

Mutations in *oxyR* and *oxyR-ahpC* Interogenic Region in Isoniazid-Resistant *Mycobacterium tuberculosis* Clinical Isolates from Chongqing, China

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Background and Aim: As a first line anti-tuberculosis drug, isoniazid (INH) has been extensively used for decades. Consequently, resistance of *Mycobacterium tuberculosis* (MTB) clinical isolates to INH is inevitably increasing and spreading, necessitating fast and accurate molecular diagnosis tools. Although many previous molecular epidemiological studies related with INH resistance included mutations in the *oxyR-ahpC* intergenic region, the role of mutations in the *oxyR-ahpC* intergenic region in INH resistance remains controversial. The present study aimed to investigate the frequency and distribution of mutations in the *ahpC* and the *oxyR-ahpC* intergenic region among INH-resistant *Mycobacterium tuberculosis* (MTB) clinical isolates in Chongqing, China.

Methods: The presence of mutations in *katG*, *ahpC* and the *oxyR-ahpC* intergenic region was analyzed in 490 MTB clinical isolates.

Results: None of the 73 INH-susceptible isolates (0%), 18 of 26 INH mono-resistant isolates (69.2%), 188 of 199 MDR (multidrug resistant) isolates (94.5%), and 176 of 192 pre-XDR/XDR (pre-extensively drug-resistant) isolates (91.7%) had mutations in *katG*. No mutations in *ahpC*, *oxyR* and the *oxyR-ahpC* intergenic region were identified in INH-susceptible and INH mono-resistant isolates. Mutations in the *oxyR-ahpC* intergenic region were rare (1.5% MDR, 1.04% pre-XDR), and only two mutations were identified (–58 G→A in MDR and –54 C→T in pre-XDR isolates). The former occurred in isolates with concurrent *katG* mutations, but the latter occurred in isolates without concurrent *katG* mutations. Notably, a novel Ala18Gly mutation in *oxyR* was identified in 3.52% of MDR and 6.77% of pre-XDR isolates.

Conclusion: Mutation in *katG* is the main cause of INH resistance in MTB clinical strains isolated from Chongqing. It is very unlikely that mutation in the *oxyR-ahpC* intergenic region alone could cause INH resistance in MTB clinical isolates. Even though mutation in the *oxyR-ahpC* intergenic region could compensate the fitness cost of *katG* mutation, our finding that it is uncommon in MTB clinical isolates from Chongqing suggests it is not the primary compensatory mechanism. Further investigation is necessary to probe the relationship between mutations in the coding region of *oxyR* and INH resistance.

Keywords: *Mycobacterium tuberculosis*, isoniazid, resistance, *katG*, *oxyR-ahpC*

Introduction

Tuberculosis (TB) remains one of the leading causes of death worldwide from a single infectious agent, and is caused by the *Mycobacterium tuberculosis* (MTB) bacillus.¹ The World Health Organization (WHO) aims to reduce mortality and incidence rates of TB by 95% and 90%, respectively, by 2035, compared with rates in 2015; however, the current TB rate of incidence remains concerning higher than the rate of TB elimination.² China was observed to have the third highest TB burden in the world (6.8%) in 2022, behind Indonesia (10%) and India (27%).² Additionally, TB is also the second leading cause of morbidity and mortality from class A and B infectious diseases in China as per The Infectious Disease Prevention and Control Acts of China.³ Despite recent advances in the management of TB, drug-resistant TB continues to be an onerous public health threat in China.² Moreover, the prevalence and molecular characteristics of multidrug

resistant TB (MDR-TB) are known to vary with geographical environment and bacterial lineage.⁴ Therefore, a thorough understanding of the genetic basis of drug resistance is necessary for the successful management of MTB-related disease.

