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ORIGINAL RESEARCH

Drug-Resistant Characteristics, Genetic Diversity, and Transmission Dynamics of Multidrug-Resistant *Mycobacterium tuberculosis* in Jiangxi, China

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Purpose: In this study, we aimed to determine the transmission pattern of multidrug-resistant tuberculosis (MDR-TB) isolates circulating in Jiangxi Province with whole-genome sequencing (WGS). In addition, we also sought to describe mutational resistome of MDR-TB isolates.

Patients and Methods: A total of 115 MDR-TB isolates determined by the phenotypic proportion method of drug susceptibility testing between January 2018 and December 2022 from provincial drug surveillance (DRS) in Jiangxi were included in our analysis. The demographic data and treatment history were extracted from the National TB Registry System. WGS was used to analyze the genotypic characteristics of drug resistance and transmissions.

Results: About 62.6% of MDR-TB strains were isolated from cases that received previous anti-tuberculosis treatment. According to the WGS results, 96.5% were genotypic MDR-TB, and more than half of MDR-TB isolates tested were also resistant to streptomycin (59.1%), ethambutol (56.5%), and fluroquinolones (53.0%), while resistance to cycloserine and linezolid was lowest, only in two (1.7%) and one (0.9%) isolate, respectively. Ser450Leu in *rpoB (57.9%)*, Ser315Thr in *katG (74.1%)*, Met306Val in *embB (40.0%)*, Lys43Arg in *rpsL (75.0%)*, Ala90Val in *gyrA (32.8%)* were predominant mutant types among the rifampin-, isoniazid-, ethambutol-, streptomycin-, fluoroquinolones-resistant isolates, respectively. Lineage 2 (East Asian genotype) occurred at the highest frequency with 97 cases (84.3%), followed by lineage 4 (Euro-American genotype) with 18 cases (15.7%). Additionally, 5 clusters consisting of 10 isolates were identified in the present study, demonstrating a clustering rate of 8.7%.

Conclusion: MDR/Rifampicin-Resistant (RR)-TB epidemic in this region is driven by lineage 2 clade that also show higher resistance to other anti-tuberculosis drugs. Lower cluster rates compared with a relatively higher proportion of new MDR-TB cases indicate that a considerable number of MDR-TB cases remain undiagnosed.

Keywords: *Mycobacterium tuberculosis*, drug resistant, whole-genome sequencing, genetic diversity, transmission dynamics

Introduction

Despite great progress during the past decades, tuberculosis (TB) remains a great public health concern worldwide, with 10.6 million new cases and 1.3 million deaths in 2022.[1] In particular, the epidemic of MDR-TB strains, which are resistant to both key first-line drugs (ie, rifampin and isoniazid), further worsens TB incidence and mortality, and contributes to an estimated 3.9% of TB deaths globally.[1] In patients infected with MDR-TB strains, appropriate diagnosis is infrequent, treatment regimens are long and expensive, and treatment success is low.[2] The extremely prolonged infectious period significantly increases the risk for transmission of MDR-TB in the community. From an epidemiological perspective, a comprehensive understanding of population structure and transmission dynamics of MDR-TB is therefore of great importance to adapt appropriate TB control strategies.

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Recent advances in molecular epidemiology of infectious diseases have made it possible to analyze the predominant lineages and genotypes and understand the transmission pattern of *Mycobacterium tuberculosis* (MTB) isolates.[3–5] Classical genotyping methods, such as restriction fragment length polymorphisms with IS6110, spoligotyping and mycobacterial interspersed repetitive unit-variable number of tandem repeat (MIRU-VNTR), have been widely used for molecular epidemiology studies.[6–8] Based on the characterization of genetic repetitive elements, these methods could successfully reconstruct complex transmission chains, which are essential for implementing effective public health interventions. Unfortunately, most of the genomic diversity and distinctives of MTB isolates are underestimated by these classical methods that are only able to resolve a small fraction of the whole genome of MTB. In contrast, WGS technology provides a vast amount of information for MTB genotyping at a genome-wide single nucleotide polymorphism (SNP)-based resolution. Sequencing data emanating from subtyping can also provide key information on mutations conferring drug resistance, which provides information on the early emergence and spread of drug-resistant TB, and guides the development of rapid diagnostic tools.[9–12]

