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Molecular epidemiology of drug-resistant tuberculosis in Jiangxi Province, China, 2022–2023

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Abstract: Drug-resistant tuberculosis (DR-TB) poses a critical public health challenge in Jiangxi Province, China, where regional resistance patterns remain understudied. This retrospective study analyzed 9,041 suspected TB patients (2022–2023), identifying 3,104 *Mycobacterium tuberculosis* (*M. tuberculosis*) cases *via* PCR-reverse blot hybridization assay (PCR-REBA). Among *M. tuberculosis*-positive cases, 19.3% exhibited drug resistance, including mono- (9.2%), double- (4.6%), triple- (3.8%), and quadruple-drug resistance (1.7%). Males had higher odds of rifampicin (OR = 1.407, 95% CI: 1.086-1.824, $p = 0.01$) and isoniazid (OR = 1.959, 95% CI: 1.538-2.495, $p < 0.001$) resistance. Dominant mutations included *rpoB* Ser531Leu (32.1%) for rifampicin and *katG* Ser315Thr (53.6%) for isoniazid resistance. Extrapulmonary TB showed higher susceptibility than pulmonary TB (*e.g.*, rifampicin: 93.47% *vs.* 87.25%, $p = 0.002$). These findings highlight the urgent need for rapid molecular diagnostics and targeted interventions in Jiangxi to address distinct DR-TB patterns and demographic disparities.

***Keywords*:** tuberculosis, drug resistance, molecular epidemiology, *rpoB*, *katG*

1. Introduction

Tuberculosis (TB), an ancient and formidable disease, remains one of the world's most lethal public health challenges (*1*). The causative agent, *Mycobacterium tuberculosis* (*M. tuberculosis*), was first isolated and identified by the pioneering Dr. Robert Koch in 1882 (*2*). Historically, TB has been the leading cause of death from a single infectious disease, only surpassed by SARS-CoV-2 during the 2020–2021 pandemic, and consistently been the foremost cause of mortality attributed to a single infectious agent (*3*). According to the World Health Organization's annual Global Tuberculosis Report, an estimated 10.6 million people worldwide contracted TB in 2022, leading to the devastating loss of 1.6 million lives, including 5.8 million men, 3.5 million women, and 1.3 million children (*4*). Despite its widespread prevalence, tuberculosis — a disease primarily affecting the lungs — continues to be a significant contributor to global morbidity and mortality. Nevertheless, its curability and the existence of preventive measures offer a glimmer of hope (*5*). Approximately 90–95% of individuals with latent tuberculosis infection (LTBI) remain asymptomatic, and it is estimated that 5–10% of these latent infections will progress to active tuberculosis (ATB) over their lifetimes (*6*). Drug-sensitive tuberculosis is typically treated with a six-month multi-drug regimen of first- and second-line antibiotics to prevent relapse and drug resistance; however, the treatment duration for drug-susceptible TB can extend up to nine months, challenging medication adherence and raising the risk of treatment failure and resistance (*7*).

The escalating issue of antimicrobial resistance, particularly in the form of multidrug-resistant tuberculosis (MDR-TB), is anticipated to significantly impact global healthcare delivery and contribute to a rise in attributable deaths, necessitating an urgent, multifaceted strategy to foster the development of new antimicrobials, enhance diagnostic capabilities, and ensure the judicious and regulated use of existing treatments (*8*). MDR-TB, characterized by resistance to at least rifampicin and isoniazid, poses a growing public health threat, particularly in Eastern Europe, Russia, Asia, and sub-Saharan Africa, where it is difficult to diagnose, leads to higher mortality and morbidity, and often results in severe post-TB lung damage, despite recent advancements in diagnostics and therapeutics (*9*). Drug susceptibility testing is crucial for

accurately diagnosing MDR-TB, traditionally achieved through phenotypic methods that involve isolating *M. tuberculosis* from patient sputum and testing its growth in the presence of anti-TB drugs, but these methods are time-consuming, costly, and complex, often rendering them inaccessible in resource-limited settings (*10*). The spoligotyping technique has been successfully applied for detecting resistance-related mutations, and molecular diagnostic tools such as the PCR-based reverse blot hybridization assay (PCR-REBA) have been developed to address these challenges (*11*). PCR-REBA offers rapid detection of antimicrobial resistance genes in clinical specimens, which is particularly advantageous for timely and accurate diagnosis of drug-resistant tuberculosis (DR-TB).

This study aims to conduct a molecular epidemiology analysis of DR-TB circulation from clinical specimens obtained from patients in Jiangxi Province, China from April 2022 to December 2023. Employing PCR reverse dot hybridization technology, it systematically identified mutant genes associated with resistance to rifampicin, isoniazid, streptomycin, and ethambutol, focusing on specific mutations in key genes. This approach specifically targeted key gene mutations, including *rpoB*, *katG*, *fabG1*, *rpsL*, and *embB*, which are known to be associated with resistance to first-line TB drugs. By elucidating the molecular epidemiology of DR-TB in Jiangxi Province, the findings aim to inform the development of more effective strategies for prevention, diagnosis, and treatment of this critical public health issue.