Isoniazid (INH) is one of the key drugs for TB treatment due to its early bactericidal effect, and is used to treat both active tuberculosis (TB) and latent TB infection (LTBI) for several decades.⁵ Both multidrug resistance and resistance to INH without concomitant rifampin resistance are associated with poor response to first-line treatment.⁶ Resistance to INH is mainly driven by mutations in the *katG* gene.⁷ The *katG* gene encodes for a catalase-peroxidase enzyme in MTB necessary for INH activation into its active form to exert its lethal effect.⁸ It is well-testified fact that *katG* gene is most frequently getting mutated and hence promotes INH resistance by diminishing the normal activity of catalase-peroxidase enzyme.⁹

The *ahpC* gene encodes alkyl-hydroperoxide reductase also involved in cellular regulation of oxidative stress.^{10,11} The loss of *katG* function in resistant strains is thought to be compensated for by mutations in the *oxyR-ahpC* intergenic region, which contains the putative promoter of *ahpC*.¹² Mutations in the *oxyR-ahpC* gene region may lead to the upregulation of *ahpC* gene expression, enhancing the cell tolerance to isoniazid-induced oxidative stress, and thereby promoting the development of drug resistance.^{13,14} However, the role of *ahpC* in INH resistance is controversial. The mutation prevalence in the intergenic region of *oxyR-ahpC* has shown regional and phenotypic differences.^{15–17} Previous studies have shown that mutations in the *oxyR-ahpC* intergenic region may compensate for the loss of catalase-peroxidase activity caused by mutations in *katG*, and thus may protect MTB bacilli against the toxic effects of organic peroxides.^{15,17} However, one subsequent study observed that mutations in *oxyR-ahpC* were not frequently seen in INH-resistant isolates with *katG* mutations.¹⁸ Baker et al reported that mutations in the *oxyR-ahpC* region did not lead to INH resistance, as these mutations can be found in 23.7% of INH-resistant clinical isolates and in 7.5% of INH-sensitive isolates.¹⁶

Chongqing is a high-incidence area for MDR-TB in China. To further verify the role of mutation in the *oxyR-ahpC* intergenic region in INH resistance, 490 clinical isolates were collected from Chongqing area, the prevalence and distribution of mutations in *katG*, *ahpC* and the *oxyR-ahpC* intergenic region was characterized.

Materials and Methods

Clinical MTB Isolates

A total of 490 clinical sputum samples were collected from patients, principally from the Chongqing and surrounding areas, attending the Chongqing Public Health Medical Center between June 2020 and March 2022. Samples were decontaminated and subjected to bacterial culture for 6 weeks using the BACTEC MGIT 960 (Becton Dickinson, Sparks, MD, United States) culture system at Chongqing Public Health Medical Center (Chongqing, China).¹⁹ According to the latest WHO guidelines,² MDR-TB was defined as resistance to at least both isoniazid and rifampicin. Pre-extensively drug-resistant TB (pre-XDR-TB) was defined as MDR-TB with additional resistance to any fluoroquinolone, moxifloxacin, levofloxacin. Furthermore, extensively drug-resistant TB (XDR-TB) is defined as MDR-TB plus resistance to one of the fluoroquinolones and to one of the second-line injectable anti-TB drugs: amikacin, kanamycin. The positive culture samples were subjected to further drug susceptibility testing using proportion method on Lowenstein-Jensen (L-J) medium slants (Encode, Zhuhai, China). The DST panel included first-line drugs (isoniazid, rifampicin) and second-line drugs (moxifloxacin, levofloxacin, amikacin, capreomycin, prothionamide, *para*-aminosalicylic acid, etc). The DST was conducted using the proportional method on L-J medium for 4–8 weeks.²⁰ Isolates were then frozen in 25% glycerol at –80°C until use.

Mycobacterial Genomic DNA

Single colonies of isolates on L-J medium slants were inoculated into 15 mL centrifuge tubes and suspended in 3 mL of Middlebrook 7H9 liquid medium with 10% (v/v) OADC, and was cultured at 37°C for 3–4 weeks. Cultures were grown to early stationary phase and subsequently used for genomic DNA preparation. Briefly, the mycobacterial cell pellet was washed twice with deionized water, and re-suspended with deionized water containing 0.45% (v/v) Triton X-100 and 0.45% (v/v) Tween-80. The bacterial cells were then incubated at 95°C for 30 min. Subsequently, 0.5 mL of the suspension was centrifuged to collect the supernatant. The extracted supernatant containing MTB genomic DNA was stored at –20°C until use.