China has the third highest TB burden in the world, it is estimated that there were 748,000 new TB cases and 30,000 cases of MDR/RR-TB in 2022, accounting for 7.1% and 7.3% of global cases, respectively.[1] However, the distribution of reported TB cases is remarkably unbalanced across the country.[13,14] A nationwide TB prevalence survey in China reported that the TB prevalence in western provinces was about three times higher than that in eastern provinces. The geographic diversity of TB prevalence undoubtedly affects the transmission pattern within the community. Jiangxi Province is located in southern China, covering an area of 167,000 square kilometers and including a population of 45 million. According to the 2010 China National Tuberculosis Epidemiologic Survey, the incidence rate of TB in the general population of Jiangxi Province was 463 cases per 100,000 population, nearly 19.8% of which were MDR/RR-TB based on a previous study[15] much higher than the average incidence rate of MDR-TB reported in China.

In this study, we aimed to determine the transmission pattern of MDR-TB isolates circulating in Jiangxi Province with WGS and to describe mutational characteristic resistome of MDR-TB isolates.

Materials and Methods

Sampling and Data Collection

In a retrospective study based on the routine provincial drug resistance surveillance in Jiangxi Province, this study collected all MDR strains isolated from sputum smear-positive suspected TB patients attending local sentinel hospitals in 6 surveillance sites as shown in Figure 1 (Shangrao, Yichun, Xinyu, Fuzhou, Ji'an and Ganzhou, Jiangxi Province, China), between January 2018 and December 2022. Mycobacterial species identification was performed for all collected strains using Lowenstein-Jensen (L-J) medium containing p-nitrobenzoic acid (PNB). A total of 115 MDR-TB strains were identified by the proportional method on L-J medium, and these sub-cultured isolates were followed by WGS. The demographic characteristics and clinical characteristics were collected from the national TB registry system. This system records registration details of tuberculosis patients, such as sex, occupation, and other relevant information.

Phenotypical Drug Susceptibility Testing

Each of the 115 MTB isolates was tested for susceptibility to the first-line drugs and second-line drugs, using 1% indirect proportion method according to World Health Organization (WHO) guidelines. The drug concentrations of each drug included in this study were as follows: isoniazid (0.2 μg/mL), rifampicin (40.0 μg/mL), ethambutol (2 μg/mL), streptomycin (4 μg/mL), ofloxacin (4 μg/mL) and kanamycin (30 μg/mL). The pan-sensitive strains H37Rv (ATCC 27294) and periodic external quality assessment of drug susceptibility testing (DST) results were provided and conducted by the Tuberculosis Reference Laboratory at the Chinese Center for Disease Control and Prevention.

DNA Extraction and Sequencing

Crude genomic DNA was extracted from fresh cultures incubated on L-J medium using the cetyltrimethylammonium bromide (CTAB) method.[16] Lysozyme was added to each tube (final concentration, 1 mg/mL), followed by incubation at 37°C for 2 h. Ten percent sodium dodecyl sulfate (final concentration, 1.1%) and proteinase K (final concentration, 0.2 mg/mL; Promega

Figure 1 Distribution of six drug resistance surveillance sites in Jiangxi province.

Inc.) were then added, and the tubes were vortexed gently and incubated for 20 min at 65°C. A mixture of N-acetyl-N,N, N-trimethyl ammonium bromide (CTAB; final concentration, 40 mM) and NaCl (final concentration, 0.1 M) was added, followed immediately by the addition of NaCl alone (final concentration, 0.6 M). The tubes were then vortexed until the suspension turned milky and were incubated for 10 min at 65°C. Seven hundred fifty microliters of chloroform-isoamyl alcohol (24:1) was added to each tube, and the tubes were vortexed and then centrifuged in a microcentrifuge at 13,000 rpm for 5 min at room temperature. The genomic DNA present in the resulting aqueous phase was then isolated by ethanol precipitation as previously described and resuspended in 30 μL nuclease-free water. The determination of nucleic acid concentration is analyzed using the Qubit 2.0 (Thermo Fisher Scientific). The qualified samples were sequenced by novaseq 6000 Sequencer (Shanghai Gene-Optimal Science & Technology Co., Ltd.) with the sequencing of 2 × 150bp double-end and 200×. The library preparation kit utilized is the FS DNA Lib Prep Kit V6 from Abclone. Performing library sequencing using the NovaSeq 6000 instrument and the accompanying NovaSeq S4 reagent kit.

