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ORIGINAL RESEARCH

Analysis of Drug-Resistance Characteristics and Genetic Diversity of Multidrug-Resistant Tuberculosis Based on Whole-Genome Sequencing on the Hainan Island, China

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Purpose: Given the high burden of Tuberculosis (TB) in China, the prevalence of multidrug-resistant tuberculosis (MDR-TB) is significant. Whole-genome sequencing (WGS) of *Mycobacterium tuberculosis* (MTB) enables the identification of lineages, drug-resistant mutations, and transmission patterns, offering valuable insights for TB control, clinical diagnosis, and treatment.

Methods: We collected 202 MDR-MTB strains from 3519 suspected pulmonary TB patients treated at The Second Affiliated Hospital of Hainan Medical University between July 2019 and June 2021. Proportional drug-susceptibility testing was performed using 8 common anti-tuberculosis drugs. Subsequently, the genotypic drug resistance and genetic characteristics were analyzed by the WGS.

Results: Lineages are identified by TB-profiler revealed 202 MDR-MTB strains, showcasing three predominant lineages, with lineage 2 being the most prevalent. Close genomic relatedness analysis and evidence of MTB transmission led to the formation of 15 clusters comprising 42 isolates, resulting in a clustering rate of 20.8%. Novelty, lineage 2.1 (non-Beijing) accounted for 27.2% of the MDR-MTB strains, which is rare in China and Neighboring countries. Regarding first-line anti-TB drugs, genes associated with rifampicin resistance, primarily the *rpoB* gene, were detected in 200 strains (99.0%). Genes conferring resistance to isoniazid, ethambutol, and streptomycin were identified in 191 (94.5%), 125 (61.9%), and 100 (49.5%) strains, respectively. Among the second-line drugs, 97 (48.0%) strains exhibited genes encoding resistance to fluoroquinolones. Comparing the results to phenotypic drug susceptibility-based testing, the sensitivity of WGS for detecting resistance to each of the six drugs (rifampicin, isoniazid, ethambutol, ofloxacin, kanamycin, capreomycin) was 90% or higher. With the exception of ethambutol, the specificity of WGS prediction for the remaining drugs exceeded 88%.

Conclusion: Our study provides crucial insights into genetic mutation types, genetic diversity, and transmission of MDR-MTB on Hainan Island, serving as a significant reference for MDR-MTB surveillance and clinical decision-making.

Keywords: tuberculosis, MDR-TB, whole-genome sequencing, WGS, genetic diversity, Hainan Island, China

Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), continues to pose a significant global public health challenge.[1–3] In 2021, there were 10.6 million new TB cases worldwide, with China accounting for 780,000 cases, making it the third highest burden country for TB globally.[4] The World Health Organization (WHO) estimates that in 2021, there were 450,000 cases of *rifampicin-resistant tuberculosis* (RR-TB) globally, of which over 70% were *multi-drug-resistant tuberculosis* (MDR-TB), characterized by resistance to both isoniazid and rifampicin simultaneously.[4] Unfortunately, only one-third of these patients receive appropriate diagnosis and treatment. Given the increased difficulty,

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cost, and poorer prognosis associated with treating MDR-TB, ongoing vigilance is essential.[5,6] Furthermore, as early diagnosis and appropriate treatment can prevent the majority of TB-related deaths, the rapid and accurate identification of MDR-TB patients is crucial for guiding early clinical interventions and improving cure rates.

Since the successful completion of the WGS of MTB H37Rv and its publication in 1998,[7] WGS has found extensive clinical applications, encompassing drug susceptibility prediction, epidemiological analysis, and transmission dynamics research.[8] WGS enables the detection of transmission events missed by epidemiological investigations, differentiation between recurrent TB and reinfection, and more reliable classification of MTB lineages and sub-lineages.[9] Moreover, it offers a comprehensive assessment of pathogen drug resistance profiles.[10–12]

TB incidence in China displays regional disparities, with substantial variations in the degree and pattern of drug resistance across different areas.[13] Hainan Island, comprising four provinces and fifteen counties, is home to an estimated population of 10.2 million.[14] Positioned within the tropical and subtropical regions, Hainan Island experiences a tropical monsoon climate and maintains a low altitude. Separated from mainland China by the Qiongzhou Strait, the island has limited population flow compared to other provinces. In 2010, the prevalence of smear-positive TB on the island was 167 cases per 100,000 people, approximately three times the national average of 59 cases per 100,000 people.[15,16] Given the unique geographical environment of Hainan Island, it is reasonable to assume that Multidrug-resistant Mycobacterium tuberculosis (MDR-MTB) exhibits distinctive genetic characteristics in this region. However, due to the island's lower socioeconomic development level and limited medical resources, research in this field has been scarce. Consequently, to comprehend the molecular resistance and genetic characteristics of MDR-MTB on Hainan Island, we employed WGS technology to analyze the strains isolated from patients diagnosed with MDR-TB admitted to the Second Affiliated Hospital of Hainan Medical University between July 2019 and June 2021. This study aimed to assess the traits of drug resistance-related mutations and genetic diversity on the island while providing a scientific foundation for MDR-TB prevention and control.

Materials and Methods

Study Subjects

According to the diagnostic criteria established by the National Health Commission for suspected TB patients, respiratory tract specimens such as sputum and alveolar lavage fluid were collected from individuals receiving treatment at the Second Affiliated Hospital of Hainan Medical University between July 2019 and June 2021. This hospital, designated as the Provincial Clinical Center for Tuberculosis and recognized as one of the designated TB hospitals in Hainan, is a 2100-bed general hospital that includes 92-bed open wards specifically dedicated to specialized TB treatment. The patients included in this study were permanent residents of Hainan Island. Clinical and epidemiological data pertaining to these patients were retrospectively retrieved from the hospital's electronic medical record management system for further analysis.

The H37Rv (ATCC27294) standard sensitive strain, provided by the Tuberculosis Reference Laboratory of China CDC, was used as the reference strain. This study was approved by the Ethics Committee of the University of the Second Affiliated Hospital of Hainan Medical University, China (No. LW2023022). This study complies with the Declaration of Helsinki. It was considered exempt from informed consent because it was secondary research, for which consent is not required.

The inclusion criteria were as follows: 1) Patients who tested positive for MTB in at least two cultures, with the identification of MDR-MTB strains. Only the first strain cultured at the beginning of the study period was recorded; 2) MDR-TB patients who had been residents of Hainan Island for a minimum of 10 years.

The exclusion criteria: Patients with incomplete clinical data and those who were non-permanent residents of Hainan Island were excluded from the analysis.

Treatment history: 1) Previously treated patients was defined as irregular anti-tuberculosis treatment for ≥1 month, failure of initial treatment and recurrence of pulmonary tuberculosis. 2) New patients were defined as those receiving anti-tuberculosis treatment for the first time. The treatment history was based on the clinical diagnosis.

Experimental Methods

Isolation, Culture, and Screening of MDR-MTB

During the study period, clinical respiratory tract specimens were utilized to isolate mycobacteria, and preliminary screening for MDR-MTB strains was conducted using the P-Nitrobenzoic acid (PNB)/2-Thiophenecarboxylic acid hydrazide (TCH) identification medium and proportional drug-susceptibility test.

Isolation and Culture of MTB

Respiratory tract specimens, including 2–3 mL of sputum, alveolar lavage fluid, and other relevant samples from suspected pulmonary tuberculosis patients, were collected. These specimens were mixed with an equal volume of 4% NaOH solution through shaking to obtain pretreatment specimens. The pretreated specimens were then left at room temperature for 15 minutes. Next, 150 μL of the pretreated specimen was inoculated into two acidic Roche media (BASO Company, Zhuhai, China) slants. The covers of the media were slightly loosened, and they were incubated at 36±1°C for 24 hours with the slants facing upward. Subsequently, the media covers were tightened and placed vertically. The growth of colonies was observed on the third and seventh day of culture, primarily to assess contamination or the growth of fast-growing mycobacteria. Weekly observations were continued until the eighth week to record culture and contamination.

MDR-MTB Screening and Drug-Susceptibility Test (DST) of Anti-Tuberculosis Drugs by Roche Proportion Method

Fresh colonies on L-J medium were scraped using a disposable sterile inoculation loop and transferred to a grinding bottle, which was then tightly closed. The colonies were mixed using a vortex shaker for 0.5 to 1 minute and left to stand for a period of time. The supernatant was then drawn and added to a culture tube containing sterilized normal saline until the turbidity matched that of a MacFarland standard turbidity tube (MacFarland No.1). This process resulted in a bacterial suspension with a concentration of 1 mg/mL, which was further serially diluted to concentrations of 10^{-2} and 10^{-4} mg/mL with sterilized saline.