2. Materials and Methods

2.1. Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki. Ethical approval was granted by the Ethics Committee of Jiangxi Province Chest Hospital (Jiangxi Third People's Hospital) with a waiver of informed consent due to the retrospective nature of the research and the use of anonymized data collected as part of routine clinical care.

2.2. Study design, site, and population

This research focused on TB and suspected TB patients admitted to the designated TB hospital in Jiangxi Province (the Third People's Hospital of Jiangxi Province, China) from April 2022 to December 2023. The patient data was collected and evaluated from archived results for the specimens detected using PCR-REBA. During the study period, patients were included if they were clinically suspected of TB and had PCR-REBA results. Only initial test results were analyzed for patients with multiple tests. Pulmonary TB was defined as *M. tuberculosis* detected in lung specimens; extrapulmonary TB involved other sites. Prior treatment history was unavailable for retrospective analysis.

2.3. Specimen collection

All specimens were obtained from patients clinically suspected of having TB, encompassing various bodily secretions or fluids such as sputum cultures, nasopharyngeal swabs, bronchoalveolar lavage, lymph node aspirates, cerebrospinal fluid, and pleural fluid, as well as tissue samples including fine-needle aspirations, pleural biopsies, and lung biopsies.

2.4. Specimen processing

Purulent specimens, such as sputum, purulent fiberoptic bronchoscopy samples, and purulent pleural fluid, were treated with an equal volume of 4% NaOH and incubated for 10 minutes. After centrifugation at 12,000g for 5 minutes, the supernatant was removed. Solid specimens, including tissue and biopsy samples, were homogenized with an equal volume of sterile water. Fluid specimens, such as urine, cerebrospinal fluid (CSF), and clear pleural fluid, were centrifuged at 12,000g for 5 minutes, followed by the removal of the supernatant. After a subsequent centrifugation at 12,000g for 3 minutes, the supernatant was discarded.

2.5. DNA isolation

The bacterial pellet was resuspended in DNA lysis solution (Yaneng BioSciences, Shenzhen, China), followed by incubation at 100 °C for 10 minutes. Subsequently, the mixture was centrifuged at 12,000 r/min for 3 minutes. The resulting supernatant, containing genomic DNA, was either utilized immediately or stored at -80°C for subsequent analysis.

2.6. Real-time polymerase chain reaction (PCR)

Five microliters of each DNA sample were carefully added to the PCR tubes provided by Yaneng BioSciences, Shenzhen, China. These tubes were pre-filled with a reaction mixture comprising primers, TaqMan polymerase, and dNTPs. The experimental protocol and the real-time PCR program on the ABI instrument were set up as detailed below: The procedure began with an initial incubation at 50°C for 2 minutes, followed by a single cycle. The temperature was gradually increased from 50°C to 95°C at a rate of 1.4°C per second. This was maintained at 95°C for 10 minutes, completing the initial cycle. Subsequently, a denaturation step was carried out at 95°C for 45 seconds, transitioning to 62°C at a rate of 1.4°C per second, and then an annealing/extension step at 60°C for 30 seconds, which was repeated for 30 cycles. Following this, the temperature was ramped up from 62°C to 95°C at 1.4°C

per second, denaturing at 95°C for 30 seconds. It then transitioned from 95°C to 54°C at a rate of 1.4°C per second, annealed at 54°C for 30 seconds, and extended at 68°C for 45 seconds, repeated for another 30 cycles. The program concluded with a final extension at 68°C for 5 minutes. The reaction mixture was then prepared to detect the amplification of the target bacterial DNA using the 7500 Real-Time PCR System from Thermo Fisher Scientific in Waltham, MA, USA. The analysis of real-time PCR results was facilitated by Real-Time PCR Software, version 1.2.3. The amplification plots were reviewed for baseline and threshold value adjustments to ensure accuracy.

2.7. PCR-reverse blot hybridization assay (PCR-REBA Myco-ID)

The amplified PCR products were analyzed for the identification of DR-TB using the PCR-REBA Myco-ID kit (Yaneng BioSciences, Shenzhen, China), in accordance with the manufacturer's protocol.

2.7.1. Hybridization

The nylon membrane strip, labeled with probes, was placed into a 15ml conical centrifuge tube. It was then gently mixed with 5ml of a hybridization buffer, which consisted of 1x SSC (Saline Sodium Citrate) and 0.1% SDS (Sodium Dodecyl Sulfate). Additionally, 25μl of PCR product was incorporated into the mixture. The tube was securely capped and submerged in a boiling water bath set at 100°C for a duration of 10 minutes, ensuring that the hybridization liquid remained below the water level. After this pre-treatment, the tube was transferred to a hybridization box and maintained at a temperature of 59°C for 1.5 hours to facilitate the hybridization process.