Genetic Lineage Analysis and Antimicrobial Resistance Prediction

Fastp (v0.20.0) was used to filter the raw FASTQ sequences by removing adaptor sequences, duplicate reads, and low-quality reads with Phred quality scores below 20 in more than 30% of the bases. The clean sequencing reads were mapped to the reference genome of H37Rv (accession number, NC_000962.2) using in-house softwares BWA (Version 0.7.17) and samtools (Version 1.7). An average of 8 million sequence reads was acquired per genome at a depth of 200× and with coverage of 98.0%. Freebayes (Version 1.3.2) was utilized for calling SNPs with filtering parameters set to include sites with at least 10 aligned reads and a mapping quality of at least 100. All genome-wide SNPs were identified by FreeBayes software by parsing the mapped genome sequence data and then excluding SNPs related to phylogeny or

located in proline-glutamate/proline-proline-glutamate (PE/PPE) gene family regions. We build maximum likelihood trees based on SNP alignment parameters using IQ-TREE (v. 2.1.3) on the basis of SNP alignment parameters.[14] Based on previous studies, the TB Profiler (v4.4.2) (https://github.com/jodyphelan/TBProfiler) was used as a tool for the prediction of known resistance-associated polymorphisms.[17]

Statistical Analysis

Chi-square test or Fisher's exact test was used for categorical data. All statistical analysis was performed in the SPSS version 18.0 software (SPSS Inc., Chicago, Illinois). $P < 0.05$ was considered statistically significant.

Ethics Statement

The study was approved by the Medical Ethics Committee of Jiangxi Center for Disease Control and Prevention (JXCDCKLS-2023-37). Informed consent was waived considering that this study presented no more than minimal risk of harm to patient subjects. This study was conducted in accordance with the Declaration of Helsinki.

Results

Demographic and Clinical Characteristics

Among the 115 MDR-TB patients 90 (78.3%) were male and 25 (21.7%) were female. Age-wise analysis displayed that the ages of the participants ranged from 11 to 84 (mean ± standard deviation [SD], 54.4 ± 17.7). Additionally, 72 (62.6%) were retreated cases (defined as cases previously treated with anti-tuberculosis drugs for more than one month, cases of relapse or cases of failure of initial treatment), 43 (37.4%) were new cases (defined as new cases not previously treated with anti-tuberculosis drugs or treated for less than one month). Detailed demographic information is shown in Table 1.

Molecular Drug-Resistance Characteristics Detected by WGS

The prevalence of resistance to six anti-tuberculosis drugs (rifampicin, isoniazid, ethambutol, streptomycin, ofloxacin and kanamycin) were compared between genetic sequencing results and phenotypic testing method, as illustrated in Figure 2. Significant overlap of the six drugs was observed between genetic sequencing and the phenotypic DST (pDST). The genotypic drug-resistance profiles are shown in Table 2. The resistance rate of pyrazinamide (40.9%) was the lowest

Table 1 Demographic Characteristics of Patients with MDR-TB

Variables		Count (N = 115)	Percentage (%)
Sex			
	Male	90	78.3
	Female	25	21.7
Age (yrs)			
	<30	15	13.0
	30–44	16	13.9
	45–59	30	26.1
	≥60	54	47.0
Occupation			
	Farmer	89	77.4
	Others	26	22.6
Ethnicity			
	Han	115	100.0
	Others	0	0.0
Previous TB treatment			
	Yes	72	62.6
	No	43	37.4

Abbreviations: MDR-TB, multidrug-resistant tuberculosis; TB, tuberculosis.

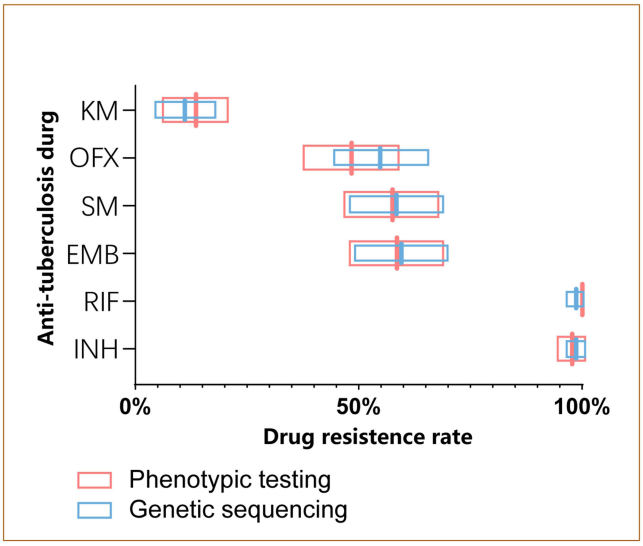

Figure 2 Prevalence of six anti-tuberculosis drugs resistance, estimated through genetic sequencing compared with phenotypic testing.