Using a disposable sterile inoculation loop, 0.01 mL of the 10^{-2} mg/mL and 10^{-4} mg/mL bacterial suspensions was uniformly inoculated into blank control medium, drug-containing media with rifampicin (RIF) at 40.0 μg/mL, isoniazid (INH) at 0.2 μg/mL, ethambutol (EMB) at 2.0 μg/mL, streptomycin (SM) at 4.0 μg/mL, ofloxacin (OFX) at 2.0 μg/mL, kanamycin (KM) at 30.0 μg/mL, capreomycin (CPM) at 40.0 μg/mL (BASO Company, Zhuhai, China), and ethionamide (ETO) at 40.0 μg/mL (Yinke Company, Zhuhai, China). Additionally, the PNB/TCH identification medium (BASO Company, Zhuhai, China) was included. The culture media were incubated at 36±1°C, and the results were observed on the third day and subsequently once a week until the fourth week. Percentage of resistance =(number of colonies grown on drug containing medium)/(number of colonies grown on control medium)×100%. If the percentage of patients with drug resistance ≥1% reported drug resistance (R), and susceptibility (S) was reported when the percentage of drug resistance was less than 1%. If the bacterial suspension at the concentration of 10–4 mg/mL grew less than 20 colonies on the control medium, the culture should be subcultured from the control medium and the experiment should be repeated.

The standard sensitive strain H37Rv (ATCC27294) should be used as the sensitive control for each batch of drug-containing medium, and all drug-containing medium should not grow and be judged as "sensitive". Roche proportional method drug susceptibility test participated in and passed the proficiency assessment of anti-tuberculosis drug susceptibility test organized by the Tuberculosis Reference Laboratory of China CDC every year.

WGS of MTB

A single ring of bacteria was scraped using a disposable sterile inoculation ring and placed in 500 μL TE buffer for high-temperature inactivation at 80°C for 30 minutes. The inactivated strains were promptly removed and frozen at −80°C. These samples were then transported on dry ice to Tb Healthcare Biotechnology (Guangdong) Co., Ltd. (China) for high-throughput sequencing and bioinformatics analysis, which allowed the acquisition of variation information such as single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels) in the target genome compared to the reference genome. The sequenced MDR-MTB strains were analyzed to determine genetic distance, lineage, and drug resistance.

Extraction and Sequencing of Genomic DNA

For the extraction of genomic DNA, 400 μL of inactivated MTB bacterial suspension was dispersed by sonication and

then subjected to the cetyltrimethylammonium bromide-lysozyme (CTAB) method.[17] The purity, concentration, and integrity of DNA were determined using an Agilent Fragment Analyzer 5400 (Agilent, USA).

Library Preparation

(1) Ultrasonic Fragmentation: Genomic DNA was extracted, quantified, and subjected to quality checks. Subsequently, it was fragmented into nucleic acid fragments ranging from 200 to 300 base pairs using a Covaris ultrasonic fragmentation machine.
(2) Library Construction: The interrupted DNA fragments were processed for library construction. The 5' protruding end or the 3' protruding end of the DNA fragment was blunted, and T4 DNA ligase was employed to ligate the connection points at both ends of the small DNA fragment. Fragment selection and purification were achieved using magnetic bead purification. PCR amplification was performed for library enrichment. To eliminate interference such as primer dimers in the reaction system, a magnetic bead purification method was utilized for further purification, resulting in a DNA library suitable for sequencing.
(3) Library Quantification: The concentration and length distribution of the libraries were determined using the Qubit Fluorometer 3.0 fluorometer and Agilent 2100, respectively.

Machine Sequencing

The qualified libraries underwent testing, and different libraries were pooled together based on the required effective concentration and target data amount. Subsequently, paired-end sequencing (PE150) was carried out on an Illumina NovaSeq 6000 machine (Illumina, USA).

Bioinformatics Analysis

Using FastQC software (version 0.11.9), reads containing adaptor sequences or exhibiting low quality were eliminated to obtain clean reads for subsequent analysis. The resulting filtered clean reads were aligned to the MTB reference genome H37Rv (NC_000962.3) using BWA (0.7.17).[18] Freebayes (1.3.2) and SnpEff (4.3t) were used for mutation site identification and mutation annotation respectively. An in-house script was employed to obtain the SNP sequence and calculate the pairwise distance between each two samples. Pairwise distance was calculated using only fixed SNPs (frequency≥75%) that were not in drug-resistance associated genes or repetitive regions of the genome (eg *PPE/PE-PGRS* family genes, phage sequence, insertion or mobile genetic elements). Samples with MTB genomes differing by no more than 12 SNPs in cluster analysis were defined as belonging to the same genomic cluster.[2,19,20]

TB Profiler software (version 2.8.12, https://github.com/jodyphelan/TB Profiler)[21,22] was employed to employed the MTB lineages and genes associated with resistance against eight anti-TB drugs (RIF, INH, SM, EMB, OFX, CPM, KM, ETO). After loading the SNP sequence in MEGA, the function "Construct/Test Neighbor-Joining Tree"under the "PHYLOGENY"tool was select to construct our phylogenetic tree.[23] The resulting phylogenetic trees were visualized and modified using the iTOL program (version 6.6) (https://itol.embl.de/).[24]

To verify the location, the residential addresses of patients within each cluster were geocoded using Baidu Map. The spatial distance between two patients represented the distance between their respective residential locations on the map.

Statistical Analysis

The statistical analysis was conducted using SPSS 26.0 software to examine the data of 202 patients diagnosed with MDR-TB. As the age distribution and clustered patient mean paired distance data did not follow a normal distribution, they were presented as medians and quartiles. A comparison of the clustering rates between lineage 2 (East Asian) and lineage 4 (Euro-American) was performed using the Chi-square test, the differences were considered statistically significant at a P-value of < 0.05.

Data Availability Statement

The raw WGS data have been submitted to the NCBI SRA database. The entry number of biological project was PRJNA1000054. The SRA entry numbers of 202 MDR-MTB strains were from SAMN36757384 to SAMN36757585. The average sequencing depth of genome was 94.6–362.7 (mean: 193.5), the 1-fold coverage of genome was 98.7–100 (mean: 99.3), and the 10-fold coverage of genome was 98.6–99.9 (mean: 99.2). The total number of SNPS ranged from 695 to 1818 (mean: 1175.7). All 202 MDR-MTB strains were interpretable by WGS.

Results

Patient Demographics

Between July 2019 and June 2021, a comprehensive investigation was conducted, yielding a total of 1522 mycobacterial strains isolated from respiratory specimens obtained from 3519 individuals suspected of having TB. Among these isolates, 1318 strains were identified as MTB, while 204 strains belonged to non-tuberculous mycobacteria (NTM). Subsequently, a meticulous screening process utilizing the proportion method for drug susceptibility testing (DST) was employed to identify 253 MDR-MTB strains. However, a number of strains were excluded due to various reasons: 12 strains were associated with non-permanent residents of Hainan Island, three strains lacked complete clinical data, 29 strains failed to undergo successful transfer and resuscitation, four strains were contaminated, and three strains did not meet the predetermined quality control parameters for WGS data. Consequently, out of the initial 253 MDR-MTB strains, a total of 202 strains (79.8%; 95% CI, 74.4–84.6%) were included in the subsequent WGS analysis. The study's overall workflow is depicted in Figure 1.

The cohort of MDR-MTB strains analyzed via WGS consisted predominantly of male individuals within the middle-aged and elderly populations. Among the included strains, 175 were derived from males with a median age of 52 (interquartile range: 42, 61) years, while the remaining 27 strains were from females with a median age of 46 (interquartile range: 25, 54) years. Type 2 diabetes mellitus was the most prevalent comorbidity, affecting 60 out of the 202 patients (29.7%; 95% CI, 23.5–36.5%). In terms of treatment history, 55 cases (27.2%; 95% CI, 21.2–33.9%) were classified as new patients, while 147 cases (72.8%; 95% CI, 66.1–78.8%) had received prior treatment. Radiographic assessments revealed the presence of lung cavities in 158 patients (78.2%; 95% CI, 71.9–83.7%) (see Table 1 for details).