2.7.2. Washing strips

A 40 mL volume of wash buffer, comprising 0.5x SSC and 0.1% SDS, was transferred into a 50 mL conical centrifuge tube equipped with a screw-top cap and preheated to 57°C within a water bath. Subsequently, the membrane strips were carefully removed from the 15 mL tube and immersed into the preheated wash buffer at 57°C, where they were subjected to gentle shaking for a duration of 15 minutes.

2.7.3. Colorimetric analysis

The strips were immersed in a 1:2,000 dilution of hybridization buffer for 30 minutes at ambient temperature. Subsequently, they were rinsed twice, each for 5 minutes, with the hybridization buffer at room temperature, followed by a brief cleaning in 0.1 mol/L sodium citrate for 2 minutes. The strips were then treated with a freshly prepared substrate solution consisting of 0.01% w/v tetramethylbenzidine dihydrochloride (TMB) (Yaneng BioSciences, Shenzhen, China) and 0.006% v/v hydrogen peroxide in 0.1M sodium citrate, while kept in the dark for 10 minutes. The color development process was halted by gently rinsing the membranes with distilled water, after which the band pattern of the colorimetric hybridization was visually assessed.

2.8. Statistical analysis

The statistical analysis was conducted using SPSS 27.0. Variables (age, sex, TB type) were selected based on known DR-TB risk factors identified in prior literature. Univariate logistic regression was performed to assess associations between demographic factors (age groups, sex) and resistance to individual drugs (rifampicin, isoniazid, streptomycin, and ethambutol), with the ≥ 65 age group and females as reference categories. Multivariable logistic regression adjusted for potential confounders, including age and sex, to isolate independent effects. Prevalence estimates were reported with 95% confidence intervals (CIs). To address multiple comparisons, Bonferroni correction was applied, and a significance threshold of $p < 0.05$ was maintained. Categorical data were presented as frequencies and percentages, and chi-square tests compared susceptibility patterns between pulmonary and extrapulmonary TB. Adjusted *p*-values and CIs for odds ratios (ORs) were included to ensure robust inference. All analyses were conducted using anonymized data to ensure patient confidentiality.

3. Results

3.1. Prevalence and patterns of DR-TB

Among the 9,041 nonrepeating patients included in the study, *M. tuberculosis* infection was identified in 3,140 cases (34.7%) *via* PCR-REBA, of which 599 (19.1%) exhibited DR-TB (Figure 1). The resistance patterns among the 3,140 *M. tuberculosis*-positive cases were as follows: 287 (9.2%) were resistant to a single first-line anti-tuberculosis drug (mono-resistant TB), 142 (4.6%) to two drugs (double-drug resistant TB), 118 (3.8%) to three drugs (triple-drug resistant TB), and 52 (1.7%) to all four first-line drugs (quadruple-drug resistant TB) (Table 1). The remaining 2,505 cases (80.6%) were susceptible to all tested drugs.

3.2. Age and gender disparities in DR-TB

Univariate logistic regression analysis revealed that, compared to the ≥ 65 years group, rifampicin resistant TB showed a significant difference in the 45–54 years group (OR = 1.497, 95% CI: 1.079–2.077, $p = 0.016$) (Table 2), but no significant differences were observed in other age groups. Similarly, compared to females,

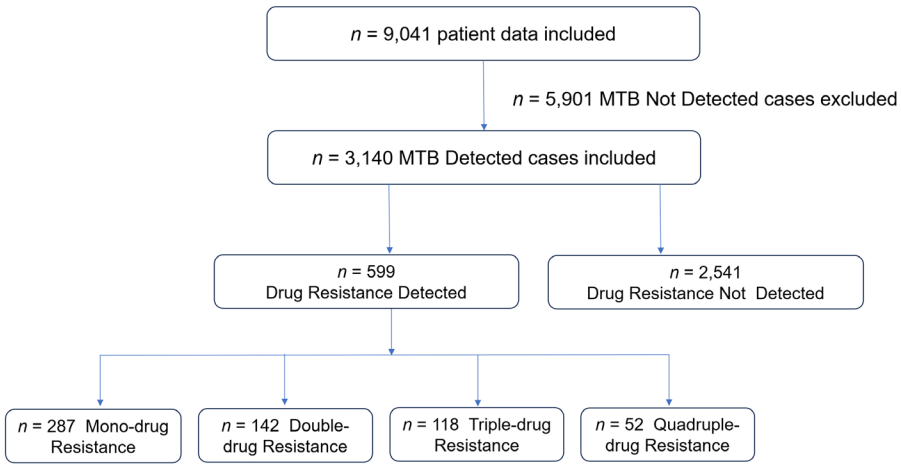

Figure 1. Schematic overview of the study's analysis on the circulation of DR-TB. DR-TB: drug-resistant tuberculosis.

