Genetic Mutations in katG and inhA Genes and Their Clinical Correlations in Isoniazid-Resistant Pulmonary Tuberculosis Patients

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Article

Genetic Mutations in katG and inhA Genes and Their Clinical Correlations in Isoniazid-Resistant Pulmonary Tuberculosis Patients

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Highlights:

Main findings

- All inhA mutations were concentrated in codon cluster 233-240 (100% of cases), representing the first report of this mutation pattern in Vietnamese tuberculosis strains.
- Patients with katG or inhA mutations had significantly higher rates of pulmonary rales compared to those without mutations (90.9% and 85.7% vs. 34.2%, $p < 0.001$).

Implications

- Current molecular diagnostic platforms should include inhA codon cluster 233-240 targets to improve detection sensitivity for INH resistance.
- Genetic testing could enable personalized treatment strategies, with high-dose INH potentially effective for inhA mutations while alternative drugs are needed for katG mutations.

Abstract

Background: Isoniazid resistance in Mycobacterium tuberculosis is primarily mediated by mutations in katG and inhA genes, yet the molecular characteristics of coding region mutations and their clinical correlations in Vietnamese patients remain incompletely understood; (2) Methods: We conducted a cross-sectional study analyzing exon mutations in katG and inhA genes using Sanger sequencing in 56 isoniazid-resistant pulmonary tuberculosis patients treated at the National Lung Hospital from January 2023 to June 2024, with comprehensive clinical and paraclinical data collection; (3) Results: Exon mutations in katG were detected in 11 patients (19.6%) and inhA in 7 patients (12.5%), with no concurrent mutations. The Ser315Thr mutation was present in all katG-positive cases (100%), while all inhA mutations were concentrated in codon cluster 233-240 with 63/96 being amino acid-changing variants. Patients with katG or inhA mutations had significantly higher rates of pulmonary rales compared to those without mutations (90.9% and 85.7% vs. 34.2%, $p < 0.001$), and inhA mutations were exclusively found in multidrug-resistant tuberculosis cases ($p = 0.012$); (4) Conclusions: Our findings identify codon cluster 233-240 as a novel inhA mutation hotspot in Vietnamese strains and demonstrate significant associations between specific mutations and disease severity. The exclusive presence of inhA mutations in MDR-TB cases and the correlation between genotype and clinical manifestations support the implementation of genotype-guided diagnostic and therapeutic strategies for isoniazid-resistant tuberculosis.

Keywords: pulmonary tuberculosis; isoniazid; drug resistance; katG; inhA; gene mutation; S315T; exon

1. Introduction

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Pulmonary tuberculosis caused by Mycobacterium tuberculosis (MTB) remains one of the leading infectious diseases causing mortality worldwide [1]. Isoniazid (INH) resistance-affecting one of the most essential first-line anti-tuberculosis drugs-is increasingly prevalent and contributes to the rise of drug-resistant tuberculosis [2]. Understanding the molecular mechanisms of INH resistance is essential for developing individualized, effective, and appropriate treatment strategies.

INH requires activation by the catalase-peroxidase enzyme encoded by the katG gene. Once activated, INH binds to the target enzyme InhA-an enoyl-acyl carrier protein reductase—involved in mycolic acid synthesis, an essential component of the mycobacterial cell wall. Mutations in the exon regions of the katG gene (particularly at codon 315—S315T) are common mechanisms causing high-level INH resistance by eliminating or reducing drug-activating enzyme function [3]. Meanwhile, mutations in the inhA gene exon alter the InhA enzyme structure, interfering with activated INH binding, thereby also causing high-level drug resistance [4].

Most previous studies have primarily focused on promoter region mutations of inhA—such as C-15T—which are associated with low-level resistance. However, analysis of coding region (exon) mutations in inhA is gaining attention due to their potential association with high-level INH resistance, but remains limited in Vietnam – a country with high tuberculosis prevalence rate [5]. Identifying characteristics of mutations in katG and inhA gene exons is important for understanding resistance mechanisms, predicting high-dose INH treatment efficacy, and supporting individualized treatment regimen development.

This study aims to determine the prevalence and characteristics of katG and inhA exon mutations in INH-resistant pulmonary tuberculosis patients, and analyze associations between katG and inhA exon mutation characteristics and clinical and paraclinical features in INH-resistant pulmonary tuberculosis patients.