Identification of the Lineage and Analysis of Genetic Distance

A total of 202 MDR-MTB strains analyzed and identified through phylogenetic analysis of transmission clusters, three major lineages were observed. The most prevalent lineage was lineage 2 (East Asian), comprising 169 strains (83.7%; 169/202; 95% CI, 77.8–88.5%). 55 strains (27.2%, 55/202; 95% CI 21.2–33.9%) were lineage 2.1 (non-Beijing). And 114 strains (56.4%, 114/202; 95% CI 49.3–63.4%) were lineage 2.2 (Beijing), of which 94 strains (46.5%, 94/202; 95% CI 39.5–53.7%) were lineage 2.2.1 (modern Beijing), 8 strains (4.0%, 8/202; 95% CI 1.7–7.7%) were lineage 2.2.1.1 (modern Beijing), 12 strains (5.9%, 12/202; 95% CI 3.1–10.1%) were lineage 2.2.2 (ancestral Beijing). Lineage 4 (Euro-American) accounted for 27 strains (13.4%; 27/202; 95% CI, 9.0–18.8%), included 6 strains (3.0%, 6/202; 95% CI 1.0–6.4%) were lineage 4.2.2, 13 strains (6.4%, 13/202; 95% CI 3.5–10.8%) were lineage 4.4.2, 8 strains (4.0%, 8/202; 95% CI 1.7–7.7%) were lineage 4.5, while lineage1 (Indo-Oceanic) was represented by 6 strains (3.0%; 6/202; 95% CI, 1.1–6.4%) (Table 2).

Based on the pairwise genetic distance analysis of MTB strains, 15 clusters consisting of 42 MDR-MTB strains were identified, resulting in a clustering rate of 20.8% (42/202; 95% CI, 15.4–27.0%). Each cluster comprised 2 to 5 MDR-MTB strains. Among the 42 MDR-MTB strains, 22 strains were isolated from new TB patients (55) and the clustering rate was 40.0% (22/55; 95% CI, 27.0–54.1%) and 20 strains were from previously treated TB patients (147) was 13.6% (20/147; 95% CI, 15.4–27.0%).

The clustering rate of lineage 2 (East Asian) was determined to be 19.5% (33/169; 95% CI, 13.8–26.3%), whereas lineage 4 (Euro-American) exhibited a clustering rate of 33.3% (9/27; 95% CI, 16.5–54.0%), with no significant difference observed between the two lineages ($\chi 2 = 2.636$, $P = 0.104$) (refer to Table 3 for detailed results). Notably, four patients which from two pairs of clustered patients differed by 0–2 SNPS (Figure 2, G15: M180 vs M212, G2:M135 vs M144), indicating a recent direct transmission events.[2] Eighteen patients showed a genetic distance of 3–5 SNPs,

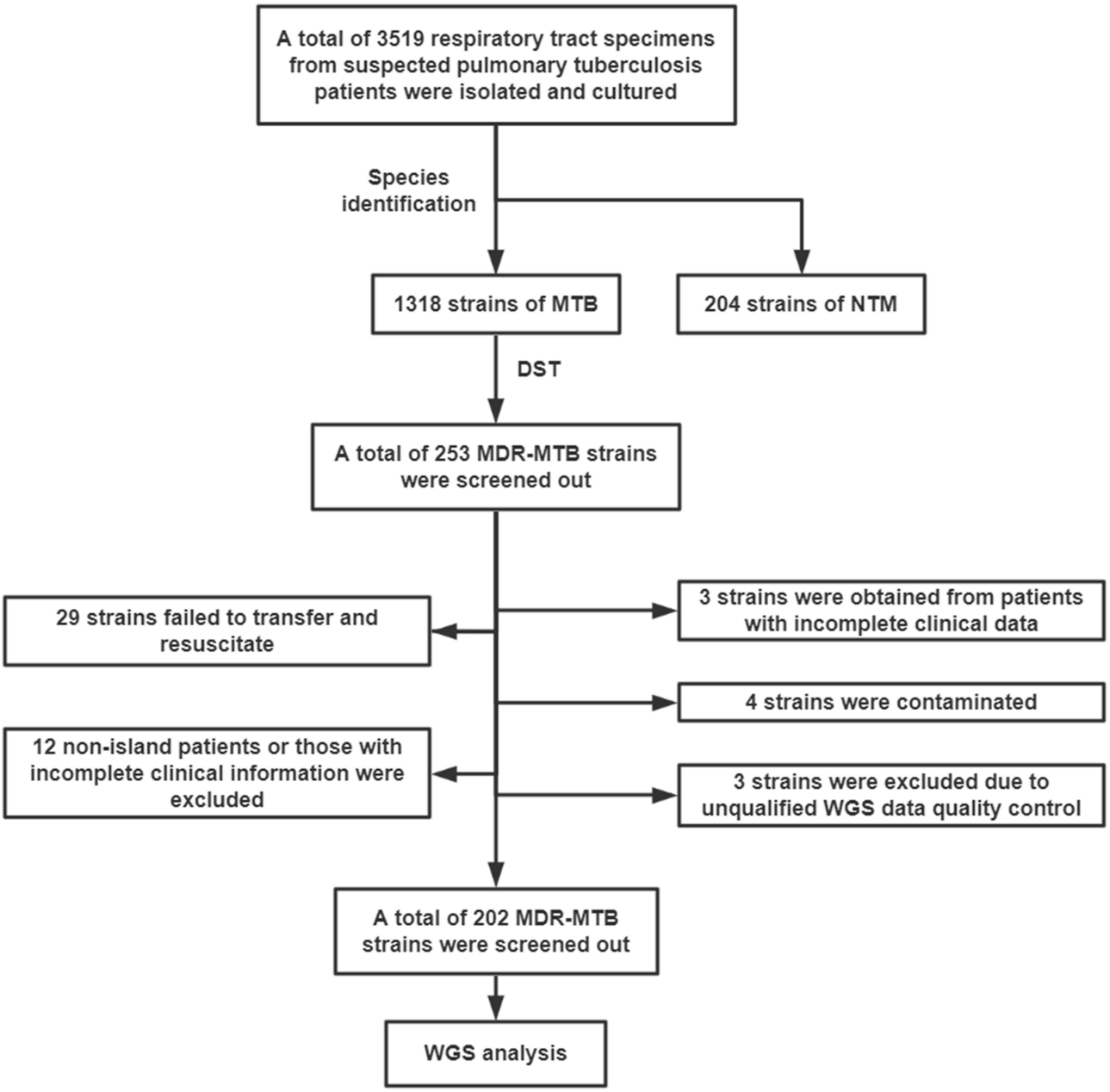

Figure 1 Flow chart summarizing the screening of strains for WGS analysis.

suggesting direct transmission. Additionally, 20 patients exhibited a genetic distance of 6–12 SNPs, indicating earlier transmission events (Figure 2).

Upon comparing the residential addresses of patients within each cluster, it was observed that the pairwise geographic distances between patients ranged from 0.5 km to 220.5 km, with an average distance of 71.9 km (31.9 km, 138.9 km).

Considering a threshold of living within two kilometers to define the same com munity, three pairs of patients within the clusters were identified as having community transmission (G4: M77 vs M108; G7: M120 vs M148; G13: M89 vs M219). Among these, only one pair of clustered patients demonstrated direct transmission, as indicated by a difference of 3 SNPs among the strains (G4: M77 vs M108) (Figure 3).

Table 1 Demographic Characteristics of 202 MDR-TB Patients

Characteristics	n=202		
	Male	Female	Total
Number	175	27	202
Age, Median	52 (42, 61)	46 (25, 54)	51 (37, 61)
<20	1	2	3
20~40	41	10	51
41–50	41	5	46
51–60	44	7	51
>60	48	3	51
Occupation			
Farmer	133	18	151
Others	42	9	51
Treatment history			
New patients	48	7	55
Previously treated patients	127	20	147
Hepatitis B			
Yes	26	3	29
No	149	24	173
Diabetes			
Yes	57	3	60
No	118	24	142
Weight monitoring			
Decline	53	9	62
Unchanged	113	15	128
Not monitored	9	3	12
Hemoptysis			
Yes	45	3	48
No	130	24	154
Smear			
Positive	146	24	170
Negative	29	3	32
Cavity			
Yes	146	12	158
No	29	15	44
Destruction of the lung			
Yes	23	4	27
No	152	23	175
Smoking			
Yes	105	1	106
No	70	26	96
Drink			
Yes	47	2	49
No	128	25	153
Nationality			
Han	163	24	187
Li	12	3	15

Phenotypic Drug Resistance Profile of MDR-MTB Strains

Out of the 202 MDR-MTB strains analyzed in this study, a total of 99 strains (49.0%, 99/202; 95% CI, 41.9–56.1%) were classified as pre-XDR strains. Pre-XDR refers to tuberculosis caused by MTB strains that meet the criteria for multidrug resistance or rifampin resistance, while also displaying resistance to any fluoroquinolone (Figure 4).