Table 1. Resistance patterns of *M. tuberculosis* strains to first-line drugs

Types of DR-TB	Cases (*n*)	Frequency (%)
Mono-drug resistant TB	287	9.2
Double-drug resistant TB	142	4.6
Triple-drug resistant TB	118	3.8
Quadruple-drug resistant TB	52	1.7
Total DR-TB	599	19.3
Total *M. tuberculosis*-positive cases	3,104	100

DR-TB: drug-resistant tuberculosis; TB: tuberculosis.

rifampicin resistant TB was significantly more prevalent in males (OR = 1.407, 95% CI: 1.086–1.824, p = 0.01). In contrast, isoniazid resistant TB did not show significant differences across age groups when compared to the ≥ 65 years group. However, isoniazid resistant TB was significantly more common in males compared to females (OR = 1.959, 95% CI: 1.538–2.495, $p < 0.001$). Additionally, streptomycin resistant TB did not exhibit significant differences across age groups or between sexes. Regarding ethambutol resistant TB, a significant difference was observed in the 35–44 years group compared to the ≥ 65 years group (OR = 2.050, 95% CI: 1.050–4.002, p = 0.035), but no such differences were found in other age groups. Similarly, ethambutol resistant TB was significantly more prevalent in males compared to females (OR = 1.748, 95% CI: 1.065–2.869, p = 0.027).

3.3. Comparative analysis of antibiotic susceptibility in pulmonary and extrapulmonary TB

In this study, it compared the distribution of susceptibility and resistance patterns to four anti-TB drugs (rifampicin, isoniazid, streptomycin, and ethambutol) in pulmonary and extrapulmonary TB cases. For rifampicin, most pulmonary cases (87.25%) were susceptible, whereas a higher proportion of extrapulmonary cases (93.47%) showed susceptibility (Table 3). Conversely, resistance was more frequent in pulmonary cases (12.75%) compared to extrapulmonary cases (6.53%). The χ^2 test confirmed a statistically significant difference (χ^2 = 9.557, p = 0.002). Similarly, for isoniazid, pulmonary cases exhibited a susceptibility rate of 86.59%, while extrapulmonary cases had a slightly higher rate of 92.03%. Resistance was more common in pulmonary cases (13.41%) than in extrapulmonary cases (7.97%), with the difference being statistically significant (χ^2 = 6.602, p = 0.01). For streptomycin, susceptibility rates were 90.99% in pulmonary cases and 94.53% in extrapulmonary cases, while resistance was more prevalent in pulmonary cases (9.01%) compared to extrapulmonary cases (5.47%). The χ^2 test showed a borderline significant difference (χ^2 = 3.926, p = 0.048). Lastly, ethambutol demonstrated high susceptibility rates in both groups, with 96.17% in pulmonary cases and 98.87% in extrapulmonary cases. Resistance was minimal, with only 3.83% and 1.13% observed in pulmonary and extrapulmonary cases, respectively. The difference was statistically significant (χ^2 = 5.119, p = 0.024).

3.4. Genetic mutations associated with rifampicin and isoniazid resistance

Analysis of 599 drug-resistant *M. tuberculosis* isolates revealed that mutations in the *rpoB* gene serve as the principal genetic basis for rifampicin resistance. Within the rifampicin resistance-determining region (RRDR), the most frequently observed mutation was *Ser531Leu*, accounting for 32.1% (192/599) of cases (Table 4). Additional mutations included *His526Asp* (11.0%, 66/599), *Asp516Val* (4.8%, 29/599), *Asp516Gly* (3.8%, 23/599), and *His526Tyr* (4.7%, 28/599). A rare variant, *Ser531Trp*, was detected in only 0.8% (5/599) of isolates. Resistance to isoniazid was primarily linked to mutations in the *katG* and *fabG1* genes. The *katG Ser315Thr* mutation, which confers high-level isoniazid resistance,

Table 2. Logistic regression analysis of socio-demographic factors associated with DR-TB