Gene Amplification and Sequencing Analysis

PCR was performed to amplify the fragments of the *katG* and *oxyR-ahpC* genes, as previously described.^{21,22} The *katG* fragment was amplified with the following primers: *katG*-F 5'-GGTCGACATTCGCGAGACGTT-3' and *katG*-R 5'-TTGTTCTCGCGACGCATCGTG-3'. The genomic DNA was used as a template for amplification. The *oxyR-ahpC* fragment was amplified with the following primers: *oxyR-ahpC*-F 5'-GCCTGGGTGTTTCGTCACCTGGT-3' and *oxyR-ahpC*-R 5'-CGCAACGTCGACTGGCTCATA-3'. PCR amplifications were performed using AceTaq Master Mix (Vazyme, P411, Nanjing, China). The reaction mixtures were subjected to 5 min at 95°C, followed by 30 cycles of 10s at 95°C, 30s at 56°C, 45s at 72°C, and were terminated by an additional 5 min at 72°C. Successful gene amplifications were confirmed by ultraviolet transillumination following electrophoresis on a 1% agarose gel stained with ethidium bromide in a 1XTAE buffer. Sequencing of PCR products was conducted at Tsingke Biotech (Chongqing, China). Gene polymorphisms were aligned with the *katG* and *oxyR-ahpC* genes of the H37Rv reference strain (GenBank ID: NC_000962.3) using SnapGene software (SnapGene software, www.snapgene.com, version 4.1).

Results

Demographic Characteristics of the Patients from Whom the MTB Clinical Isolates Were Collected

A total of 490 clinical isolates from different patients with TB were collected, which included 73 INH-susceptible isolates, 26 INH-mono-resistant isolates, 199 MDR isolates, 190 pre-XDR isolates, and two XDR isolates. Three hundred and forty-one isolates (69.6%) were sampled from male patients and 149 isolates (30.4%) were taken from female patients, resulting in a sex ratio of 2.29. The median patient age was 47 years (range 13–83) (Table 1).

Drug Sensitivity Tests (DSTs)

DSTs were conducted on 490 MTB clinical isolates, and we categorized 417 of the 490 isolates (85.10%) to have resistance against INH, including 26 of 417 (6.24%) mono-INH resistant, 199 of 417 (47.72%) MDR, 190 of 417 (45.56%) pre-XDR, and two of 417 (0.48%) XDR-MTB isolates. The remaining 73 (14.90%) of the 490 isolates were observed to consistently be susceptible to all the fourteen anti-tuberculosis drugs used in the test panel (Figure 1A).

Resistance to rifapentine, streptomycin, *para*-aminosalicylic acid, rifabutin, amikacin, ethambutol, capreomycin, prothionamide, clofazimine and clarithromycin was observed in 184 (92.5%), 164 (82.4%), 62 (31.2%), 24 (12.1%), 20 (10.1%), 10 (5.0%), 3 (1.5%), 1 (0.5%), 2 (1.0%), and 2 (1.0%) isolates, respectively, among the 199 MDR isolates (Figure 1B). Additionally, resistance to rifapentine, streptomycin, *para*-aminosalicylic acid, amikacin, ethambutol, rifabutin, prothionamide, clarithromycin, clofazimine, capreomycin, and linezolid was observed in 181 (94.3%), 161 (83.9%), 85 (44.3%), 38 (19.8%), 21 (10.9%), 16 (8.3%), 9 (4.7%), 4 (2.1%), 3 (1.6%), and 2 (1.0%) isolates, respectively, among the 190 pre-XDR and 2 XDR isolates (Figure 1B).