Table 2 Lineages of 202 MDR-MTB Strains

Lineage	Number	Hypotype	Number	Percentage
Lineage 1 (Indo-Oceanic)	6	Lineage 1.1.1	2	1.0%
		Lineage 1.1.1.1	1	0.5%
		Lineage 1.1.2	1	0.5%
		Lineage 1.2.2	1	0.5%
		Lineage 1.2.2.2	1	0.5%
Lineage 2 (East Asian)	169	Lineage 2.1	55	27.2%
		Lineage 2.2.1	94	46.5%
		Lineage 2.2.1.1	8	4.0%
		Lineage 2.2.2	12	5.9%
Lineage 4 (Euro-American)	27	Lineage 4.2.2	6	3.0%
		Lineage 4.4.2	13	6.4%
		Lineage 4.5	8	4.0%

Table 3 Comparison of Clustering Rates Between lineage 2 and lineage 4

Lineages	Clustered Cases	Unclustered Cases	χ^2	*P*
Lineage 2 (East Asian)	33	136	2.636	0.104
Lineage 4 (Euro-American)	9	18		

Drug-Resistance Mutation Spectrum of MDR-MTB Strains

The drug resistance profile of the 202 MDR-MTB strains analyzed in this study was determined for four first-line anti-TB drugs (RIF, INH, EMB, and SM) and four second-line anti-TB drugs (OFX, KM, CPM, and ETO). Table 4 provides a summary of the findings.

Regarding the first-line anti-TB drugs, RIF-resistance genes were identified in 200 strains (99.0%, 200/202), with 98.5% (197/200) displaying mutations in the rifampicin-resistance determining region (RRDR) of the *rpoB* gene. The most prevalent mutation type was *rpoB* S450L, observed in 56.5% (113/200) of the RIF-resistant strains. Among these 200 strains, 42 (21.0%) exhibited combined mutations at two or more sites. Furthermore, two strains (1.0%) presented insertions or deletions in the *rpoB* gene (*rpoB* 1295_1303_delAATTCATGG and *rpoB* 1299_1304_delCATGGA).

INH-resistance genes were detected in 191 strains (94.5%, 191/202), with the most frequent mutation being *katG* S315T, observed in 74.9% (143/191) of the INH-resistant strains. Nineteen strains (9.9%, 19/191) exhibited combined mutations at two or more sites, primarily involving *katG* and *fabG1* promoter mutations. Additionally, 13 strains (6.8%, 13/191) presented *katG* frameshift mutations, 1 strain (0.5%, 1/191) had a *katG* gene deletion, and 2 strains (1.0%, 2/191) displayed *katG* 1_1251_del and *katG* 1080_2156111_del mutations.

EMB-resistance-related genes were detected in 125 strains (61.9%, 125/202), primarily associated with mutations in the *embB* gene and *embA* promoter region. Among these, *embB* M306I (30.4%, 38/125) and M306V (24.8%, 31/125) were the most common mutations observed. Moreover, 20 strains (16.0%, 20/125) exhibited combined mutations at two or more sites.

Mutations associated with SM resistance were identified in 100 strains (49.5%, 100/202), predominantly affecting the *rpsL* and *rrs* genes. The primary mutation type observed was *rpsL* K43R, accounting for 58.0% (58/100) of the SM-resistant strains. Two SM-resistant strains (2.0%) exhibited combined mutations, while six strains (6.0%) displayed insertions or deletions in the *gid* gene.

Among the second-line drugs, 97 strains (48.0%, 97/202) harbored resistance genes related to fluoroquinolones (FQs), such as OFX. The mutation rate in the quinolone-resistance-determining region (QRDR) for FQs was as high as 99.0% (96/97). The primary mutation types identified in the *gyrA* gene were D94G, observed in 44.3% (43/97) of the strains, and A90V, observed in 33.3% (29/97) of the strains. Furthermore, 13 strains (13.4%, 13/97) exhibited combined

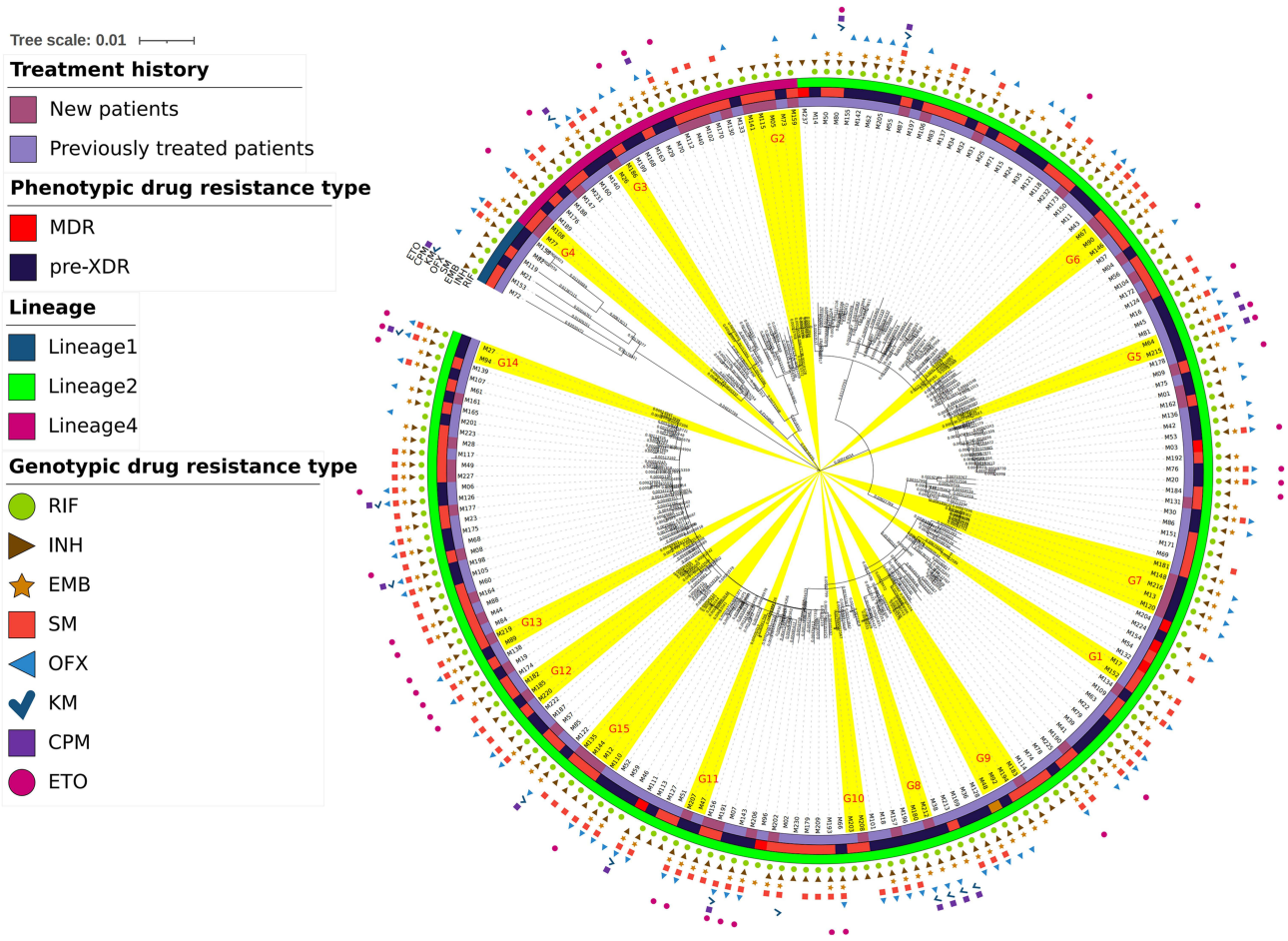

Figure 2 Phylogenetic tree of 202 MDR-MTB strains.
Notes: The inner circles marked in yellow are 15 clusters (G1-G15) with an SNP distance of no more than 12. The drugs labeled from the inside to the outside of the outer circle resistance spectrum are: RIF, INH, EMB, SM, OFX, KM, CPM, ETO.

mutations at two sites. For aminoglycosides, mutations in the *rrs* promoter region were prevalent, with *rrs* A1401G accounting for 93.3% (14/15) and 77.8% (14/18) of KM and CPM-resistant strains, respectively. ETO-resistant mutations primarily affected the *ethA* frameshift (47.4%, 18/38) and *fabG1* C-15T (39.5%, 15/38) genes. Combined mutations were observed in five ETO-resistant strains, and one strain exhibited an insertion or deletion in the *ethA* gene.

Consistency of Phenotypic and Genotypic Drug Resistance in MDR-MTB Strains

Drug sensitivity was determined through WGS by assessing the presence or absence of mutations in resistance-related genes. A strain was classified as genotype resistant if a drug resistance gene mutation was detected, while it was considered genotype sensitive if no such mutation was found. The accuracy of WGS predictions for drug resistance was evaluated using phenotypic drug susceptibility results as the reference standard, as depicted in Table 5. When compared to phenotypic results, WGS exhibited a sensitivity of 90% or higher for detecting resistance to each of the six drugs (RIF, INH, EMB, OFX, KM, CPM). Except for EMB, the specificity of WGS predictions for the remaining drugs exceeded 88%. Overall, agreement between the two methods in determining strain sensitivity or resistance was observed in more than 85% of cases for all drugs, excluding EMB.