among the first-line drugs. The resistance rate for the other drugs was much lower than those for the first-line drugs in the following ranking order (from low to high): linezolid (0.9%) < cycloserine (1.7%) < aminoglycosides (9.6%) = para-aminosalicylic acid (9.6%) < ethionamide (15.7%) < fluoroquinolones (53.0%).

Distribution of drug-resistant tuberculosis strains with co-resistance was further investigated, and the results revealed that more than half of MDR strains were resistant to at least 2 anti-tuberculosis drugs, with more than 20% of MDR strains resistant to at least 5 drugs.

Table 2 Drug-Resistant Profiles of 115 MDR-TB Strains by WGS

Drugs/Drug-Resistant Patterns	Resistance (Gene Mutations) (N = 115) No (%).	Drug Resistance Genes	Major Mutation Characteristics of Drug-Associated Genes No.
First-line drugs			
Rifampicin	114 (99.1)	*rpoB*	Ser450Leu: 66
			His445Asp: 7
			His445Leu: 5
			Leu430Pro: 5
			Val170Phe: 1
			Ile491Phe: 1
		rpoB+rpoC	3
Isoniazid	112 (97.4)	*KatG*	Ser315Thr: 83
			Ser315Arg: 3
		fabG1	c.-15C>T: 10
		ahpC	c.-52C>T: 4
Streptomycin	68 (59.1)	*rpsL*	Lys43Arg: 51
			Lys88Arg: 10
		rrs	5
Ethambutol	65 (56.5)	*embB*	Met306Val: 26
			Met306Ile: 12
		embB+embA-P	11
Pyrazinamide	47 (40.9)	*pncA*	47

(*Continued*)

Table 2 (Continued).

Drugs/Drug-Resistant Patterns	Resistance (Gene Mutations) (N = 115) No (%).	Drug Resistance Genes	Major Mutation Characteristics of Drug-Associated Genes No.
Second-line drugs			
Fluoroquinolones	61 (53.0)	*gyrA*	Ala90Val: 20
			Asp94Gly: 19
			Asp94Asn: 7
			Asp94Ala: 4
			Asp94Tyr: 4
			Asp94His: 1
		gyrB	3
		gyrA+gyrB	3
Ethionamide	18 (15.7)	*fabG1*	c.-15C>T: 10
Para-aminosalicylic acid	11 (9.6)	*thyX*	c.-16C>T: 6
Aminoglycosides	11 (9.6)	*rrs*	a-1401g: 11
Cycloserine	2 (1.7)	*alr*	Leu113Arg: 1
		ald	c.433_434insGC: 1
Linezolid	1 (0.9)	*rplC*	Cys154Arg
MDR-TB[a]	111 (96.5%)	/	/
Pre-XDR-TB[b]	59 (51.3%)	/	/
XDR-TB[c]	1 (0.9%)	/	/

Notes: [a]MDR, Defined as resistance to at least isoniazid and rifampicin;. [b]Pre-XDR, Pre-extensively drug-resistant, defined as MDR/RR isolates with additional resistance to either a fluoroquinolone. [c]XDR, extensively drug-resistant, defined as resistance to rifampicin, any fluoroquinolone and at least one of bedaquiline or linezolid.
Abbreviations: MDR-TB, multidrug-resistant tuberculosis; Pre-XDR-TB, pre-extensively drug resistant tuberculosis; XDR, extensively drug-resistant.