Types of DR-TB	Socio-demographic characteristics	Univariable Analysis*		Multivariable Analysis**	
		Odds Ratio (95% CI)	*p*-value	Odds Ratio (95% CI)	*p*-value
Rifampicin Resistant	Age				
	0–14	1.077 (0.318–3.655)	0.905	1.249 (0.366–4.266)	0.723
	15–24	0.833 (0.555–1.252)	0.380	0.890 (0.590–1.341)	0.576
	25–34	1.050 (0.719–1.534)	0.799	1.114 (0.761–1.632)	0.578
	35–44	1.331 (0.908–1.952)	0.142	1.410 (0.959–2.073)	0.080
	45–54	1.471 (1.061–2.039)	0.021	1.497 (1.079–2.077)	0.016
	55–64	1.275 (0.935–1.740)	0.125	1.266 (0.927–1.727)	0.138
	≥ 65	1.000		1.000	
	Sex				
	Male	1.418 (1.100–1.828)	0.007	1.407 (1.086–1.824)	0.010
	Female	1.000		1.000	
Isoniazid Resistant	Age				
	0–14	1.376 (0.465–4.076)	0.564	1.626 (0.571–4.63)	0.362
	15–24	0.732 (0.482–1.112)	0.144	0.842(0.559–1.269)	0.412
	25–34	1.005 (0.687–1.470)	0.981	1.108 (0.764–1.605)	0.589
	35–44	1.322 (0.902–1.937)	0.153	1.270 (0.878–1.837)	0.205
	45–54	1.433 (1.036–1.982)	0.030	1.038 (0.760–1.417)	0.814
	55–64	1.278 (0.942–1.735)	0.115	1.038 (0.773–1.394)	0.803
	≥ 65	1.000		1.000	
	Sex				
	Male	1.310 (1.021–1.68)	0.033	1.959 (1.538–2.495)	< 0.001
	Female	1.000		1.000	
Streptomycin Resistant	Age				
	0–14	0		0	
	15–24	0.680 (0.420–1.102)	0.118	0.702 (0.431–1.141)	0.153
	25–34	0.680 (0.420–1.102)	0.118	0.699 (0.430–1.136)	0.149
	35–44	1.111 (0.713–1.731)	0.643	1.139 (0.729–1.781)	0.567
	45–54	1.280 (0.883–1.856)	0.193	1.289 (0.888–1.869)	0.181
	55–64	1.007 (0.702–1.445)	0.969	1.003 (0.699–1.438)	0.989
	≥ 65	1.000		1.000	
	Sex				
	Male	1.245 (0.928–1.670)	0.144	1.168 (0.865–1.577)	0.310
	Female	1.000		1.000	
Ethambuto Resistant	Age				
	0–14	1.440 (0.187–11.108)	0.726	1.807 (0.232–14.098)	0.573
	15–24	1.337 (0.669–2.671)	0.410	1.481 (0.737–2.973)	0.270
	25–34	1.407 (0.715–2.767)	0.323	1.549 (0.784–3.061)	0.208
	35–44	1.885 (0.970–3.665)	0.062	2.050 (1.050–4.002)	0.035
	45–54	1.665 (0.911–3.043)	0.098	1.708 (0.934–3.125)	0.082
	55–64	1.003 (0.531–1.894)	0.993	0.989 (0.524–1.869)	0.974
	≥ 65	1.000		1.000	
	Sex				
	Male	1.587 (0.976–2.579)	0.062	1.748 (1.065–2.869)	0.027
	Female	1.000		1.000	

*Univariate analysis: Crude odds ratios for demographic factors associated with DR-TB. **Multivariable analysis: Adjusted for age, sex, and tuberculosis type. DR-TB: drug-resistant tuberculosis.

Table 3. Antibiotic susceptibility patterns in pulmonary and extra-pulmonary TB cases

Drugs	Groups	Pulmonary Cases (%)	Extra pulmonary Cases (%)	Total	χ^2	*p*-value
Rifampicin	Susceptible	2,477 (87.25)	272 (93.47)	2,749	9.557	0.002
	Resistant	362 (12.75)	19 (6.53)	381		
Isoniazid	Susceptible	2,356 (86.59)	254 (92.03)	2,610	6.602	0.010
	Resistant	365 (13.41)	22 (7.97)	387		
Streptomycin	Susceptible	2,484 (90.99)	259 (94.53)	2,743	3.926	0.048
	Resistant	246 (9.01)	15 (5.47)	261		
Ethambuto	Susceptible	2,533 (96.17)	263 (98.87)	2,796	5.119	0.024
	Resistant	101 (3.83)	3 (1.13)	104		

TB: tuberculosis.

Table 4. Frequency of SNPs in *rpoB*, *katG*, and *fabG*1 genes among rifampicin- and isoniazid-resistant TB

Gene	SNP*	Cases (*n*)	Frequency (%)
rpoB	*Asp516Val*	29.0	4.8
rpoB	*Asp516Gly*	23.0	3.8
rpoB	*His526Tyr*	28.0	4.7
rpoB	*His526Asp*	66.0	11
rpoB	*Ser531Leu*	192.0	32.1
rpoB	*Ser531Trp*	5.0	0.8
katG	*Ser315Thr*	321	53.6
fabG1	*c.-15C>T*	67	11.2
Total		599	100

*SNP: single nucleotide polymorphism.

was the most prevalent (53.6%, 321/599). Meanwhile, the f*abG1 c.-15C>T* variant — associated with low-level resistance due to *inhA* promoter upregulation — was identified in 11.2% (67/599) of isolates.