Discussion

The identification and treatment of close contacts with TB-infected patients play a crucial role in controlling the spread of the disease.[25] Obtaining epidemiological data for TB in China can be challenging due to its high burden. However, WGS

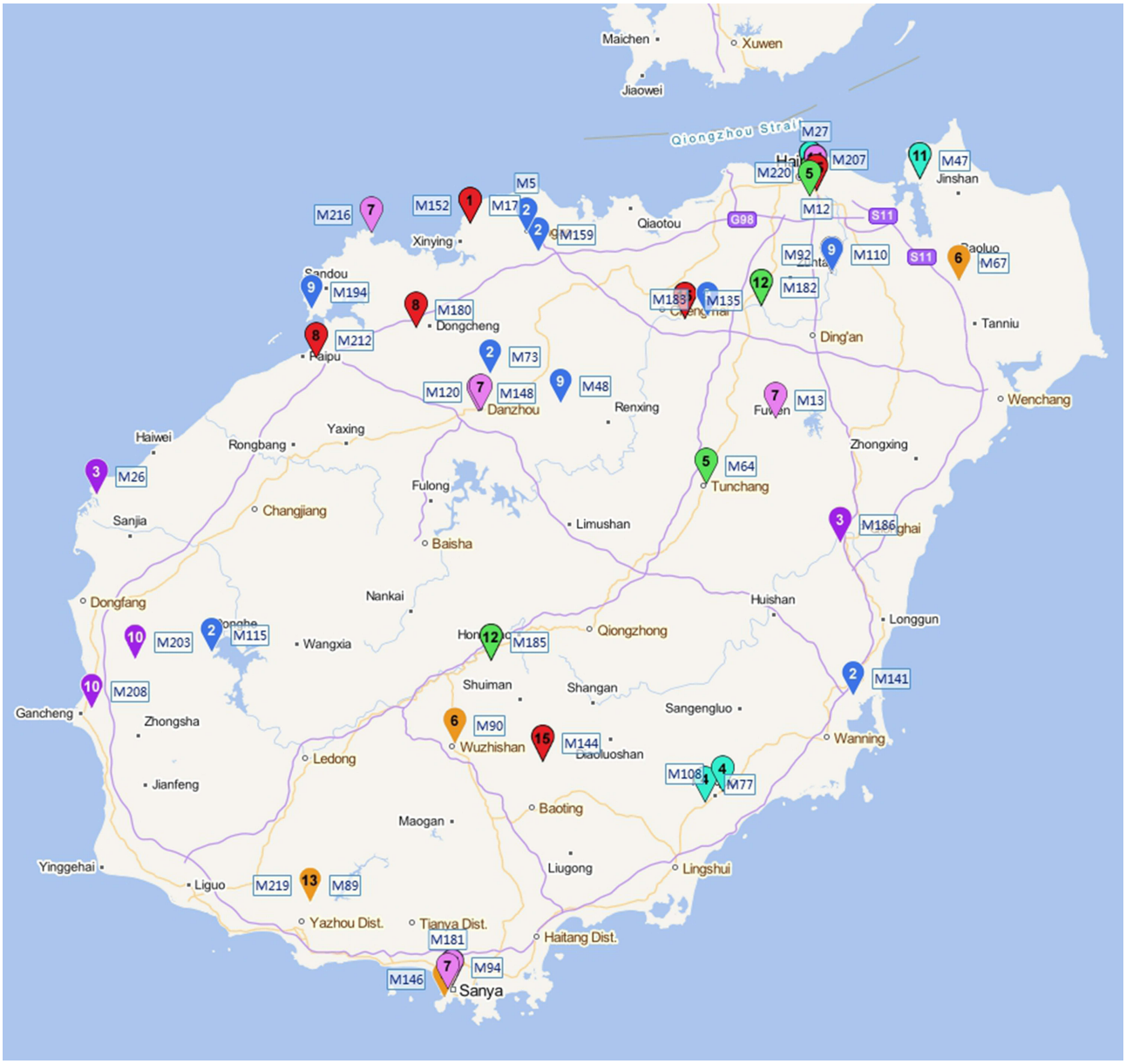

Figure 3 Spatial distribution of clustered strains.

can aid in identifying transmission events in patients even without epidemiological data.[26,27] In this study, WGS was employed to detect drug resistance patterns, assess genetic diversity, and analyze the drug resistance of potential MDR-MTB strains, thereby shedding light on the molecular epidemiology of MDR-MTB on Hainan Island, China. Consistent with previous reports from various regions in China,[6,27–29] lineage 2 (East Asian) was found to be the predominant genotype among MDR-MTB strains on Hainan Island, followed by lineage 4 (Euro-American). Interestingly, a small proportion (3.0%) of MDR-MTB strains belonged to lineage 1 (Indo-Oceanic), resembling the genetic profile observed in Indonesia, a tropical island in the South China Sea.[30] A case of MDR-TB with lineage 1.1.1.1 was also identified, which was only considered to be an endemic lineage in Vietnam.[31] Although the lineage 2.2 (Beijing) was the dominant lineage 2 (East Asian) sublineage (56.4%), we were more surprised to find that lineage 2.1 (non-Beijing) accounted for 27.2% of the MDR-MTB strains in Hainan Island, which was unheard of before. Only 9.6% reported in Guangxi[32] and 5.35% reported in Thailand[33] were found in other areas of China and neighboring countries of Hainan Island, which indicated

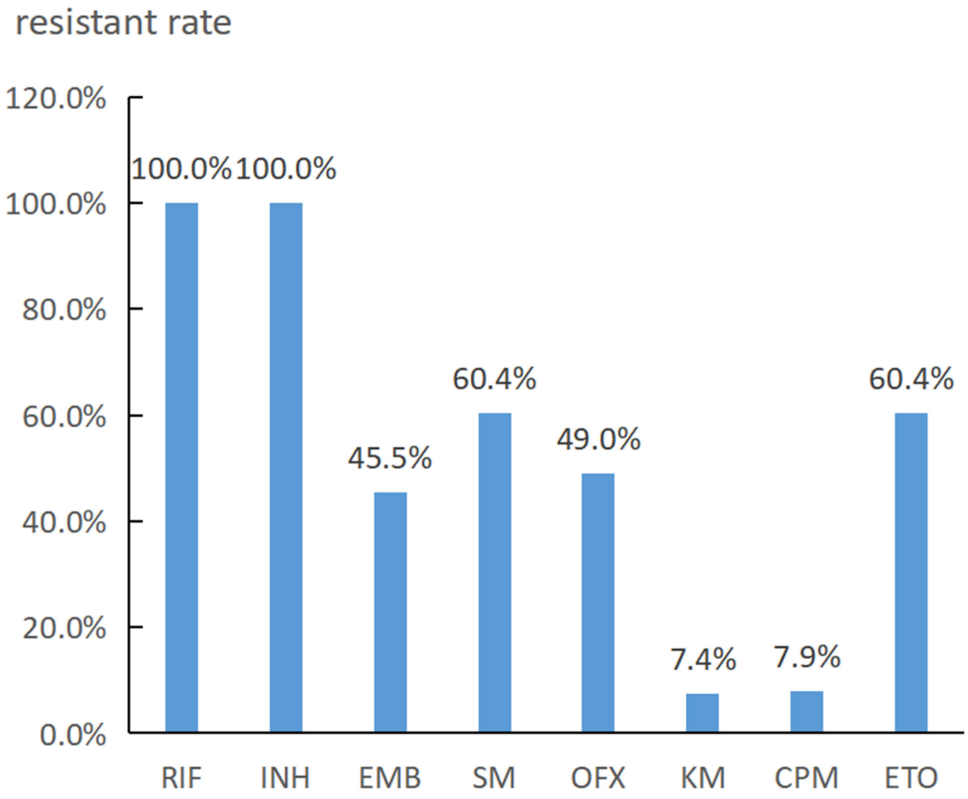

Figure 4 Phenotypic drug resistance rate of 202 MDR-MTB strains.
Abbreviations: RIF, rifampicin; INH, isoniazid; EMB, ethambutol; SM, streptomycin; OFX, ofloxacin; KM, kanamycin; CPM, capreomycin; ETO, ethionamide.

that Hainan Island had unique genetic diversity and was obviously different from other areas of China and neighboring countries.