Among 115 phenotypic MDR-TB strains, 114 strains had detectable mutations in the *rpo*B gene. The most prevalent drug-resistant mutations were Ser450Leu (57.9%, 66/114), followed by His445Asp (6.1%, 7/114), His445Leu (4.4%, 5/114) and Leu430Pro (4.4%, 5/114). Two strains (1.8%, 2/114) harbored gene mutations out of rifampicin resistance-determining region (RRDR), namely Val170Phe and Ile491Phe, respectively. Double mutations were detected in 9 strains (7.9%, 9/114), three (2.6%, 3/114) of which also carried complementary mutations in the *rpoC* gene. Additionally, 112 strains had detectable mutations related to isoniazid resistance. *KatG* Ser315Thr (83/112, 74.1%) was the most common mutations, followed by *fabG*1 c.-15C>T (8.9%, 10/112), *ahpC* c.-52C>T (3.6%, 4/112) and *KatG*Ser315Arg (2.7%, 3/112). Fourteen strains (12.5%) carried combined mutations, 7 of which revealed *katG* mutations combined with *ahpc* mutations (6.3%). Among the 47 MTB strains with genotypically pyrazinamide-resistance, 41 mutant forms in the *pncA* gene were detected, and no hot spot regions were identified. Genotypically ethambutol-resistant isolates were mainly related to the mutation *embB*Met306Val (40.0%, 26/65) and Met306Ile (18.5%, 12/65). Notably, 11 MTB strains carried combinations of mutations in the *embB* gene and the *embA* promoter region. Interestingly, 6 of these 11 strains carried combined mutations in *embA* promoter region (c.-12C>T) and *embB* (Gly406Ala). Most of the genotypically streptomycin-resistant strains were detected with Lys43Arg and Lys88Arg mutation in *rpsL* gene, accounting for 75.0% (51/68) and 14.7% (10/68), respectively. Five MTB strains with streptomycin-resistant genotype carried mutation in *rrs* gene (7.4%, 5/68). Additionally, all fluoroquinolone-resistant MTB strains were identified with mutations in the *gyrA* and/or *gyrB* gene, mainly linked to the mutation of Ala90Val (32.8%, 20/61) and Asp94Gly (31.1%, 19/61) in *gyrA* gene. In addition to this, 16 strains (26.2%) with mutations occurring in codon 94 in *gyrA* gene (7 with Asp94Asn, 4 with Asp94Ala, 4 with Asp94Tyr and one with Asp94His). Eleven strains were detected with the a-1401g mutation in *rrs* gene associated with aminoglycosides-resistance. Among 18 ethionamide-resistant isolates, 10 carried mutations in *fabG1*c.-15C>T (55.6%, 10/18). Para-aminosalicylic acid-resistance-related genes were detected in 11 strains, of which 6 had mutation in *thyX* c.-16C>T (54.5%). Mutations associated with cycloserine resistance were identified in two strains with

Leu113Arg in *alr* gene and *ald* c.433_434insGC. Moreover, one strain exhibited mutation in *rplC* Cys154Arg. The molecular drug-resistant characteristics are shown in Table 2.

Lineage Distribution of Multidrug-Resistant TB Strains

Potential interaction of host genetic factors with genetic nature of MTB may influence the susceptibility of MTB to anti-tuberculosis drugs. The results of lineage analysis showed that among 115 isolates of MDR-TB collected in this study, lineage 2 (East Asian genotype) occurred at the highest frequency with 97 cases (84.3%), followed by lineage 4 (Euro-American genotype) with 18 cases (15.7%). Furthermore, lineage 2.2.1 (Beijing genotype) was the dominant sub lineage with 94 cases (81.7%), followed by lineage 4.4 (8 cases, 7.0%), lineage 4.2 and lineage 4.5 (5 cases, 4.3%). Only 3 isolates belonged to lineage 2.2.2 (2.6%), as shown in Figure 3. Five clusters, consisting of 10 isolates were identified in the present study, demonstrating a clustering rate of 8.7%. Further analysis of the association between the two lineages and the emergence of resistance revealed that lineage 2 was more likely to be streptomycin resistant ($P < 0.05$) (Table 3).