Table 1 Classification of 490 MTB Clinical Isolates Used in This Study

Drug Susceptibility Profile	Number of Isolates	Median Age (Range)	Male	Female
Pan-susceptible	73	50 (15–83)	49	24
Mono-INH	26	49(17–78)	18	8
MDR	199	45(13–79)	142	57
Pre-XDR	190	47(14–77)	131	59
XDR	2	44(25–63)	1	1

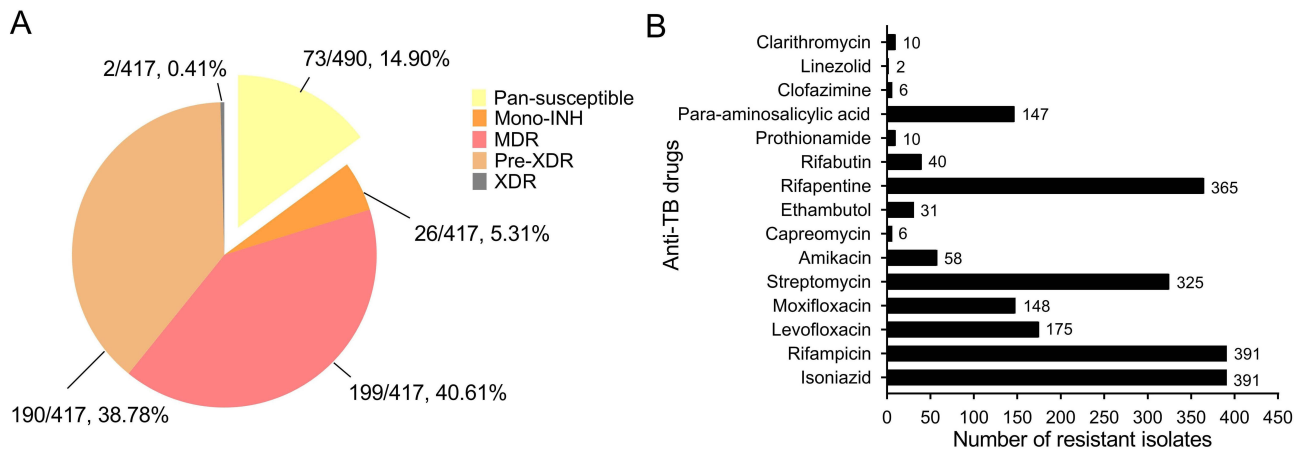


Figure 1 Drug resistance profile of 490 *Mycobacterium tuberculosis* clinical isolates. **(A)** Distribution of drug susceptibility profiles among the collected isolates. The pie chart shows the proportion of pan-susceptible, Mono-INH, MDR, pre-XDR, and XDR isolates. **(B)** Drug resistance patterns among MDR and pre-XDR/XDR isolates. The bar graph depicts the number of MDR (n=199) and pre-XDR/XDR (n=192) isolates that are resistant to each of the fourteen anti-tuberculosis drugs tested.

Mutations in *katG*, *oxyR* and the *oxyR-ahpC* Intergenic Region

Among the 73 pan-susceptible clinical isolates, 69 of 73 isolates (94.5%) had no mutations, but four (5.5%) harbored two synonymous mutations (Pro375Pro, Phe272Phe) and two non-synonymous mutations (Ile364Val, Asn508Ser) in the *katG* gene (Table 2). Among the 417 INH-resistant clinical isolates, 382 (91.6%) had a canonical mutation in *katG* at codon 315, of which S315T (G944C) substitution was the most common (379 of 382, 99.2%).

Among the 26 INH-mono-resistant isolates, 18 of 26 (69.23%) had a canonical mutation in *katG* (S315T) (Table 2). Three other *katG* mutations were identified in three INH-mono-resistant isolates, ie, G→T at 859 (Glu287STOP), G insertion at 1002, and G→T at 1390 (Arg465Leu).