Molecular evolution studies have suggested that lineage 2 (East Asian) has a higher propensity for propagation compared to lineage 4 (Euro-American).[34,35] However, despite no significant association between MDR-MTB genotypes

Table 4 Drug Resistance Gene Mutation Spectrum of 202 MDR-MTB Strains

Drug	Number of Drug-Resistant Strains (Percentage)	Mutation Type	Number of Mutant Strains	Mutation Percentage
Rifampicin (RIF)	200 (99.0%)	*rpoB* S450L	113	56.5%
		rpoB L430P	16	8.0%
		rpoB H445D	16	8.0%
		rpoB D435V	11	5.5%
		Other	91	45.5%
Isoniazid (INH)	191 (94.5%)	*katG* S315T	143	74.9%
		fabG1 C-15T	15	7.9%
		katG frameshift	13	6.8%
		Other	39	20.4%
Ethambutol (EMB)	125 (61.9%)	*embB* M306I	38	30.4%
		embB M306V	31	24.8%
		embB G406A	16	12.8%
		embB Q497R	11	8.8%
		embB G406D	8	6.4%
		embA C-16T	7	5.6%
		Other	35	28.0%

(*Continued*)

Table 4 (Continued).

Drug	Number of Drug-Resistant Strains (Percentage)	Mutation Type	Number of Mutant Strains	Mutation Percentage
Streptomycin (SM)	100(49.5%)	*rpsL* K43R	58	58.0%
		rpsL K88R	21	21.0%
		rrs A514C	6	6.0%
		rrs C517T	5	5.0%
		Other	12	12.0%
Ofloxacin (OFX)	97 (48.0%)	*gyrA* D94G	43	44.3%
		gyrA A90V	29	29.9%
		gyrA D94N	9	9.3%
		gyrA D94A	8	8.2%
		gyrA D94Y	6	6.2%
		Other	15	15.5%
Kanamycin (KM)	15 (7.4%)	*rrs*_A1401G	14	93.3%
		*eis*_G-12A	1	6.7%
Capreomycin (CPM)	18 (8.9%)	rrs_A1401G	14	77.8%
		*tlyA*_Frameshift	4	22.2%
Ethionamide (ETO)	38 (18.8%)	*ethA*_Frameshift	18	47.4%
		*fabG1*_C-15T	15	39.5%
		*fabG1*_T-8C	4	10.5%
		*inhA*_S94A	3	7.9%
		*ethA*_large_deletion	2	5.3%
		*fabG1*_G-17T	2	5.3%
		*inhA*_I21T	1	2.6%

Note: Other: Is the sum of the types in which the single mutation type is less than 5% in the drug.

Table 5 Comparison of Genotypic and Phenotypic Resistance Results of WGS

Drug	Genotypic Drug Sensitivity	Phenotypic Drug Sensitivity		Concordance (%)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Positive Predictive Value	Negative Predictive Value
		R	S					
Rifampicin (RIF)	R	200	0	99.0%	99.0%	NA	100.0%	0.0%
	S	2	0		(96.1–99.8%)		(97.7–100%)	(0.0–80.2%)
Isoniazid (INH)	R	191	0	94.6%	94.6%	NA	100.0%	0.0%
	S	11	0		(90.2–%97.1)		(97.6–100.0%)	(0.0–32.1%)
Ethambutol (EMB)	R	86	39	77.7%	93.5%	64.5%	68.8%	92.2%
	S	6	71		(85.8–97.3%)	(54.8–73.3%)	(59.8–76.6%)	(83.2–96.8%)
Streptomycin (SM)	R	98	2	87.1%	80.3%	97.5%	98.0%	76.5%
	S	24	78		(71.9–86.8%)	(90.4–99.6%)	(92.3–99.7%)	(66.8–84.1%)
Ofloxacin (OFX)	R	91	6	93.1%	91.9%	94.2%	93.8%	92.4%
	S	8	97		(84.2–96.2%)	(87.2–97.6%)	(86.5–97.5%)	(85.1–96.4%)
Kanamycin (KM)	R	15	0	100.0%	100.0%	100.0%	100.0%	100.0%
	S	0	187		(74.7–100%)	(97.5–100%)	(74.7–100.0%)	(97.5–100.0%)
Capreomycin (CPM)	R	15	3	98.0%	93.8%	98.4%	83.3%	98.4%
	S	1	183		(67.7–99.7%)	(95.0–99.6%)	(57.7–95.6%)	(96.5–100.0%)
Ethionamide (ETO)	R	17	21	86.6%	73.9%	88.3%	44.7%	96.3%
	S	6	158		(51.3–88.9%)	(82.4–92.4%)	(29.0–61.5%)	(91.8–98.5%)

Abbreviations: S, sensitive; R, resistant; NA, not available data.

and clustering rate ($\chi 2$=2.636, P=0.104) in this study, lineage 4 (Euro-American) exhibited a higher clustering rate (33.3%) than lineage 2 (East Asian) (19.5%).

Among the 202 MDR-MTB strains analyzed in this study, only 42 (20.8%) exhibited clustering, including 22 strains from new TB patients and 20 strains from previously treated TB patients. Furthermore, 33 MDR-MTB strains without unique clusters were identified among new patients. According to this rule,[27] it can be inferred that 75 (37.1%) MDR-TB patients on Hainan Island potentially transmitted MDR-MTB strains during the study period, consistent with findings from other studies where more than one-third of MDR-TB cases were suspected to be transmission events.[20] Several factors, including sample size, study duration, and different genomic cluster thresholds, may contribute to variations in these results.[36]

In addition, this study investigated the correlation among 42 patients with MDR-TB in 15 clusters by integrating conventional epidemiological data with WGS information. We found that the geographical distance of clustered patients in Hainan Island was uneven, with an average pairing distance of 71.9 km. Among these clusters, only patients within two specific clusters were identified as having a community transmission connection, as they were known to each other (G4: M77 vs M108; G13: M89 vs M219), these two pairs of clustered patients also had clear epidemiological investigation evidence. No transmission associations were observed in the remaining clusters, suggesting that these cases may have resulted from incidental transmission events. This indicates that with the construction of international tourism island and free trade port in Hainan Island in the past decade, economic development and increased personnel flow have also brought pressure to local tuberculosis prevention and control.

Numerous studies have demonstrated that over 96% of rifampicin (RIF)-resistant strains exhibit mutations in the rifampicin-resistance determining region (RRDR) of the *rpoB* gene.[37] Consistent with these findings, our study revealed RRDR mutations in the *rpoB* gene in 97.5% of the MDR-MTB strains on Hainan Island, China, with only three MDR-MTB strains lacking RRDR mutations. The predominant mutation observed was *rpoB* S450L (56.5%). Resistance to isoniazid (INH) can be attributed to various gene mutations, and the specific mutations determine the extent of resistance. Notably, the *katG* gene mutation indicates high-level INH resistance, while mutations in the *fabG1* promoter region are associated with low-level resistance to INH. Our study identified the *katG* S315T mutation (74.9%) as the most prevalent in MDR-MTB strains, and previous research has established its association with multidrug resistance.[38] The primary mutation types of RIF and INH in MDR-MTB strains on Hainan Island were similar to those reported in other regions of China, although the mutation frequencies differed. For example, *rpoB* S450L (58.2%) and *katG* S315T (73.8%) in Beijing,[39] *rpoB* S450L (55.1%) and *katG* S315T (75.9%) in Chongqing,[27] *rpoB* S450L (54.8%) and *katG* S315T (86.0%) in Shanghai,[28] *rpoB* S450L (45.2%) and *katG* S315T (56.3%) in Hunan,[6] *rpoB*_S450L (57.7%) and *katG* S315T (53.7%) in Hebei,[6] *rpoB* S450L (44.2%) and *katG* S315T (55.8%) in Jiangxi.[40]

Regarding streptomycin (SM) resistance, our study revealed *rpsL* K43R (58.0%) as the primary mutation in MDR-MTB strains, consistent with findings in Shanghai, China,[28] whereas *rpsL* L43A (76.7%) was predominant in Hunan.[6] The significance of *embB* gene mutations, particularly at codon 306, has been subject to debate due to their presence in both ethambutol (EMB)-resistant and EMB-sensitive strains. In our study, the most common *embB* gene mutations were M306I (30.4%) and M306V (24.8%), whereas M306V was the prevailing mutation type in Hunan and Shanghai strains.[6,28] The primary fluoroquinolone-resistance gene identified was *gyrA* mutation. In our study, the mutation rate in the quinolone-resistance-determining region (QRDR) reached 99.0%, with the main mutation types being D94G (44.3%) and A90V (33.3%) in the *gyrA* gene, although previous studies reported the highest frequency of A94G mutation.[6,39,40] Cross-resistance between ethionamide (ETO) and INH has been well-documented, and *fabG1* mutation serves as a primary mechanism of ETO resistance.[41–43] Notably, *ethA* frameshift (47.4%) and *fabG1* C-15T (39.5%) were identified as major drug-resistant genes, distinguishing them from other studies conducted in China.[44] The *rrs* A1401G mutation site plays a crucial role in the resistance to injectable aminoglycosides. In our study, we found that 14 strains with *rrs* A1401G mutation exhibited resistance to kanamycin (KM) and capreomycin (CPM). Overall, the primary mutation types associated with anti-tuberculosis drug resistance in different regions of China are generally consistent, albeit with potential variations in mutation frequencies that may be influenced by sample size, genotype differences, medication practices, and patient adherence.