4. Discussion

The molecular epidemiology of DR-TB in Jiangxi Province, China, presents distinct characteristics that both align with and diverge from patterns observed in other regions of China and globally. The present study, conducted from April 2022 to December 2023, revealed a DR-TB prevalence of 19.3% among *M. tuberculosis* positive cases, aligning with Hubei Province's reported rate (19.3%) but markedly higher than the 10.5% observed among students in Henan Province (*12,13*). However, Jiangxi's resistance profile demonstrates unique traits. Notably, mono-resistant TB accounted for 9.2% of cases in this study, mirroring a rising trend observed in neighboring Hubei — where mono-resistance increased from 8.5% (2020) to 12.9% during a similar period (*12*). These parallels between the two adjacent provinces suggest potential regional epidemiological linkages. Additionally, while the study data showed the *rpoB Ser531Leu* mutation as the most prevalent rifampicin resistance determinant (32.1%), studies from Zhejiang Province reported codon 526 mutations as dominant (> 75% of cases), with *Ser531Leu* being rare (< 5%) (*14*). These regional variations underscore the importance of localized surveillance and tailored intervention strategies.

The demographic patterns of DR-TB in Jiangxi provide further insights into the province's unique epidemiological landscape. The present findings confirmed the global trend of higher TB incidence among males (*15,16*), with male patients showing significantly greater susceptibility to both rifampicin (OR = 1.407, $p = 0.01$) and isoniazid resistance (OR = 1.959, $p < 0.001$). This gender disparity may be exacerbated in Jiangxi by factors such as higher smoking rates and occupational exposures among male populations. Age-specific analysis revealed that individuals aged 45–54 years had significantly higher rifampicin resistance (OR = 1.497, $p = 0.016$) compared to those ≥ 65 years, a pattern that aligns with studies from Shandong Province (*17*); but contrasts with data from Western populations where elderly patients typically show higher resistance rates (*18*). This suggests that middle-aged adults in Jiangxi may represent a particularly vulnerable group, possibly due to chronic infection or treatment interruption during peak working years.

The mutation profile of DR-TB strains in Jiangxi offers critical insights for both diagnosis and treatment. The high frequency of the *katG Ser315Thr* mutation (53.6%), associated with high-level isoniazid resistance, has important clinical implications. This mutation renders standard isoniazid therapy ineffective (*19*), necessitating alternative regimens for affected patients. Particularly concerning is that this mutation reduces the efficacy of bedaquiline and linezolid-containing short-course MDR-TB regimens (*20*), which are increasingly used in China. The *fabG1 c.-15C>T* mutation, though less common (11.2%), presents a distinct challenge. This mutation confers low-level isoniazid resistance through inhA promoter upregulation (*21*) and responds to higher isoniazid doses, suggesting that routine detection of this mutation could guide more effective, personalized treatment strategies in Jiangxi. For instance, clinicians could consider escalating isoniazid doses or combining it with other agents like ethionamide, which targets the same pathway, to overcome resistance in cases harboring this mutation. The clinical significance of these findings is heightened by this observation that 8.7% of DR-TB cases showed quadruple-drug resistance, indicating the emergence of particularly challenging strains that may require novel therapeutic approaches.

Comparative analysis with national and global data reveals both consistencies and unique aspects of Jiangxi's DR-TB situation. While the *rpoB Ser531Leu* mutation's dominance in Jiangxi (32.1%) aligns with findings from Guizhou (*22*) and Changchun (*23*), its prevalence is markedly higher than in Zhejiang (*14*) and lower than in Northern Morocco (34.46%) (*24*) or Uganda (40%) (*25*). These differences likely reflect variations in treatment practices, strain genotypes, and population immunity. The rare occurrence of *Ser531Trp* (0.8%) in Jiangxi compared to Brazil (*26*) further suggests distinct evolutionary pressures or transmission patterns. Such regional variations emphasize the need for Jiangxi-specific resistance databases to inform clinical decision-making and public health planning.

The differential drug susceptibility between pulmonary and extrapulmonary TB cases in Jiangxi has important clinical implications. This study found significantly higher susceptibility rates in extrapulmonary cases across all first-line drugs, most notably for rifampicin (93.47% *vs.* 87.25%, $p = 0.002$) and isoniazid (92.03% *vs.* 86.59%, $p = 0.01$). This pattern, consistent with global reports (*27*), may reflect lower bacterial burdens in extrapulmonary sites or better drug penetration (*28*). These findings suggest that

standard first-line regimens may be more effective for extrapulmonary TB in Jiangxi, while pulmonary cases may require more aggressive monitoring and tailored regimens. The minimal resistance to ethambutol in both groups (3.83% pulmonary, 1.13% extrapulmonary) supports its continued use as a cornerstone of TB treatment in Jiangxi (*29*), though the emergence of even low-level resistance warrants vigilance.