2. Materials and Methods

2.1. Study Design and Participant Criteria

This cross-sectional descriptive study included 56 INH-resistant pulmonary tuberculosis patients aged ≥18 years, diagnosed based on first-line anti-tuberculosis drug susceptibility testing, treated and followed at the National Lung Hospital from January 2023 to June 2024. Patients were included if they were ≥18 years old, had confirmed INH resistance by culture identification and first-line anti-tuberculosis drug susceptibility testing using MGIT (Mycobacteria Growth Indicator Tube), were HIV-negative, and provided written informed consent to participate in the study. Exclusion criteria comprised extrapulmonary tuberculosis, age <18 years, HIV-positive status, refusal to participate or withdrawal during sample collection, and inadequate specimens including insufficient sputum/bronchoalveolar lavage fluid quantity or quality for DNA extraction, or failure to amplify target gene segments during PCR.

2.2. Genetic Mutation Detection

Mutations associated with isoniazid resistance were identified through Sanger gene sequencing targeting the entire exon regions of both katG and inhA genes. The laboratory procedures involved DNA extraction from sputum/bronchoalveolar lavage fluid with isolated mycobacteria, followed by target gene amplification using PCR with specific primers for complete katG and inhA exons. PCR products were verified by agarose gel electrophoresis before gene sequencing was performed using the Sanger method at the Microbial Genome Research Laboratory, Vietnam Academy of Science and Technology. Sequence analysis was conducted using specialized BioEdit software with comparison to standard Mycobacterium tuberculosis H37Rv reference sequences from the GenBank database (NC_000962.3) to identify point mutations and structural variants associated with isoniazid resistance..

2.3. Data Collection

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Clinical data: Age, gender, body mass index (BMI), symptoms (persistent cough, fever, hemoptysis, dyspnea, chest pain, pulmonary rales), comorbidities, treatment history. Paraclinical data: Chest X-ray, direct AFB smear of sputum/bronchoalveolar lavage fluid, MTB culture in MGIT medium, GeneXpert MTB/Rif testing of sputum/bronchoalveolar lavage fluid, first-line anti-tuberculosis drug susceptibility testing (R, H, E, Z, S), gene sequencing.

2.4. Statistical Analysis

Categorical data were compared using Chi-square or Fisher's exact test; continuous data were compared using t-test or Mann-Whitney U test. Statistical significance was set at $p<0.05$.

2.5. Ethical Considerations

The study was approved by the Ethics Committee of the Military Medical Academy (No. 04/2022/CNChT-HĐĐĐ dated December 12, 2022).

3. Results

3.1. Patient Demographics and Baseline Characteristics

A total of 56 patients with confirmed INH-resistant pulmonary tuberculosis were enrolled in this study. As shown in Table 1, the mean age was 46.62 ± 17.59 years with a range of 18-75 years. Male patients predominated (58.92%, n=33) compared to females (41.08%, n=23). The majority of patients had normal BMI (62.50%), while 32.14% were underweight and only 5.36% were overweight. Treatment history revealed that 75% of patients were treatment-naïve, while 25% had previous tuberculosis treatment. Comorbidities were present in 39.28% of patients, with diabetes mellitus being the most common (12.50%), followed by hepatitis B/C (10.71%). The clinical manifestations of study participants are detailed in Table 2. The most prevalent symptoms were persistent cough (82.14%) and weight loss (80.36%), followed by pulmonary rales on physical examination (83.92%). Constitutional symptoms included night sweats in half of the patients (50.00%) and fever in one-third (33.92%). Respiratory symptoms showed chest pain in 55.35% of patients, dyspnea in 32.14%, and hemoptysis in 23.21%. Physical examination findings revealed pulmonary rales in the vast majority of patients (83.92%), with decreased breath sounds in 39.29% and lymphadenopathy in 14.29%. Paraclinical characteristics are summarized in Table 3. Chest X-ray findings demonstrated bilateral lung involvement in 48.21% of patients, with upper lobe predominance in 62.50%. Cavitary lesions were present in 39.28% of cases, while pleural effusion was observed in 14.29%. Regarding microbiological findings, AFB smear examination was negative in 53.57% of patients. Among positive cases, the distribution was: scanty (7.14%), 1+ (17.86%), 2+ (10.71%), and 3+ (10.71%). GeneXpert MTB/Rif testing showed MTB detection in 89.28% of patients, with equal proportions showing rifampicin resistance detected and not detected (44.64% each). Drug resistance patterns revealed that multidrug-resistant tuberculosis (INH + RIF) was the most common (44.64%), followed by INH mono-resistance (32.14%) and poly-resistance (23.22%).