Among the 199 MDR isolates, 190 (95.48%) possessed mutations in *katG* and *oxyR-ahpC* (Table 2). The most frequent change observed in *katG* was S315T (187 of 199, 93.97%). Four isolates were observed to have synonymous mutations in either *katG* or *oxyR*, ie, Pro501Pro (one isolate) and Gly428Gly (three isolates), and seven isolates contained a mutation (Ala18Gly) in *oxyR*. We also observed that four isolates contained double mutations, ie, Ser315Asn with Ile317Val in *katG* (one isolate), and S315T in *katG* with -58 G→A in *oxyR* (three isolates).

Table 2 Mutations in *katG*, *oxyR* and *oxyR-ahpC* Intergenic Region of the Clinical Isolates

Resistance Profile	Nucleotide Change(s) of <i>katG</i>	Amino Acid Change(s)	No (%). of Isolates	Other Mutations
Pan-susceptible	A→G at 1090	Ile364Val	1 (1.37)	
	G→T at 1125	Pro375Pro	1 (1.37)	
	A→G at 1523	Asn508Ser	1 (1.37)	
	C→T at 816	Phe272Phe	1 (1.37)	
	No	No	69 (94.5)	
INH-mono-resistant	G→C at 944	Ser315Thr	18 (69.23)	
	G→T at 859	Glu287STOP	1 (3.85)	
	G insertion at 1002		1 (3.85)	
	G→T at 1390	Arg465Leu	1 (3.85)	
	No	No	5 (19.23)	

(Continued)

Table 2 (Continued).

Resistance Profile	Nucleotide Change(s) of <i>katG</i>	Amino Acid Change(s)	No (%). of Isolates	Other Mutations
MDR	G→C at 944	Ser315Thr	170 (85.42)	
	G→A at 944	Ser315Asn	2 (1.01)	
	G→C at 944 C→A at 945	Ser315Thr	3 (1.50)	
	G→C at 944	Ser315Thr	6 (3.02)	Ala18→Gly of <i>oxyR</i>
	G→C at 944	Ser315Thr	3 (1.50)	G→A at -58 of <i>oxyR</i>
			1 (0.50)	Ala18→Gly of <i>oxyR</i>
	G→A at 944 A→G at 949	Ser315Asn Ile317Val	1 (0.50)	
	G→C at 944 A→G at 1503	Ser315Thr Pro501Pro	1 (0.50)	
	G→C at 944 G→A at 1284	Ser315Thr Gly428Gly	2 (1.01)	
	G→A at 947	Gly316Asp	1 (0.50)	
	G→A at 899 G→A at 1032	No	1 (0.50)	
	No	No	8 (4.02)	
Pre-XDR and XDR	G→C at 944	Ser315Thr	160 (83.33)	
	G→C at 944	Ser315Thr	13 (6.77)	Ala18→Gly of <i>oxyR</i>
	G→C at 944 G→A at 1284	Ser315Thr Gly428Gly	1 (0.52)	
	G→A at 944	Ser315Asn	1 (0.52)	
	G→T at 944	Ser315Ile	1 (0.52)	
	G→A at 947	Gly316Asp	1 (0.52)	
			2 (1.04)	C→T at -54 of <i>oxyR</i>
	No	No	13 (6.77)	

With respect to the 192 pre-XDR and XDR isolates, 179 of 192 (93.22%) possessed mutations in *katG* and *oxyR-ahpC* (Table 2). Among these 179 isolates, 176 contained the *katG* S315T mutation (176 of 192, 91.67%). Only one isolate contained a synonymous mutation (Gly428Gly) in *katG*, and 13 isolates had a mutation (Ala18Gly) in *oxyR*.