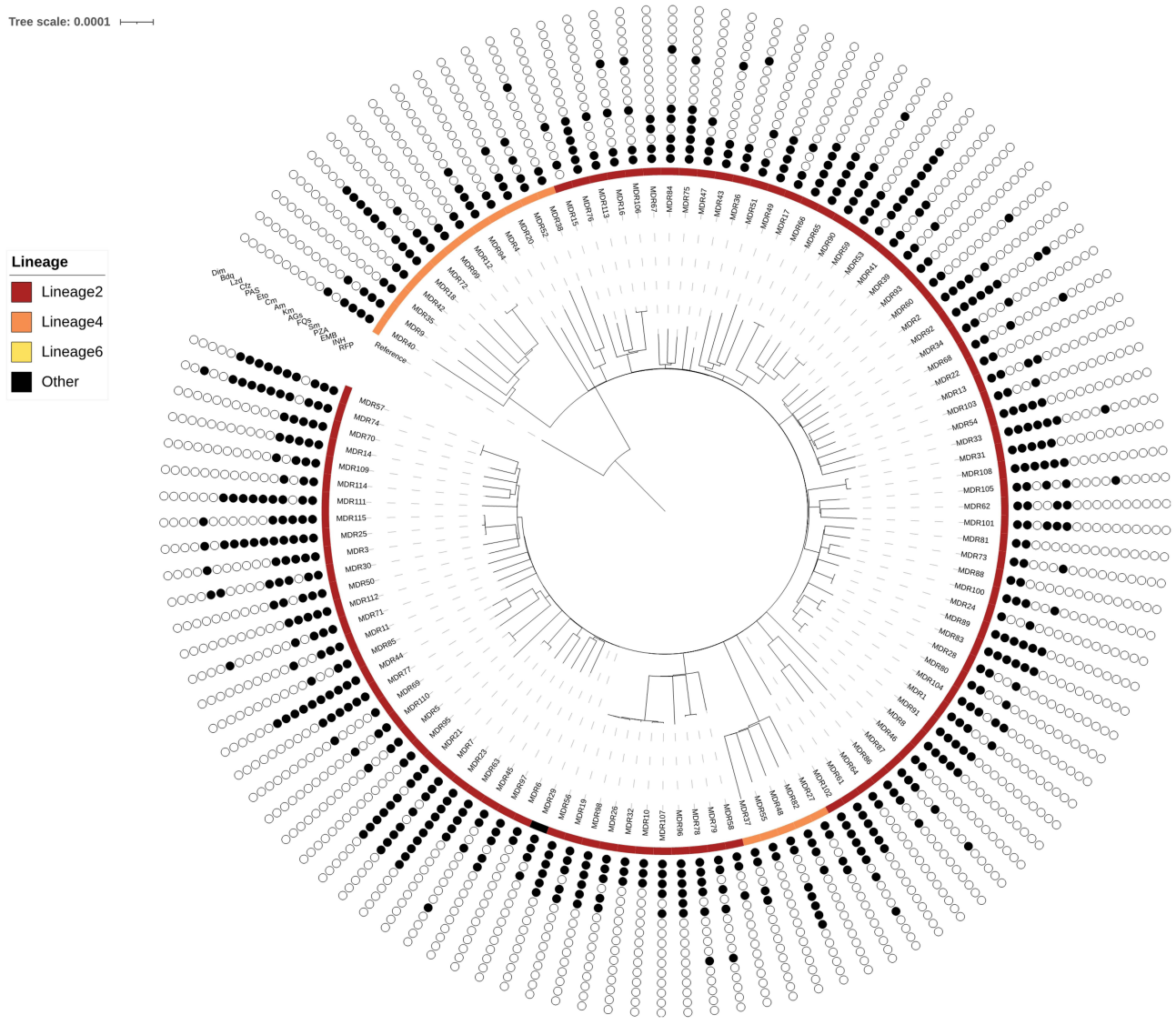

Figure 3 Maximum-likelihood tree of 115 MDR-TB strains, lineages.
Notes: Scale bar indicates the genetic distance proportional to the total number of single nucleotide polymorphisms.

Table 3 Comparison of Genotypic Drug-Resistant Profiles Between Lineage 2 and Lineage 4