The public health implications of these findings are substantial. The high prevalence of specific mutations like *rpoB Ser531Leu* and *katG Ser315Thr* suggests that molecular diagnostics should be prioritized in Jiangxi's TB control program. Techniques like PCR-REBA (*11*), used in this study, offer rapid detection of these mutations, and could significantly improve treatment outcomes. The data also highlight the need for targeted interventions for high-risk groups, particularly middle-aged males, who showed elevated resistance rates. The presence of *fabG*1 mutations, though less common, underscores the value of comprehensive genetic testing to detect all resistance mechanisms, as these mutations may respond differently to treatment adjustments.

Looking forward, the study identifies several critical research needs for Jiangxi Province. Whole-genome sequencing could elucidate transmission networks and evolutionary pathways of resistant strains, as demonstrated in studies from Santa Catarina, Brazil (*26*). Investigation of social determinants, particularly factors driving the high resistance rates among middle-aged adults, could inform targeted interventions. The development of localized treatment algorithms incorporating Jiangxi's specific mutation patterns would optimize therapeutic outcomes. Additionally, continuous surveillance is essential to monitor emerging resistance patterns, especially given the province's economic development and increasing population mobility.

In conclusion, the present study provides a comprehensive molecular epidemiological profile of DR-TB in Jiangxi Province, revealing both shared and unique characteristics compared to other regions. The high prevalence of specific resistance mutations, demographic disparities in resistance patterns, and differential susceptibility between pulmonary and extrapulmonary cases all have immediate implications for TB control policies in Jiangxi. These findings underscore the necessity of implementing rapid molecular diagnostics, developing mutation-specific treatment guidelines, and tailoring interventions to high-risk populations. As DR-TB continues to evolve in Jiangxi, ongoing surveillance and research will be crucial to maintain progress toward TB elimination goals. The distinct patterns observed in the study highlight the importance of region-specific approaches in the global fight against DR-TB.

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References

1. Rungelrath V, Ahmed M, Hicks L, Miller SM, Ryter KT, Montgomery K, Ettenger G, Riffey A, Abdelwahab WM, Khader SA, Evans JT. Vaccination with Mincle agonist UM-1098 and mycobacterial antigens induces protective Th1 and Th17 responses. NPJ Vaccines. 2024; 9:100.
2. Negi K, Bhaskar A, Dwivedi VP. Progressive host-directed strategies to potentiate BCG vaccination against tuberculosis. Front Immunol. 2022; 13:944183.
3. Pitaloka DAE, Syaputri Y, Nurlilasari P, Khairunnisa SF, Saallah S. Promising ursolic acid as a novel antituberculosis agent: Current progress and challenges. Drug Des Devel Ther. 2024; 18:1969-1979.
4. Kantipudi K, Gu J, Bui V, Yu H, Jaeger S, Yaniv Z. Automated pulmonary tuberculosis severity assessment on chest X-rays. J Imaging Inform Med. 2024; 37:2173-2185.
5. Debnath A, Singh N, Gupta S, Kishore J. Burden of tuberculosis among tribal populations of India: A systematic review and meta-analysis. Lung India. 2024; 41:121-129.
6. Niu L, Wang H, Luo G, Zhou J, Hu Z, Yan B. Advances in understanding immune homeostasis in latent tuberculosis infection. WIREs Mech Dis. 2024; 16:e1643.
7. Singh V. Tuberculosis treatment-shortening. Drug Discov Today. 2024; 29:103955.
8. Lange C, Dheda K, Chesov D, Mandalakas AM, Udwadia Z, Horsburgh CR Jr. Management of drug-resistant tuberculosis. Lancet. 2019; 394:953-966.
9. Lecai J, Mijiti P, Chuangyue H, Mingzhen L, Qian G, Weiguo T, Jihong C. Predictors and trends of MDR/RR-TB in Shenzhen China: A retrospective 2012-2020 period analysis. Infect Drug Resist. 2021; 14:4481-4491.
10. Sanchini A, Lanni A, Giannoni F, Mustazzolu A. Exploring diagnostic methods for drug-resistant tuberculosis: A comprehensive overview. Tuberculosis (Edinb). 2024; 148:102522.
11. Wan L, Guo Q, Wei JH, Liu HC, Li MC, Jiang Y, Zhao LL, Zhao XQ, Liu ZG, Wan KL, Li GL, Guan CX. Accuracy of a reverse dot blot hybridization assay for simultaneous detection of the resistance of four anti-tuberculosis drugs in Mycobacterium tuberculosis isolated from China. Infect Dis Poverty. 2020; 9:38.
12. Wang F-L, Chen R, Xu Q, Wang X-Q, Tao F-X, Huang Z-K, Zhang Y-T, Chen S-Q, Wu X-J, Cao H-Y, Jiang Q. Longitudinal phenotypic and genomic evidence revealing increased risk of drug resistance accumulation in tuberculosis patients in the counties of central China. Microbiol Spectr. 2025; 13:e0038025.
13. Xu J, Zhang Y, Wang G, Jiang J, Du W, Chen S, Meng D, Suo W, Zhuang Y, Wang K, Wang W, Zhao D. Drug resistance among students with pulmonary tuberculosis:

a study based on screening in Henan, China. BMC Infect Dis. 2025; 25:1002.
14. Sheng J, Li J, Sheng G, Yu H, Huang H, Cao H, Lu Y, Deng X. Characterization of *rpoB* mutations associated with rifampicin resistance in *Mycobacterium tuberculosis* from eastern China. J Appl Microbiol. 2008; 105:904-911.
15. Bai W, Ameyaw EK. Global, regional and national trends in tuberculosis incidence and main risk factors: A study using data from 2000 to 2021. BMC Public Health. 2024; 24:12.
16. Yang H, Ruan X, Li W, Xiong J, Zheng Y. Global, regional, and national burden of tuberculosis and attributable risk factors for 204 countries and territories, 1990-2021: A systematic analysis for the Global Burden of Diseases 2021 study. BMC Public Health. 2024; 24:3111.
17. An Q, Song W, Liu J, Tao N, Liu Y, Zhang Q, Xu T, Li S, Liu S, Li Y, Yu C, Li H. Primary drug-resistance pattern and trend in elderly tuberculosis patients in Shandong, China, from 2004 to 2019. Infect Drug Resist. 2020; 13:4133-4145.
18. Caraux-Paz P, Diamantis S, de Wazières B, Gallien S. Tuberculosis in the elderly. J Clin Med. 2021; 10:5888.
19. Lempens P, Meehan CJ, Vandelannoote K, Fissette K, de Rijk P, Van Deun A, Rigouts L, de Jong BC. Isoniazid resistance levels of Mycobacterium tuberculosis can largely be predicted by high-confidence resistance-conferring mutations. Sci Rep. 2018; 8:3246.
20. Yadav S. Primary Isoniazid mono-resistant pulmonary tuberculosis in a COVID-19-positive male: World's first case of its kind in the present pandemic. Cureus. 2022; 14:e27163.
21. Faïon L, Djaout K, Frita R, *et al*. Discovery of the first Mycobacterium tuberculosis MabA (FabG1) inhibitors through a fragment-based screening. Eur J Med Chem. 2020; 200:112440.
22. Chen L, Gan X, Li N, Wang J, Li K, Zhang H. *rpoB* gene mutation profile in rifampicin-resistant *Mycobacterium tuberculosis* clinical isolates from Guizhou, one of the highest incidence rate regions in China. J Antimicrob Chemother. 2010; 65:1299-1301.
23. Zhang MJ, Ren WZ, Sun XJ, Liu Y, Liu KW, Ji ZH, Gao W, Yuan B. GeneChip analysis of resistant Mycobacterium tuberculosis with previously treated tuberculosis in Changchun. BMC Infect Dis. 2018; 18:234.
24. Karimi H, Oudghiri A, En-Nanei L, Mzibri ME, Laglaoui A, Chaoui I, Abid M. Frequency of genomic mutations mediating resistance of Mycobacterium tuberculosis isolates to rifampicin in Northern Morocco. Rev Inst Med Trop Sao Paulo. 2020; 62:e37.
25. Kigozi E, Kasule GW, Musisi K, Lukoye D, Kyobe S, Katabazi FA, Wampande EM, Joloba ML, Kateete DP. Prevalence and patterns of rifampicin and isoniazid resistance conferring mutations in Mycobacterium tuberculosis isolates from Uganda. PLoS One. 2018; 13:e0198091.
26. Prim RI, Schörner MA, Senna SG, Nogueira CL, Figueiredo AC, Oliveira JG, Rovaris DB, Bazzo ML. Molecular profiling of drug resistant isolates of Mycobacterium tuberculosis in the state of Santa Catarina, southern Brazil. Mem Inst Oswaldo Cruz. 2015; 110:618-623.
27. Arnedo-Pena A, Romeu-Garcia MA, Meseguer-Ferrer N, Vivas-Fornas I, Vizcaino-Batllés A, Safont-Adsuara L, Bellido-Blasco JB, Moreno-Muñoz R. Pulmonary versus extrapulmonary tuberculosis associated factors: A case-case study. Microbiol Insights. 2019; 12:1178636119840362.
28. Mohammadnabi N, Shamseddin J, Emadi M, Bodaghi AB, Varseh M, Shariati A, Rezaei M, Dastranj M, Farahani A. Mycobacterium tuberculosis: The mechanism of pathogenicity, immune responses, and diagnostic challenges. J Clin Lab Anal. 2024; 38:e25122.
29. Olivença F, Pires D, Silveiro C, Gama B, Holtreman F, Anes E, Catalão MJ. Ethambutol and meropenem/clavulanate synergy promotes enhanced extracellular and intracellular killing of Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2024; 68:e0158623.

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