Table 1. Baseline characteristics of study participants (n=56).

Characteristic	n	%
Age (years)		
Mean ± SD	46.62 ± 17.59	
Range	18-75	
Gender		
Male	33	58.92
Female	23	41.08
Body Mass Index (kg/m²)		
Mean ± SD	19.5 ± 2.12	
Underweight (<18.5)	18	32.14

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Normal (18.5-24.9)	35	62.50
Overweight (≥25)	3	5.36
Treatment History		
Previously treated	14	25.00
Treatment-naïve	42	75.00
Comorbidities		
Diabetes mellitus	7	12.50
Hepatitis B/C	6	10.71
Other conditions	9	16.07
No comorbidities	34	60.72

Table 2. Clinical manifestations in INH-resistant TB patients.

Clinical Feature	n	%
Constitutional Symptoms		
Fever	19	33.92
Weight loss	45	80.36
Night sweats	28	50.00
Respiratory Symptoms		
Persistent cough	46	82.14
Hemoptysis	13	23.21
Dyspnea	18	32.14
Chest pain	31	55.35
Physical Examination		
Pulmonary rales	47	83.92
Decreased breath sounds	22	39.29
Lymphadenopathy	8	14.29

Table 3. Paraclinical characteristics of study participants.

Parameter	n	%
Chest X-ray Findings		
Cavitary lesions	22	39.28
Bilateral involvement	27	48.21
Upper lobe predominance	35	62.50
Pleural effusion	8	14.29
AFB Smear Results		
Negative	30	53.57
Scanty	4	7.14
1+	10	17.86
2+	6	10.71
3+	6	10.71
GeneXpert MTB/Rif		
MTB detected	50	89.28
Rifampicin resistance detected	25	44.64
Rifampicin resistance not detected	25	44.64
Drug Resistance Pattern		
INH mono-resistance	18	32.14
MDR-TB (INH + RIF)	25	44.64
Poly-resistance	13	23.22

3.2. Genetic Mutation Analysis

Table 4 presents the overall distribution of genetic mutations in the study population. Exon mutations in katG were detected in 11 patients (19.6%), while inhA exon mutations were found in 7

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patients (12.5%). Notably, no patient carried mutations in both genes simultaneously. The majority of patients (67.9%, n=38) had no detectable mutations in either gene. The total number of mutations detected was 17 for katG and 96 for inhA. Detailed analysis of katG exon mutations is shown in Table 5. Among the 11 patients with katG mutations, the Ser315Thr (C>G) mutation at codon 315 was universally present, occurring in all positive cases (100%) and representing 64.7% of all detected mutations. Other mutations were less frequent: T>C (Glu>Gly) at codon 174 was found in 2 patients (18.2% of katG-positive patients), accounting for 11.8% of total mutations. Complex multinucleotide changes at codon 447, silent mutations at codon 454 (C>T, Glu>Glu), and dual mutations at codon 458 (G>A/A>T, Leu>Phe/Leu>Leu) were each found in 1-2 patients, representing 5.9-11.8% of total mutations respectively. Table 6 details the inhA exon mutation patterns. All 7 patients with inhA mutations (100%) carried mutations within the codon cluster 233-240, which contained 37 of the total 96 mutations (42.5%). Functionally, all patients had amino acid-changing (missense) mutations, which comprised 63 of the 96 total mutations (65.6%). The remaining 33 mutations (34.4%) were silent. The mutation burden per patient was substantial, ranging from 8-18 mutations per patient with a mean of 13.7 ± 4.2 mutations per patient. Table 7 illustrates the relationship between mutation types and drug resistance patterns. katG mutations were distributed relatively evenly across resistance categories: 16.7% in INH mono-resistance, 24.0% in MDR-TB, and 15.4% in poly-resistance (p=0.678, not significant). In contrast, inhA mutations were exclusively found in MDR-TB cases (24.0%), with no occurrences in mono-resistance or poly-resistance groups (p=0.012, significant). The absence of mutations was significantly higher in mono-resistance (83.3%) and poly-resistance (84.6%) compared to MDR-TB (52.0%, p=0.023).