Discussion

In this study, a total of 490 clinical samples were collected from different patients afflicted with TB, with a male–female ratio of 2.29, which is a similar relative proportion of TB prevalence to that observed by the WHO globally, and also to that seen in the national survey of China.^{2,13}

From our sample of 417 INH^R clinical isolates, 382 (91.6%) could be explained by mutations in the 315 codon of *katG*, of which 379 (99.2%) contained the S315T transition. It has been previously shown that MTB strains possessing the S315T mutation are fully virulent in mice,²³ and this may be related to the increased capacity for transmission of the

S315T strain.²⁴ Consequently, the S315T mutation is more frequently observed in MDR-TB patients than in INH mono-resistant clinical isolates,²⁵ and is considered as a harbinger mutation that often precedes MDR.²⁶ Our results further support this hypothesis. A clear trend was observed in our study, where the proportion of *katG* 315 mutations in MDR (93.97%) and Pre-XDR/XDR (91.67%) isolates was significantly higher than that in INH-mono-resistant (69.23%) isolates. Cohen et al, also showed that the overwhelming majority of MDR-TB and XDR-TB organisms evolved resistance to isoniazid prior to resistance to rifampicin.²⁷ Additionally, analysis of phenotypic drug susceptibility testing by Izu et al, has revealed that isoniazid resistance is acquired before rifampicin resistance.²⁸ Collectively, the preceding observations suggest that some MDR-TB bacilli may have evolved from isoniazid-resistant strains. De novo emergence and reinfection by MDR-TB strains contribute equally to MDR development.²⁷

In the present study, a total of 6 mutation sites (in 10 strains, and other than at the 315 site) in the *katG* gene were observed in 417 INH-resistant isolates, five of which were also accompanied by mutation at the 315 site. We have thus shown that the *katG* 315 gene mutation remains a major contributor to the earlier evolution of isoniazid resistance.

The low frequency (1.5%, 3/199 and 1.04%, 2/192) of *oxyR-ahpC* intergenic mutations observed in our study, even among MDR and pre-XDR/XDR isolates, provides important epidemiological context. While these compensatory mutations are theoretically advantageous for strains bearing the fitness cost of *katG* mutations,²⁹ their low prevalence in our high MDR/XDR burden setting implies that they may not be the prerequisite for the evolution and success of complex drug-resistant strains. This also indicates that other, as yet uncharacterized, genetic or metabolic pathways might be more critical or common in compensating the *katG* defect in this population, allowing MDR/XDR strains to maintain transmissibility and virulence.

While our data firmly establish *katG* S315T (91.6%, 382/417) as the dominant mechanism of INH resistance in this population, the role of other genetic regions remains critical to a comprehensive understanding. Mutations in the *katG* gene usually confer a high level of INH resistance, which causes decreased INH substrate affinity at the expense of a reduction in overall catalase activity.⁷ Since the MTB *katG* enzyme (catalase peroxidase) is one of the major participants in the detoxification of reactive oxygen species (ROS) in MTB, *katG* mutations that confers INH resistance have been shown to influence bacterial pathogenesis and responses to antibiotic treatment.^{30–32} This implies that INH resistance-conferring *katG* mutations have fitness costs for MTB. Thus, it is reasonable to assume that INH-resistant MTB clinical isolates with *katG* mutations may have other compensatory mutations. Previous studies have shown that mutations in the *oxyR-ahpC* intergenic region may compensate for the loss of *katG* or catalase activity caused by *katG* mutations, which may result in over-expression of *ahpC*.²⁹ The *ahpC* gene encodes another important component of the ROS scavenger system in MTB,³³ and over-expression of the gene results in low-level INH resistance.¹¹ Additionally, mutations in the *oxyR-ahpC* intergenic region have been observed to contribute to 10–15% of INH resistance.^{34–36} However, the frequency of mutation in the *oxyR-ahpC* area was observed to be rather low (1.5% in MDR and 1.04% in pre-XDR/XDR) in the present study, and all the preceding mutations were identified in MDR isolates with the *katG* mutation. This finding is consistent with the controversial and likely secondary role of *oxyR-ahpC* region in INH resistance, supporting the view that these mutations may act as one potential compensatory mechanism for the loss of catalase activity induced by *katG* mutations in some strains. The complete absence of these mutations in INH-susceptible and INH mono-resistant isolates in our study further supports the model that they do not significantly contribute to isoniazid resistance by themselves, but may play a minor, compensatory role in strains already harboring primary resistance mutations such as *katG* S315T. However, over-expression of *ahpC* via mutations in *oxyR-ahpC* might not be the primary route that compensates for the deficiency caused by the *katG* mutation, suggesting that the bacterium may compensate for the deficiency caused by the *katG* mutation via other mechanisms, and this merits further investigation. Beyond confirming the established role of *katG* and the limited contribution of the *oxyR-ahpC* intergenic region, our study brings to light a previously underreported genetic change. A considerable proportion of INH-resistant isolates (3.52% in MDR isolates and 6.77% in pre-XDR isolates) with the *katG* mutation were observed to have a novel mutation in the *oxyR* gene (Ala18Gly of *oxyR*), which, to our knowledge, has not been previously observed in the contemporary literature, it is possible that this specific mutation may also compensate for the loss of catalase activity caused by *katG* mutations. Although the functional consequence of this specific amino acid change is unknown, its location in the *oxyR* gene, which encodes a redox-sensitive transcription regulator, suggests it could potentially alter the expression of

