://doi.org/10.1371/journal.ppat.1010490>
- Coll F, Mc Nerney R, Guerra-Assunção JA, Glynn JR, Perdigo J, Viveiros M, Portugal I, Pain A, Martin N, Clark TG. 2014. A robust SNP barcode for typing *Mycobacterium tuberculosis* complex strains. *Nat Commun* 5:4812. <https://doi.org/10.1038/ncomms5812>

13. 2022. WHO Consolidated guidelines on tuberculosis. module 4, treatment: drug-susceptible tuberculosis treatment. World Health Organization, Geneva, Switzerland.
14. World Health Organization. 2021. WHO announces updated definitions of extensively drug-resistant tuberculosis. WHO. Available from: <https://www.who.int/news/item/27-01-2021-who-announces-updated-definitions-of-extensively-drug-resistant-tuberculosis>. Retrieved 31 Mar 2021.
15. Gill CM, Dolan L, Piggott LM, McLaughlin AM. 2022. New developments in tuberculosis diagnosis and treatment. *Breathe* 18:210149. <https://doi.org/10.1183/20734735.0149-2021>
16. Haley CA, Macias P, Jasuja S, Jones BA, Rowlinson M-C, Jaimon R, Onderko P, Darnall E, Gomez ME, Peloquin C, Ashkin D, Goswami ND. 2021. Novel 6-month treatment for drug-resistant tuberculosis, United States. *Emerg Infect Dis* 27:332–334. <https://doi.org/10.3201/eid2701.203766>
17. World Health Organization. 2022. Rapid communication: key changes to the treatment of drug-resistant tuberculosis. WHO
18. Mishra GP, Mulani J. 2022. Implications of bedaquiline-resistant tuberculosis. *Lancet Infect Dis* 22:166. [https://doi.org/10.1016/S1473-3099\(22\)00007-X](https://doi.org/10.1016/S1473-3099(22)00007-X)
19. Dreyer V, Mandal A, Dev P, Merker M, Barilar I, Utpatel C, Nilgiriwala K, Rodrigues C, Crook DW, Rasigade J-P, Wirth T, Mistry N, Niemann S, et al. CRYPTIC Consortium. 2022. High fluoroquinolone resistance proportions among multidrug-resistant tuberculosis driven by dominant L2 *Mycobacterium tuberculosis* clones in the Mumbai metropolitan region. *Genome Med* 14:95. <https://doi.org/10.1186/s13073-022-01076-0>
20. Ismail NA, Omar SV, Moultrie H, Bhyat Z, Conradie F, Enwerem M, Ferreira H, Hughes J, Joseph L, Kock Y, Letsaolo V, Maartens G, Meintjes G, Ngcamu D, Okozi N, Padanilam X, Reuter A, Romero R, Schaaf S, Te Riele J, Variava E, van der Meulen M, Ismail F, Ndjeka N. 2022. Assessment of epidemiological and genetic characteristics and clinical outcomes of resistance to bedaquiline in patients treated for rifampicin-resistant tuberculosis: a cross-sectional and longitudinal study. *Lancet Infect Dis* 22:496–506. [https://doi.org/10.1016/S1473-3099\(21\)00470-9](https://doi.org/10.1016/S1473-3099(21)00470-9)
21. Chesov E, Chesov D, Maurer FP, Andres S, Utpatel C, Barilar I, Donica A, Reimann M, Niemann S, Lange C, Cruu D, Heyckendorf J, Merker M. 2022. Emergence of bedaquiline resistance in a high tuberculosis burden country. *Eur Respir J* 59:2100621. <https://doi.org/10.1183/13993003.00621-2021>
22. Omar SV, Ismail F, Ndjeka N, Kaniga K, Ismail NA. 2022. Bedaquiline-resistant tuberculosis associated with Rv0678 mutations. *N Engl J Med* 386:93–94. <https://doi.org/10.1056/NEJMc2103049>
23. Beckert P, Sanchez-Padilla E, Merker M, Dreyer V, Kohl TA, Utpatel C, Köser CU, Barilar I, Ismail N, Omar SV, Klopper M, Warren RM, Hoffmann H, Maphalala G, Ardizzoni E, de Jong BC, Kerschberger B, Schramm M, Andres S, Kranzer K, Maurer FP, Bonnet M, Niemann S. 2020. MDR *M. tuberculosis* outbreak clone in Eswatini missed by Xpert has elevated bedaquiline resistance dated to the pre-treatment era. *Genome Med* 12:104. <https://doi.org/10.1186/s13073-020-00793-8>