In this study, we evaluated the performance of WGS in predicting drug resistance using phenotypic drug susceptibility as the reference standard. Our findings demonstrate that WGS exhibited high sensitivity and specificity in predicting resistance to rifampicin (RIF), isoniazid (INH), streptomycin (SM), ofloxacin (OFX), kanamycin (KM), and capreomycin (CPM), surpassing the 80.0% threshold. Notably, the concordance between WGS predictions and phenotypic susceptibility results for RIF, INH, OFX, KM, and CPM exceeded 90%, consistent with previously reported findings.[22,44–46] However, it is important to highlight that the specificity of WGS in predicting ethambutol (EMB) resistance was notably lower compared to other drugs. This discrepancy may arise from the presence of single mutations, such as *embA* or M306I/G406A in *embB*, which can be found in both EMB-resistant and EMB-sensitive strains. The reliance on phenotypic drug susceptibility testing may be less reliable for EMB when single gene locus mutations are involved.[44,47,48] Moreover, the relatively low sensitivity of WGS in predicting ethionamide (ETO) resistance could be attributed to the inherent limitations of phenotypic drug susceptibility testing and the complex nature of resistance mechanisms.[49–52]

In this study, we employed WGS data from MDR-TB strains obtained from Hainan Island, the sole tropical island in China, to perform cluster analysis based on single nucleotide polymorphism (SNP) differences. This approach allowed for more accurate identification of clustered strains. Nevertheless, it is important to acknowledge certain limitations associated with this study. Firstly, the number of recent transmission events may have been underestimated since only patients with culture-positive MDR-TB over a two-year period were included, and transmissibility among culture-negative MDR-TB patients was not assessed. Secondly, the lack of recorded treatment outcomes for TB patients precluded the analysis of correlations between resistance characteristics and clinical outcomes. Thirdly, the limited resistance rates of certain drugs resulted in inadequate data, such as the low resistance rates of aminoglycosides, leading to a higher margin of error. Consequently, the high specificity observed for these drugs may not be entirely representative. It is important to acknowledge the study's limitations, particularly regarding drugs with low resistance rates. Lastly, the small sample size prevented the examination of correlations between MTB strain sub-lineages and drug resistance characteristics.

Conclusion

This study focused on the examination of genetic diversity and drug-resistant mutation characteristics in prevalent MDR-MTB strains on Hainan Island, China, utilizing WGS. The findings revealed that lineage 2 (East Asian) and its sublineage lineage 2.2 (Beijing) played a dominant role in the MDR-TB prevalence on the Hainan Island. It was also found that the proportion of lineage 2.1 (non-Beijing) was significantly higher than that in other regions of China and surrounding countries of Hainan Island, which has unique genetic characteristics. Providing insights into the primary types of drug-resistant mutations observed in MDR-MTB strains for both first-line and second-line anti-tuberculosis drugs. Furthermore, the study demonstrated the favorable performance of WGS in predicting resistance to anti-TB drugs, with the exception of ethambutol. Given the significant impact of recent transmission on MDR-TB incidence, targeted interventions such as improved TB case management and active case detection are urgently required to prevent further transmission of MDR-TB on the Hainan Island. With its potential to predict resistance and identify strain clusters, WGS emerges as a promising tool for guiding treatment regimen design and informing tailored public interventions.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Bakhtiyariniya P, Khosravi AD, Hashemzadeh M, et al. Genetic diversity of drug-resistant Mycobacterium tuberculosis clinical isolates from Khuzestan province, Iran. *AMB Express*. 2022;12(1):85. doi:10.1186/s13568-022-01425-7
2. Dohal M, Dvořáková V, Šperková M, et al. Whole genome sequencing of multidrug-resistant Mycobacterium tuberculosis isolates collected in the Czech Republic, 2005–2020. *Sci Rep*. 2022;12(1):7149. doi:10.1038/s41598-022-11287-5
3. Lin M, Zhong Y, Chen Z, et al. High incidence of drug-resistant Mycobacterium tuberculosis in Hainan Island, China. *Trop Med Int Health*. 2019;24(9):1098–1103. doi:10.1111/tmi.13285
4. World Health Organisation. Global tuberculosis report 2022; 2022. Available from: http://www.who.int/tb/publications/global_report/en/. Accessed August 25, 2023.
5. Gandhi NR, Nunn P, Dheda K, et al. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. *Lancet*. 2010;375(9728):1830–1843. doi:10.1016/S0140-6736(10)60410-2
6. He W, Tan Y, Liu C, et al. Drug-resistant characteristics, genetic diversity, and transmission dynamics of rifampicin-resistant Mycobacterium tuberculosis in Hunan, China, Revealed by Whole-Genome Sequencing. *Microbiol Spectr*. 2022;10(1):e0154321. doi:10.1128/spectrum.01543-21
7. Cole ST, Brosch R, Parkhill J, et al. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. *Nature*. 1998;393(6685):537–544. doi:10.1038/31159
8. Vaziri F, Kohl TA, Ghajavand H, et al. Genetic diversity of multi- and extensively drug-resistant Mycobacterium tuberculosis isolates in the capital of Iran, revealed by whole-genome sequencing. *J Clin Microbiol*. 2019;57(1). doi:10.1128/JCM.01477-18
9. Rendon-Bautista LA, Álvarez-maya I, Sandoval-Díaz M, et al. Characterization of genetic diversity and clonal complexes by whole genome sequencing of Mycobacterium tuberculosis isolates from Jalisco, Mexico. *Tuberculosis*. 2021;129:102106. doi:10.1016/j.tube.2021.102106
10. Satta G, Atzeni A, McHugh TD. Mycobacterium tuberculosis and whole genome sequencing: a practical guide and online tools available for the clinical microbiologist. *Clin Microbiol Infect*. 2017;23(2):69–72. doi:10.1016/j.cmi.2016.09.005
11. Faksri K, Tan JH, Chaiprasert A, et al. Bioinformatics tools and databases for whole genome sequence analysis of Mycobacterium tuberculosis. *Infect Genet Evol*. 2016;45:359–368. doi:10.1016/j.meegid.2016.09.013
12. Nikolayevskyy V, Kranzer K, Niemann S, et al. Whole genome sequencing of Mycobacterium tuberculosis for detection of recent transmission and tracing outbreaks: a systematic review. *Tuberculosis*. 2016;98:77–85. doi:10.1016/j.tube.2016.02.009
13. Liu L, Zhao X, Wu X, et al. Prevalence and molecular characteristics of drug-resistant Mycobacterium tuberculosis in Hainan, China: from 2014 to 2019. *BMC Microbiol*. 2021;21(1):185. doi:10.1186/s12866-021-02246-7
14. Wang J, Chen Z, Xu Y, et al. Screening and drug resistance analysis of non-tuberculous mycobacteria in patients with suspected pulmonary tuberculosis on the Hainan Island, China. *Infect Drug Resist*. 2023;16:463–476. doi:10.2147/IDR.S396050
15. Du J, Wang C, Zhang G, et al. Epidemiological sampling survey of tuberculosis in Hainan province in 2010. *Chin Trop Med*. 2012;12:1323–1326.
16. Wang L, Zhang H, Raun Y, et al. Tuberculosis prevalence in China, 1990–2010; a longitudinal analysis of national survey data. *Lancet*. 2014;383 (9934):2057–2064. doi:10.1016/S0140-6736(13)62639-2
17. Larsen MH, Biermann K, Tandberg S, Hsu T, Jacobs WR. Genetic manipulation of Mycobacterium tuberculosis. *Curr Protoc Microbiol*. 2007;6 (1):10A–12A. doi:10.1002/9780471729259.mc10a02s6
18. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25(14):1754–1760. doi:10.1093/ bioinformatics/btp324
19. Zhou Z, Alikhan N-F, Sergeant MJ, et al. GrapeTree: visualization of core genomic relationships among 100,000 bacterial pathogens. *Genome Res*. 2018;28(9):1395–1404. doi:10.1101/gr.232397.117
20. Yang C, Luo T, Shen X, et al. Transmission of multidrug-resistant Mycobacterium tuberculosis in Shanghai, China: a retrospective observational study using whole-genome sequencing and epidemiological investigation. *Lancet Infect Dis*. 2017;17(3):275–284. doi:10.1016/S1473-3099(16) 30418-2
21. Phelan JE, O'Sullivan DM, Machado D, et al. Integrating informatics tools and portable sequencing technology for rapid detection of resistance to anti-tuberculous drugs. *Genome Med*. 2019;11(1):41. doi:10.1186/s13073-019-0650-x
22. Coll F, McNerney R, Preston MD, et al. Rapid determination of anti-tuberculosis drug resistance from whole-genome sequences. *Genome Med*. 2015;7(1):51. doi:10.1186/s13073-015-0164-0
23. Tamura K, Stecher G, Kumar S, Battistuzzi FU. MEGA11: molecular evolutionary genetics analysis version 11. *Mol Biol Evol*. 2021;38 (7):3022–3027. doi:10.1093/molbev/msab120
24. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res*. 2021;49(W1): W293–W296. doi:10.1093/nar/gkab301
25. Walker TM, Monk P, Grace Smith E, et al. Contact investigations for outbreaks of Mycobacterium tuberculosis: advances through whole genome sequencing. *Clin Microbiol Infect*. 2013;19(9):796–802. doi:10.1111/1469-0691.12183
26. Tagliani E, Anthony R, Kohl TA, et al. Use of a whole genome sequencing-based approach for Mycobacterium tuberculosis surveillance in Europe in 2017–2019: an ECDC pilot study. *Eur Respir J*. 2021;57(1):2002272. doi:10.1183/13993003.02272-2020
27. Zhao B, Liu C, Fan J, et al. Transmission and drug resistance genotype of multidrug-resistant or rifampicin-resistant Mycobacterium tuberculosis in Chongqing, China. *Microbiol Spectr*. 2022;10(5):e0240521. doi:10.1128/spectrum.02405-21
28. Sun W, Gui X, Wu Z, et al. Prediction of drug resistance profile of multidrug-resistant Mycobacterium tuberculosis (MDR-MTB) isolates from newly diagnosed case by whole genome sequencing (WGS): a study from a high tuberculosis burden country. *BMC Infect Dis*. 2022;22(1):499. doi:10.1186/s12879-022-07482-4
29. Lin S, Wei S, Zhao Y, et al. Genetic diversity and drug susceptibility profiles of multidrug-resistant tuberculosis strains in Southeast China. *Infect Drug Resist*. 2021;14:3979–3989. doi:10.2147/IDR.S331516
30. Tania T, Sudarmono P, Kusumawati RL, et al. Whole-genome sequencing analysis of multidrug-resistant Mycobacterium tuberculosis from Java, Indonesia. *J Med Microbiol*. 2020;69(7):1013–1019. doi:10.1099/jmm.0.001221
31. Holt KE, McAdam P, Thai PVK, et al. Frequent transmission of the Mycobacterium tuberculosis Beijing lineage and positive selection for the EsxW Beijing variant in Vietnam. *Nat Genet*. 2018;50(6):849–856. doi:10.1038/s41588-018-0117-9