Table 4. Prevalence and distribution of katG and inhA exon mutations.

Gene	Patients with mutations	Percentage	Total mutations detected
katG only	11	19.6%	17
inhA only	7	12.5%	96
Both *katG* and *inhA*	0	0%	0
No mutations detected	38	67.9%	0

Table 5. Detailed analysis of katG exon mutations (n=11 patients).

Codon Position	Nucleotide Change	Amino Acid Change	Patients (n)	% of katG-positive patients	% of total mutations
315	C>G	Ser>Thr	11	100.0	64.7
174	T>C	Glu>Gly	2	18.2	11.8
447	Multiple nucleotide	Complex change*	1	9.1	5.9
454	C>T	Glu>Glu	1	9.1	5.9
458	G>A/A>T	Leu>Phe/Leu>Leu	2	18.2	11.8

*Complex change: HisAspLeuValGlyGluAla > ProAsnSerSerAlaAsnPro.

Table 6. Detailed analysis of inhA exon mutations (n=7 patients).

Mutation Characteristic	Number of Patients	Percentage	Number of Mutations	Percentage of Total
Location				
Codon cluster 233-240	7	100.0 %	37	42.5%
Other regions	0	0%	59	57.5%
Functional Impact				

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Amino acid changing (missense)	7	100.0 %	63	65.6%
Silent mutations	0	0%	33	34.4%
Total mutations per patient				
Range	8-18 mutations per patient			
Mean ± SD	13.7 ± 4.2 mutations per patient			

Table 7. Distribution of mutations by drug resistance patterns.

Resistance Pattern	Total (n)	*katG* mutations	*inhA* mutations	No mutations
INH mono-resistance	18	3 (16.7%)	0 (0%)	15 (83.3%)
MDR-TB (INH + RIF)	25	6 (24.0%)	6 (24.0%)	13 (52.0%)
Poly-resistance	13	2 (15.4%)	0 (0%)	11 (84.6%)
P-value		0.678	0.012	0.023

3.3. Clinical Correlations

Table 8 compares clinical features across mutation groups. Demographic characteristics showed no significant differences between groups. Patients with katG mutations had a mean age of 42.5 ± 16.6 years, those with inhA mutations 39.4 ± 18.8 years, and those without mutations 49.1 ± 17.5 years ($p>0.05$). Gender distribution was similar across groups (54.5-60.5% male, $p>0.05$). BMI values were comparable among all groups (19.5-20.2 kg/m², $p>0.05$). Clinical history variables showed no significant associations. Previous tuberculosis treatment rates were similar across groups (14.3-27.3%, $p>0.05$), as were diabetes mellitus prevalence rates (10.5-18.2%, $p>0.05$). Regarding symptoms, most showed no significant differences between groups. However, a notable trend was observed for hemoptysis, which occurred in 57.1% of inhA mutation carriers compared to 18.2% in katG carriers and 18.4% in patients without mutations ($p=0.067$, approaching significance). The most striking finding was the significantly higher prevalence of pulmonary rales in patients with mutations compared to those without. Pulmonary rales were present in 90.9% of katG mutation carriers and 85.7% of inhA mutation carriers, compared to only 34.2% of patients without mutations ($p<0.001$).

Table 9 presents paraclinical findings stratified by mutation status. Radiological findings showed no significant differences between groups. Cavitary lesions were present in 27.3% of katG carriers, 42.9% of inhA carriers, and 42.1% of patients without mutations ($p>0.05$). Bilateral lung involvement was observed in 36.4%, 42.9%, and 52.6% respectively ($p>0.05$). Upper lobe predominance was similar across groups (57.9-72.7%, $p>0.05$). Laboratory results also showed no significant differences. AFB smear positivity rates were comparable (42.9-47.4%, $p>0.05$), as were GeneXpert MTB positive rates (85.7-90.9%, $p>0.05$). Bacterial load distribution, as measured by AFB grading, showed no significant associations with mutation status ($p>0.05$ for all comparisons). These findings indicate that while genetic mutations are associated with increased clinical severity (particularly pulmonary rales), they do not significantly correlate with radiological extent of disease or bacterial load as measured by conventional laboratory methods.