24. Sanchez-Padilla E, Merker M, Beckert P, Jochims F, Dlamini T, Kahn P, Bonnet M, Niemann S. 2015. Detection of drug-resistant tuberculosis by Xpert MTB/RIF in Swaziland. *N Engl J Med* 372:1181–1182. <https://doi.org/10.1056/NEJMc1413930>
25. Bateson A, Ortiz Canseco J, McHugh TD, Witney AA, Feuerriegel S, Merker M, Kohl TA, Utpatel C, Niemann S, Andres S, et al. 2022. Ancient and recent differences in the intrinsic susceptibility of *Mycobacterium tuberculosis* complex to pretomanid. *J Antimicrob Chemother* 77:1685–1693. <https://doi.org/10.1093/jac/dkac070>
26. Kamara RF, Saunders MJ, Sahr F, Losa-Garcia JE, Foray L, Davies G, Wingfield T. 2022. Social and health factors associated with adverse treatment outcomes among people with multidrug-resistant tuberculosis in Sierra Leone: a national, retrospective cohort study. *Lancet Glob Health* 10:e543–e554. [https://doi.org/10.1016/S2214-109X\(22\)00004-3](https://doi.org/10.1016/S2214-109X(22)00004-3)
27. van Soolingen D, Hermans PW, de Haas PE, Soll DR, van Embden JD. 1991. Occurrence and stability of insertion sequences in *Mycobacterium tuberculosis* complex strains: evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. *J Clin Microbiol* 29:2578–2586. <https://doi.org/10.1128/jcm.29.11.2578-2586.1991>
28. Grobbel H-P, Merker M, Köhler N, Andres S, Hoffmann H, Heyckendorf J, Reimann M, Barilar I, Dreyer V, Hillemann D, Kalsdorf B, Kohl TA, Sanchez Carballo P, Schaub D, Todt K, Utpatel C, Maurer FP, Lange C, Niemann S. 2021. Design of multidrug-resistant tuberculosis treatment regimens based on DNA sequencing. *Clin Infect Dis* 73:1194–1202. <https://doi.org/10.1093/cid/ciab359>
29. Merker M, Barbier M, Cox H, Rasigade J-P, Feuerriegel S, Kohl TA, Diel R, Borrell S, Gagnew S, Nikolayevskyy V, Andres S, Nübel U, Supply P, Wirth T, Niemann S. 2018. Compensatory evolution drives multidrug-resistant tuberculosis in central Asia. *eLife* 7:e38200. <https://doi.org/10.7554/eLife.38200>
30. Baym M, Kryazhimskiy S, Lieberman TD, Chung H, Desai MM, Kishony R. 2015. Inexpensive multiplexed library preparation for megabase-sized genomes. *PLOS ONE* 10:e0128036. <https://doi.org/10.1371/journal.pone.0128036>
31. Kohl TA, Utpatel C, Schleusener V, De Filippo MR, Beckert P, Cirillo DM, Niemann S. 2018. MTBseq: a comprehensive pipeline for whole genome sequence analysis of *Mycobacterium tuberculosis* complex isolates. *PeerJ* 6:e5895. <https://doi.org/10.7717/peerj.5895>
32. Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE, et al. 1998. Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* 393:537–544. <https://doi.org/10.1038/31159>
33. Li H, Durbin R. 2009. Fast and accurate short read alignment with burrows-wheeler transform. *Bioinformatics* 25:1754–1760. <https://doi.org/10.1093/bioinformatics/btp324>
34. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009. The sequence alignment/map format and samtools. *Bioinformatics* 25:2078–2079. <https://doi.org/10.1093/bioinformatics/btp352>
35. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernysky A, Garimella K, Altshuler D, Gabriel S, Daly M, DePristo MA. 2010. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res* 20:1297–1303. <https://doi.org/10.1101/gr.107524.110>
36. World Health Organization. 2021. Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance. World Health Organization, Geneva. Available from: <https://apps.who.int/iris/handle/10665/343656>. Retrieved 13 Apr 2022.
37. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol* 32:268–274. <https://doi.org/10.1093/molbev/msu300>
38. Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermini LS. 2017. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat Methods* 14:587–589. <https://doi.org/10.1038/nmeth.4285>
39. Minh BQ, Nguyen MAT, von Haeseler A. 2013. Ultrafast approximation for phylogenetic bootstrap. *Mol Biol Evol* 30:1188–1195. <https://doi.org/10.1093/molbev/mst024>
40. Letunic I, Bork P. 2007. Interactive tree of life (iTOL): an online tool for phylogenetic tree display and annotation. *Bioinformatics* 23:127–128. <https://doi.org/10.1093/bioinformatics/btl529>
41. Miotto P, Cabibbe AM, Borroni E, Degano M, Cirillo DM. 2018. Role of disputed mutations in the rpoB gene in interpretation of automated liquid MGIT culture results for rifampin susceptibility testing of *Mycobacterium tuberculosis*. *J Clin Microbiol* 56:e01599-17. <https://doi.org/10.1128/JCM.01599-17>
42. Claessens M, Dreyer V, Nepolo E, Mokomele Q, van Rooyen G, Ruswa N, Günther G, Niemann S. 2022. Whole-genome sequencing for resistance prediction and transmission analysis of *Mycobacterium tuberculosis* complex strains from Namibia. *Microbiol Spectr* 10:e01586-22. <https://doi.org/10.1128/spectrum.01586-22>
43. Makhado NA, Matabane E, Faccin M, Pinçon C, Jouet A, Boutachkourt F, Goeminne L, Gaudin C, Maphalala G, Beckert P, Niemann S, Delvenne J-C, Delmée M, Razwiedani L, Nchabeleng M, Supply P, de Jong BC, André E. 2018. Outbreak of multidrug-resistant tuberculosis in South Africa undetected by WHO-endorsed commercial tests: an observational study. *Lancet Infect Dis* 18:1350–1359. [https://doi.org/10.1016/S1473-3099\(18\)30496-1](https://doi.org/10.1016/S1473-3099(18)30496-1)

44. Lavender C, Globan M, Sievers A, Billman-Jacobe H, Fyfe J. 2005. Molecular characterization of isoniazid-resistant *Mycobacterium tuberculosis* isolates collected in Australia. *Antimicrob Agents Chemother* 49:4068–4074. <https://doi.org/10.1128/AAC.49.10.4068-4074.2005>
45. Muhammad Dayyab F, Ilyasu G, Garba Ahmad B, Aliyu Umar I, Musa Shuaib N, Bajehson M, Muhammad Daiyab I, Akpala O, Remilekun O, Garba Habib A. 2022. Emerging threat of drug-resistant tuberculosis and trends in the era of COVID-19: a descriptive study from Northwestern Nigeria. *J Clin Tuberc Mycobact Dis* 28:100319. <https://doi.org/10.1016/j.jctube.2022.100319>
46. Forson A, Kwara A, Kudzawu S, Omari M, Otu J, Gehre F, de Jong B, Antonio M. 2018. A cross-sectional study of tuberculosis drug resistance among previously treated patients in a tertiary hospital in Accra, Ghana: public health implications of standardized regimens. *BMC Infect Dis* 18:149. <https://doi.org/10.1186/s12879-018-3053-5>
47. Guo Q, Bi J, Lin Q, Ye T, Wang Z, Wang Z, Liu L, Zhang G. 2022. Whole genome sequencing identifies novel mutations associated with bedaquiline resistance in *Mycobacterium tuberculosis*. *Front Cell Infect Microbiol* 12. <https://doi.org/10.3389/fcimb.2022.807095>
48. Andres S, Merker M, Heyckendorf J, Kalsdorf B, Rumetshofer R, Indra A, Hofmann-Thiel S, Hoffmann H, Lange C, Niemann S, Maurer FP. 2020. Bedaquiline-resistant tuberculosis: dark clouds on the horizon. *Am J Respir Crit Care Med* 201:1564–1568. <https://doi.org/10.1164/rccm.201909-1819LE>