Drugs/Drug Resistant Patterns	Lineage 2 (*n* = 97) No. (%)	Lineage 4 (*n* = 18) No. (%)	χ^2	*P*
First-line drugs				
Rifampicin	96 (99.0)	18 (100.0)	/	/
Isoniazid	94 (96.9)	18 (100.0)	/	/
Pyrazinamide	43 (44.3)	4 (22.2)	3.071	0.080
Ethambutol	58 (59.8)	7 (38.9)	2.700	0.100
Streptomycin	64 (66.0)	4 (22.2)	12.029	0.001
Second-line drugs				
Fluoroquinolones	52 (53.6)	9 (50.0)	0.079	0.778
Aminoglycosides	9 (9.3)	2 (11.1)		0.682 [a]
Ethionamide	16 (16.5)	2 (11.1)		0.734 [a]
Para-aminosalicylic acid	11 (11.3)	0 (0.0)		0.209 [a]
Cycloserine	2 (2.1)	0 (0.0)		>0.999 [a]
Linezolid	1 (1.0)	0 (0.0)		>0.999 [a]
MDR-TB[b]	93 (95.9)	18 (100.0)	/	/
Pre-XDR-TB[c]	50 (51.5)	9 (50.0)	0.015	0.904
XDR-TB[d]	1 (1.0)	0		0.843[a]

Notes: [a]indicates that *P* value was calculated by Fisher exact test. [b]MDR, Defined as resistance to at least isoniazid and rifampicin. [c]Pre-XDR, Pre-extensively drug-resistant, defined as MDR/RR isolates with additional resistance to either a fluoroquinolone. [d]XDR, Extensively drug-resistant, defined as resistance to rifampicin, any fluoroquinolone and at least one of bedaquiline or linezolid.
Abbreviations: MDR-TB, multidrug-resistant tuberculosis; Pre-XDR-TB, pre-extensively drug resistant tuberculosis; XDR, extensively drug-resistant.

Discussion

Transmission of MDR/RR-TB represents the greatest threat to global TB control. Molecular epidemiological investigation of drug-resistant TB is essential to identify its transmission dynamics, thereby providing important insights for the formulation of effective TB control strategies.[18] In this study, we explored the role of bacterial genetics in the MDR/RR-TB epidemic in Jiangxi province of China, one of the MDR-TB hotspots recognized by WHO. Our findings demonstrate that the MDR/RR-TB epidemic in Jiangxi province is driven by lineage 2 clade that are resistant to various anti-tuberculosis drugs and are also predominant in other countries of East Asia. Studies have reported that 'Lineage 2', also referred to as 'East Asian lineage' and almost entirely consisting of lineage 2.2.1 (Beijing genotype strains).[19,20] In a recent nationwide survey, approximately half of MTB isolates tested in Southern China belong to Beijing genotype, which was significantly lower than our observation among MDR/RR-TB isolates, indicating the association between Beijing genotype and drug resistance.[21,22] Increasing evidence has demonstrated that the Beijing genotype of MTB has increased the opportunity to develop drug resistance compared to other lineages of the bacteria. On the one hand, the Beijing genotype has been associated with a higher frequency of certain genetic mutations, leading to a greater likelihood of developing drug resistance. On the other hand, this prevailing genotype has shown a high potential to evade host immune response, effectively facilitating the accumulation of genetic mutations conferring drug resistance. Our research shows that lineage 2 was more likely to be streptomycin resistant than lineage 4, Zhou et al also found that MDR Beijing strains were more likely to be resistant to streptomycin than non-MDR Beijing strains and other genotypes.[23]

Another important finding of our study is that 37.4% of MDR/RR-TB patients had no previous exposure history to anti-tuberculosis drugs, emphasizing the contribution of recent transmission in the epidemic of drug-resistant TB in Jiangxi province. Conversely, only 8.7% of MDR/RR-TB patients were in clusters based on WGS results. This great gap highlights that a considerable number of MDR-TB remain undiagnosed due to poor access to drug susceptibility testing. In line with our hypothesis, only 40% of an estimated 30,000 MDR/RR-TB cases were reported in China. Patients with MDR-TB are more likely to generate new secondary cases compared with patients with drug-susceptible tubercle bacilli, which emphasizes the high risk of MDR-TB nosocomial transmission due to the missed cases in the community.[24] In

Jiangxi province, conventional DST remains the major methodology used for the diagnosis of drug-resistant TB, and there is an urgent need to scale up molecular diagnostics to facilitate the timely detection of MDR-TB in this region.