32. Liang D, Song Z, Liang X, et al. Whole genomic analysis revealed high genetic diversity and drug-resistant characteristics of Mycobacterium tuberculosis in Guangxi, China. *Infect Drug Resist*. 2023;16:5021–5031. doi:10.2147/IDR.S410828
33. Chee C, Lim LKY, Ong RTH, et al. Whole genome sequencing analysis of multidrug-resistant tuberculosis in Singapore, 2006–2018. *Eur J Clin Microbiol Infect Dis*. 2021;40(5):1079–1083. doi:10.1007/s10096-020-04100-6
34. Nonghanphithak D, Chaiprasert A, Smithtikarn S, et al. Clusters of drug-resistant Mycobacterium tuberculosis detected by whole-genome sequence analysis of nationwide sample, Thailand, 2014–2017. *Emerg Infect Dis*. 2021;27(3):813–822. doi:10.3201/eid2703.204364
35. Guerra-Assuncao JA, Crampin AC, Houben R, et al. Large-scale whole genome sequencing of M. tuberculosis provides insights into transmission in a high prevalence area. *Elife*. 2015;4. doi:10.7554/eLife.05166
36. Yang C, Shen X, Peng Y, et al. Transmission of Mycobacterium tuberculosis in China: a population-based molecular epidemiologic study. *Clin Infect Dis*. 2015;61(2):219–227. doi:10.1093/cid/civ255
37. Jaksuwan R, Tharavichikul P, Patumanond J, et al. Genotypic distribution of multidrug-resistant and extensively drug-resistant tuberculosis in northern Thailand. *Infect Drug Resist*. 2017;10:167–174. doi:10.2147/IDR.S130203
38. Marttila HJ, Soini H, Eerola E, et al. A Ser315Thr substitution in KatG is predominant in genetically heterogeneous multidrug-resistant Mycobacterium tuberculosis isolates originating from the St. Petersburg Area in Russia. *Antimicrob Agents Chemother*. 1998;42(9):2443–2445. doi:10.1128/AAC.42.9.2443
39. Jian J, Yang X, Yang J, et al. Evaluation of the GenoType MTBDRplus and MTBDRsl for the detection of drug-resistant Mycobacterium tuberculosis on isolates from Beijing, China. *Infect Drug Resist*. 2018;11:1627–1634. doi:10.2147/IDR.S176609
40. Yuan X, Zhang T, Kawakami K, et al. Molecular characterization of multidrug- and extensively drug-resistant Mycobacterium tuberculosis strains in Jiangxi, China. *J Clin Microbiol*. 2012;50(7):2404–2413. doi:10.1128/JCM.06860-11
41. Sandoval R, Monteghirfo M, Salazar O, et al. Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence [Cross-resistance between isoniazid and ethionamide and its strong association with mutation C-15T in Mycobacterium tuberculosis isolates from Peru]. *Rev Argent Microbiol*. 2020;52(1):36–42. Spanish. doi:10.1016/j.ram.2019.03.005
42. Vilcheze C, Jacobs WJ, Hatfull GF, Jacobs WR. Resistance to isoniazid and ethionamide in Mycobacterium tuberculosis: genes, mutations, and causalities. *Microbiol Spectr*. 2014;2(4). doi:10.1128/microbiolspec.MGM2-0014-2013
43. Bollela VR, Namburete EI, Feliciano CS, et al. Detection of katG and inhA mutations to guide isoniazid and ethionamide use for drug-resistant tuberculosis. *Int J Tuberc Lung Dis*. 2016;20(8):1099–1104. doi:10.5588/ijtld.15.0864
44. Wu X, Tan G, Sha W, et al. Use of whole-genome sequencing to predict Mycobacterium tuberculosis complex drug resistance from early positive liquid cultures. *Microbiol Spectr*. 2022;10(2):e0251621. doi:10.1128/spectrum.02516-21
45. Wu X, Gao R, Shen X, et al. Use of whole-genome sequencing to predict Mycobacterium tuberculosis drug resistance in Shanghai, China. *Int J Infect Dis*. 2020;96:48–53. doi:10.1016/j.ijid.2020.04.039
46. Shea J, Halse TA, Lapierre P, et al. Comprehensive whole-genome sequencing and reporting of drug resistance profiles on clinical cases of Mycobacterium tuberculosis in New York State. *J Clin Microbiol*. 2017;55(6):1871–1882. doi:10.1128/JCM.00298-17
47. Van Deun A, Wright A, Zignol M, et al. Drug susceptibility testing proficiency in the network of supranational tuberculosis reference laboratories. *Int J Tuberc Lung Dis*. 2011;15(1):116–124.
48. Madison B, Robinson-Dunn B, George I, et al. Multicenter evaluation of ethambutol susceptibility testing of Mycobacterium tuberculosis by agar proportion and radiometric methods. *J Clin Microbiol*. 2002;40(11):3976–3979. doi:10.1128/JCM.40.11.3976-3979.2002
49. Malinga L, Brand J, Jansen van Rensburg C, et al. Investigation of isoniazid and ethionamide cross-resistance by whole genome sequencing and association with poor treatment outcomes of multidrug-resistant tuberculosis patients in South Africa. *Int J Mycobacteriol*. 2016;5(Suppl 1):S36–S37. doi:10.1016/j.ijmyco.2016.11.020
50. Morlock GP, Metchock B, Sikes D, et al. ethA, inhA, and katG loci of ethionamide-resistant clinical Mycobacterium tuberculosis isolates. *Antimicrob Agents Chemother*. 2003;47(12):3799–3805. doi:10.1128/AAC.47.12.3799-3805.2003
51. Hicks ND, Carey AF, Yang J, et al. Bacterial genome-wide association identifies novel factors that contribute to ethionamide and prothionamide susceptibility in Mycobacterium tuberculosis. *mBio*. 2019;10(2). doi:10.1128/mBio.00616-19
52. Colangeli R, Jedrey H, Kim S, et al. Bacterial factors that predict relapse after tuberculosis therapy. *N Engl J Med*. 2018;379(9):823–833. doi:10.1056/NEJMoa1715849

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