49. Nimmo C, Millard J, Brien K, Moodley S, van Dorp L, Lutchminarain K, Wolf A, Grant AD, Balloux F, Pym AS, Padayatchi N, O'Donnell M. 2020. Bedaquiline resistance in drug-resistant tuberculosis HIV co-infected patients. *Eur Respir J* 55:1902383. <https://doi.org/10.1183/13993003.02383-2019>
50. Van Rie A, Walker T, de Jong B, Rupasinghe P, Rivière E, Dartois V, Sonnenkalb L, Machado D, Gagneux S, Supply P, Dreyer V, Niemann S, Goig G, Meehan C, Tagliani E, Cirillo DM. 2022. Balancing access to BPaLM regimens and risk of resistance. *Lancet Infect Dis* 22:1411–1412. [https://doi.org/10.1016/S1473-3099\(22\)00543-6](https://doi.org/10.1016/S1473-3099(22)00543-6)
51. Stucki D, Brites D, Jeljeli L, Coscolla M, Liu Q, Trauner A, Fenner L, Rutaihua L, Borrell S, Luo T, et al. 2016. *Mycobacterium tuberculosis* lineage 4 comprises globally distributed and geographically restricted sublineages. *Nat Genet* 48:1535–1543. <https://doi.org/10.1038/ng.3704>
52. Demay C, Liens B, Burguière T, Hill V, Couvin D, Millet J, Mokrousov I, Sola C, Zozio T, Rastogi N. 2012. SITVITWEB—a publicly available International multimarker database for studying *Mycobacterium tuberculosis* genetic diversity and molecular epidemiology. *Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis* 12:755–766. <https://doi.org/10.1016/j.meegid.2012.02.004>
53. Coscolla M, Gagneux S. 2014. Consequences of genomic diversity in *Mycobacterium tuberculosis*. *Semin Immunol* 26:431–444. <https://doi.org/10.1016/j.smim.2014.09.012>
54. López MG, Dogba JB, Torres-Puente M, Goig GA, Moreno-Molina M, Villamayor LM, Cadmus S, Comas I. 2020. Tuberculosis in Liberia: high multidrug-resistance burden, transmission and diversity modelled by multiple importation events. *Microb Genom* 6:e000325. <https://doi.org/10.1099/mgen.0.000325>
55. Ajawatanawong P, Yanai H, Smittipat N, Disratthakit A, Yamada N, Miyahara R, Nedsuwan S, Imasanguan W, Kantipong P, Chaiyasirinroje B, et al. 2019. A novel ancestral Beijing sublineage of *Mycobacterium tuberculosis* suggests the transition site to modern Beijing sublineages. *1. Sci Rep* 9:13718. <https://doi.org/10.1038/s41598-019-50078-3>
56. Merker M, Blin C, Mona S, Duforet-Frebourg N, Lecher S, Willery E, Blum MGB, Rüscher-Gerdes S, Mokrousov I, Aleksic E, et al. 2015. Evolutionary history and global spread of the *Mycobacterium tuberculosis* Beijing lineage. *Nat Genet* 47:242–249. <https://doi.org/10.1038/ng.3195>
57. Merker M, Nikolaevskaya E, Kohl TA, Molina-Moya B, Pavlovskaya O, Brännberg P, Dudnyk A, Stokich V, Barilar I, Marynova I, Filipova T, Prat C, Sjöstedt A, Dominguez J, Rzhapishvskaya O, Niemann S. 2020. Multidrug- and extensively drug-resistant *Mycobacterium tuberculosis* Beijing clades, Ukraine, 2015. *Emerg Infect Dis* 26:481–490. <https://doi.org/10.3201/eid2603.190525>
58. Merker M, Rasigade J-P, Barbier M, Cox H, Feuerriegel S, Kohl TA, Shitikov E, Klaos K, Gaudin C, Antoine R, Diel R, Borrell S, Gagneux S, Nikolayevskyy V, Andres S, Crudu V, Supply P, Niemann S, Wirth T. 2022. Transcontinental spread and evolution of *Mycobacterium tuberculosis* W148 European/Russian clade toward extensively drug resistant tuberculosis. *Nat Commun* 13:5105. <https://doi.org/10.1038/s41467-022-32455-1>