We also observed an extremely high proportion of rifampicin-resistant MTB isolates with mutation *rpoB*S450L, accounting for 57.9% of isolates in our study. It is well recognized that this amino substitution is always confers high-level rifampicin resistance in MTB.[25] Previous experimental studies have revealed that the genetic mutation conferring high-level drug resistance led to the significant loss of fitness. However, in a recent report, Loiseau et al[26] found that the MTB isolates carrying the mutation *rpoB*S450L was associated with a minimal fitness cost (the relative transmission fitness of MDR in a drug resistance hotspot). One explanation for this seemingly contradictory result is that the fitness cost of this mutation depends on the phylogenetic lineage of MTB isolates. Data from previous genotyping studies also support our hypothesis that lineage 4 MDR-TB strains suffer a significant reduction in transmission fitness compared to drug-susceptible counterparts, but lineage 2 MDR-TB strains do not.[26,27] Many rifampicin-resistant mutants with *rpoB* mutations harbor the second mutations in *rpoA, rpoB*, or *rpoC* to compensate for the fitness cost caused by the primary mutation in *rpoB* gene. In the present study, the fitness-compensatory mutations were only recorded in three isolates, which echoes the aforementioned results of the predominance of lineage 2 MDR-TB strains with the mutation *rpoB*S450L.

Increasing resistance to fluoroquinolone (FQ) agents in MTB isolates has attracted more attention in view of their cornerstone role in the treatment of MDR-TB. Approximate half of MDR/RR-TB isolates were resistant to FQ. In line with our observation, a recent study by Yao and colleagues revealed that FQ resistance was noted in three quarters of MDR-TB isolates in China, and the increasing FQ resistance among MDR-TB patients may be majorly attributed to poorly controlled use of FQs in empirical therapy of respiratory infections.[28] FQ resistance among MDR-TB patients significantly increases the risk of unfavorable treatment outcomes. Thus, our findings highlight the potential for the high rate of adverse clinical outcomes in patients afflicted with MDR-TB. In addition, a detailed analysis of WGS data revealed that linezolid and bedaquiline resistance were extremely low in this region, indicating the anticipated usage of WGS technology in the diagnosis of MDR-TB. Recently, WHO has endorsed the use of WGS for surveillance of resistance to anti-tuberculosis drugs.[29] Considering that pDST to the majority of second-line anti-tuberculosis drugs almost inaccessible in Jiangxi province, WGS would provide a superior solution due to its endorsable utilities and affordable cost.

We also acknowledged several obvious limitations of this study. First, despite the enrolment of MDR/RR-TB isolates in the pilots, the limited sample size weakened the significance of our conclusion. Second, the present study lacks the epidemiological links of the clustered cases, which hampered us to confirm the genotype clusters. Third, in view of the small number of MDR/RR-TB isolates, we could not identify the risk factors associated with the transmission of drug-resistant TB in this region.

Conclusion

In conclusion, we firstly investigate the genetic diversity of MDR/RR-TB in Jiangxi Province. Our findings demonstrate that the MDR/RR-TB epidemic in this region is driven by lineage 2 clade that are resistant to many anti-tuberculosis drugs. Lower cluster rates compared with a relatively higher proportion of new MDR-TB cases indicate that a considerable number of MDR-TB cases remain undiagnosed.

Abbreviations

MDR-TB, multidrug-resistant tuberculosis; WGS, whole-genome sequencing; DRS, drug surveillance; TB, tuberculosis; RR-TB, rifampicin-resistant tuberculosis; MTB, *Mycobacterium tuberculosis*; MIRU-VNTR, mycobacterial interspersed repetitive unit-variable number of tandem repeat; L-J, Lowenstein-Jensen; DST, drug susceptibility testing; PNB, p-nitrobenzoic acid; WHO, World Health Organization; CTAB, cetyltrimethylammonium bromide; SNP, single-nucleotide polymorphism; PE/PPE, proline-glutamate/proline-proline-glutamate; pDST, phenotypic susceptibility testing; RRDR, rifampicin resistance-determining region; Pre-XDR-TB, pre-extensively drug-resistant tuberculosis; XDR, extensively drug-resistant; FQ, fluoroquinolone.

Data Sharing Statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request. The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive[30] in National Genomics Data Center,[31] China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences (GSA: CRA014147) that are publicly accessible at https://ngdc.cncb.ac.cn/gsa.

Ethics Approval and Informed Consent

The study was approved by the Medical Ethics Committee of Jiangxi Center for Disease Control and Prevention (JXCDCKLS-2023-37). Informed consent was waived considering that this study presented no more than minimal risk of harm to patient subjects. This study was conducted in accordance with the Declaration of Helsinki.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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