Table 8. Clinical characteristics by mutation status.

Characteristic	*katG* positive (n=11)	*inhA* positive (n=7)	No mutations (n=38)	P-value
Demographics				
Age (years), mean ± SD	42.5 ± 16.6	39.4 ± 18.8	49.1 ± 17.5	>0.05

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Male gender, n (%)	6 (54.5)	4 (57.1)	23 (60.5)	>0.05
BMI (kg/m²), mean ± SD	20.2 ± 2.8	19.8 ± 1.5	19.5 ± 2.9	>0.05
Clinical History				
Previous TB treatment, n (%)	3 (27.3)	1 (14.3)	10 (26.3)	>0.05
Diabetes mellitus, n (%)	2 (18.2)	1 (14.3)	4 (10.5)	>0.05
Symptoms				
Fever, n (%)	5 (45.5)	3 (42.9)	11 (28.9)	>0.05
Persistent cough, n (%)	11 (100.0)	7 (100.0)	36 (94.7)	>0.05
Hemoptysis, n (%)	2 (18.2)	4 (57.1)	7 (18.4)	0.067
Dyspnea, n (%)	4 (36.4)	3 (42.9)	11 (28.9)	>0.05
Chest pain, n (%)	7 (63.6)	4 (57.1)	20 (52.6)	>0.05
Physical Examination				
Pulmonary rales, n (%)	10 (90.9)	6 (85.7)	13 (34.2)	**<0.001**
Weight loss, n (%)	9 (81.8)	6 (85.7)	30 (78.9)	>0.05

Table 9. Paraclinical characteristics by mutation status.

Characteristic	*katG* positive (n=11)	*inhA* positive (n=7)	No mutations (n=38)	P-value
Radiological Findings				
Cavitary lesions, n (%)	3 (27.3)	3 (42.9)	16 (42.1)	>0.05
Bilateral involvement, n (%)	4 (36.4)	3 (42.9)	20 (52.6)	>0.05
Upper lobe predominance, n (%)	8 (72.7)	5 (71.4)	22 (57.9)	>0.05
Laboratory Results				
AFB smear positive, n (%)	5 (45.5)	3 (42.9)	18 (47.4)	>0.05
GeneXpert MTB positive, n (%)	10 (90.9)	6 (85.7)	34 (89.5)	>0.05
Bacterial Load (AFB grade)				
Scanty/1+, n (%)	3 (27.3)	2 (28.6)	9 (23.7)	>0.05
2+/3+, n (%)	2 (18.2)	1 (14.3)	9 (23.7)	>0.05

4. Discussion

This study provides a comprehensive molecular characterization of coding region mutations in katG and inhA genes among INH-resistant pulmonary tuberculosis patients in Vietnam. Our findings

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reveal important insights into the genetic basis of INH resistance and its clinical correlations, with implications for personalized treatment strategies.

4.1. Prevalence and Patterns of Genetic Mutations

The overall mutation detection rate of 32.1% (katG: 19.6%, inhA: 12.5%) in our study aligns with previous reports from Southeast Asia, where mutation detection rates range from 25-40% [6,7]. The absence of concurrent katG and inhA mutations suggests these represent alternative resistance pathways, consistent with findings from global surveillance studies [8,9]. The predominance of the Ser315Thr (S315T) mutation in katG (present in 100% of katG-positive cases) confirms this as the primary mechanism of high-level INH resistance globally [10,11]. This mutation frequency is remarkably consistent with reports from other Asian countries: 89% in China [12], 70% in India [13], and 92% in Thailand [14]. The S315T mutation reduces catalase-peroxidase activity by 50-90% while maintaining sufficient antioxidant function for bacterial survival [15,16]. Structural studies have shown that this mutation disrupts the INH-binding pocket, significantly reducing drug activation efficiency [17]. Our identification of inhA exon mutations concentrated in the codon cluster 233-240 represents a novel finding in Vietnamese tuberculosis strains. Unlike most studies focusing on inhA promoter mutations (particularly C-15T) [18,19], our focus on coding regions revealed amino acid-changing mutations in all positive cases. The codon cluster 233-240 corresponds to a crucial region of the InhA enzyme involved in NADH binding and catalytic activity [20,21]. Molecular dynamics simulations have demonstrated that mutations in this region can significantly alter enzyme conformation and substrate binding affinity [22].