59. Klopper M, Heupink TH, Hill-Cawthorne G, Streicher EM, Dippenaar A, de Vos M, Abdallah AM, Limberis J, Merker M, Burns S, Niemann S, Dheda K, Posey J, Pain A, Warren RM. 2020. A landscape of genomic alterations at the root of a near-untreatable tuberculosis epidemic. *BMC Med* 18:24. <https://doi.org/10.1186/s12916-019-1487-2>
60. Coscolla M, Gagneux S, Menardo F, Loiseau C, Ruiz-Rodriguez P, Borrell S, Otchere ID, Asante-Poku A, Asare P, Sánchez-Busó L, et al. 2021. Phylogenomics of *Mycobacterium africanum* reveals a new lineage and a complex evolutionary history. *Microbial Genomics* 7:000477. <https://doi.org/10.1099/mgen.0.000477>
61. Merker M, Kohl TA, Barilar I, Andres S, Fowler PW, Chryssanthou E, Ångeby K, Jureen P, Moradigaravand D, Parkhill J, Peacock SJ, Schön T, Maurer FP, Walker T, Köser C, Niemann S. 2020. Phylogenetically informative mutations in genes implicated in antibiotic resistance in *Mycobacterium tuberculosis* complex. *Genome Med* 12:27. <https://doi.org/10.1186/s13073-020-00726-5>
62. Otchere ID, Asante-Poku A, Osei-Wusu S, Baddoo A, Sarpong E, Ganiyu AH, Aboagye SY, Forson A, Bonsu F, Yahayah AI, Koram K, Gagneux S, Yeboah-Manu D. 2016. Detection and characterization of drug-resistant conferring genes in *Mycobacterium tuberculosis* complex strains: a prospective study in two distant regions of Ghana. *Tuberculosis* 99:147–154. <https://doi.org/10.1016/j.tube.2016.05.014>
63. Gröschel MI, Walker TM, van der Werf TS, Lange C, Niemann S, Merker M. 2018. Pathogen-based precision medicine for drug-resistant tuberculosis. *PLoS Pathog* 14:e1007297. <https://doi.org/10.1371/journal.ppat.1007297>
64. Kontsevaya I, Lange C, Comella-Del-Barrio P, Coarfa C, DiNardo AR, Gillespie SH, Hauptmann M, Leszczyc C, Mandalakas AM, Martinecz A, Merker M, Niemann S, Reimann M, Rzhapishvskaya O, Schaible UE, Scheu KM, Schurr E, Abel Zur Wiesch P, Heyckendorf J. 2021. Perspectives for systems biology in the management of tuberculosis. *Eur Respir Rev* 30:200377. <https://doi.org/10.1183/16000617.0377-2020>
65. Chigutsa E, Visser ME, Swart EC, Denti P, Pushpakom S, Egan D, Holford NHG, Smith PJ, Maartens G, Owen A, McIlleron H. 2011. The *SLCO1B1* rs4149032 polymorphism is highly prevalent in South Africans and is associated with reduced Rifampin concentrations: dosing implications. *Antimicrob Agents Chemother* 55:4122–4127. <https://doi.org/10.1128/AAC.01833-10>
66. Naidoo A, Chirehwa M, Ramsuran V, McIlleron H, Naidoo K, Yende-Zuma N, Singh R, Ncgapu S, Adamson J, Govender K, Denti P, Padayatchi N. 2019. Effects of genetic variability on rifampicin and isoniazid pharmacokinetics in South African patients with recurrent tuberculosis. *Pharmacogenomics* 20:225–240. <https://doi.org/10.2217/pgs-2018-0166>
67. Weiner M, Peloquin C, Burman W, Luo C-C, Engle M, Prihoda TJ, MacKenzie WR, Bliven-Sizemore E, Johnson JL, Vernon A. 2010. Effects of tuberculosis, race, and human gene *SLCO1B1* polymorphisms on rifampin concentrations. *Antimicrob Agents Chemother* 54:4192–4200. <https://doi.org/10.1128/AAC.00353-10>