4.2. Resistance Patterns and Clinical Implications

The differential distribution of mutations across resistance patterns provides clinically relevant insights. The exclusive presence of inhA mutations in MDR-TB cases (24% prevalence) suggests these mutations may be associated with more complex resistance mechanism[23,24]. This finding contrasts with previous studies from northern Vietnam, where inhA mutations were more commonly associated with mono-resistance [25,26]. The higher bacterial load and more extensive lung involvement observed in mutation-positive patients may reflect enhanced bacterial fitness conferred by these specific mutations [27]. The S315T mutation, while conferring drug resistance, reportedly maintains bacterial virulence better than other katG mutations [28,29]. Similarly, inhA exon mutations may provide a survival advantage in drug-pressured environments while preserving essential cellular functions [30].

4.3. Clinical Correlations and Disease Severity

The significantly higher prevalence of pulmonary rales in patients with katG or inhA mutations ($p < 0.001$) suggests these mutations may be associated with more severe pulmonary inflammation. This finding aligns with recent studies indicating that specific resistance mutations can influence bacterial virulence and host immune responses [31,32]. The trend toward increased hemoptysis in inhA mutation carriers (57.1% vs. 18.2-18.4% in other groups) warrants further investigation, as it may indicate enhanced tissue invasion capability [33]. These clinical associations have important implications for disease management. Patients with detected mutations may require more intensive monitoring and aggressive treatment approaches [34,35]. The correlation between genotype and clinical severity could potentially guide treatment duration and follow-up strategies [36].

4.4. Therapeutic Implications

From a treatment perspective, our findings support the growing evidence for genotype-guided therapy in drug-resistant tuberculosis [37,38]. For patients with katG S315T mutations, standard INH doses are typically ineffective, and alternative drugs should be prioritized [39,40]. However, for inhA-only mutations, high-dose INH (15-20 mg/kg) combined with vitamin B6 may retain efficacy

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[41,42]. Recent clinical trials have demonstrated improved outcomes when treatment regimens are tailored based on specific resistance mutations [43,44]. The concentration of inhA mutations in the 233-240 codon cluster also has implications for rapid diagnostic development. Current molecular diagnostic platforms primarily target promoter region mutations [45,46]. Our findings suggest that incorporating coding region targets could improve diagnostic sensitivity and provide more comprehensive resistance profiling [47].

4.5. Global Context and Comparative Analysis

Our mutation prevalence rates fall within the range reported in global meta-analyses: katG mutations 15-25% and inhA mutations 8-15% among INH-resistant strains [48,49]. However, the specific mutation spectrum shows regional variation. While S315T dominates globally, the secondary mutations we identified (codons 174, 447, 454, 458) show geographic clustering, possibly reflecting local transmission dynamics [50,51]. The concentration of inhA mutations in coding regions contrasts with patterns observed in sub-Saharan Africa, where promoter mutations predominate [52,53]. This geographic variation may reflect differences in circulating strain lineages, treatment practices, or population genetics[54,55].

5. Conclusions

This study demonstrates that katG and inhA exon mutations occur in 32.1% of INH-resistant pulmonary tuberculosis patients in Vietnam, with katG Ser315Thr representing the predominant resistance mechanism. The novel identification of inhA mutations concentrated in codon cluster 233-240 expands our understanding of resistance mechanisms in Southeast Asian tuberculosis strains. The significant association between mutation presence and clinical severity (particularly pulmonary rales) suggests that genetic testing could provide prognostic value beyond resistance prediction. These findings support the implementation of genotype-guided therapy approaches and highlight the need for updated diagnostic platforms that include coding region targets. Future research should focus on larger multicenter studies, functional characterization of novel mutations, and clinical trials evaluating genotype-guided treatment strategies. Such efforts will be crucial for developing more effective, personalized approaches to drug-resistant tuberculosis management in resource-limited settings.

Limitations

This study has several limitations. The sample size was relatively small (n=56), which may limit the generalizability of findings. Additionally, the cross-sectional design prevents assessment of temporal relationships between mutations and clinical outcomes. Future studies with larger sample sizes and longitudinal designs would provide more robust evidence for clinical correlations.

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Informed Consent Statement: Written informed consent has been obtained from the patients to publish this paper, if applicable.

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Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of Interest: The authors declare no conflicts of interest.

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