oxidative stress response genes including *ahpC*. This hypothesis positions the *oxyR* Ala18Gly mutation as a candidate compensatory mutation, warranting dedicated functional validation in future studies.

A key limitation of this study is the lack of paired phenotypic data (such as catalase activity assays or in vitro growth kinetics) so that we could perform direct functional comparisons between strains carrying only *katG* mutations and strains carrying both *katG* and *oxyR/oxyR-ahpC* mutations. These experiments will be critical to determine the compensatory effects of these mutations. Furthermore, a retrospective analysis of DST data showed that no specific resistance patterns were found among isolates carrying both *katG* and *oxyR-ahpC* mutations compared with isolates carrying only *katG* mutations ([Supplementary Tables S1](#) and [S2](#)). This observation suggests that potential compensatory effects of the *oxyR-ahpC* mutation may not translate directly into broad changes in the phenotypic resistance spectrum detected by standard DST, but may instead fine-tune bacterial fitness. Future studies are needed to combine these phenotypic assays to elucidate the precise biological costs and compensatory mechanisms involved.

In our study, although the incidence of *katG* mutations was higher, the *oxyR-ahpC* intergenic mutation rate was significantly lower (1.5% for MDR and 1.04% for pre-XDR/XDR), indicating that *ahpC* upregulation through this promoter region is not a major compensatory pathway in the clinical MTB strains prevalent in Chongqing. This observation raises the question of how these adapted drug-resistant strains overcome the vulnerability to oxidative stress caused by *katG* deficiency. It is possible that other, more potent genetic or metabolic adaptations are at work. These may include mutations in other regulators of the oxidative stress response, enhanced activity of additional detoxifying enzymes such as superoxide dismutase.^{17,37} Identifying these alternative mechanisms represents an important goal for future studies of the evolution of drug-resistant tuberculosis.

Conclusion

The present study supports the utility of the *katG* 315 mutation as a predictive molecular marker for INH resistance. Our findings support the primacy of *katG* S315T in molecular diagnosis in Chongqing and suggest that routine screening of the *oxyR-ahpC* intergenic region may have limited added value due to its low prevalence. Mutations in the *oxyR-ahpC* intergenic region exist selectively and in low frequency in INH-resistant isolates with *katG* mutations, thus supporting their putative role in compensating for the catalase deficiency caused by *katG* mutations. However, mutation in the *oxyR-ahpC* intergenic region seems unlikely to be the principal route which compensates for the catalase deficiency caused by *katG* mutations in MTB clinical isolates. Furthermore, one novel mutation *oxyR* Ala18Gly identified in this study warrants further investigation to elucidate its potential compensatory function. Future research directions include: 1) verifying the compensatory function by testing the in vitro antioxidant capacity of the *oxyR* Ala18Gly mutant; 2) conducting transcriptomic studies in strains carrying mutations in the *oxyR-ahpC* intergenic region to explore the expression of a wider range of oxidative stress regulators; 3) comprehensive genome sequencing to identify other unknown compensatory mutations. The outcomes of the present study contribute substantially to our knowledge with respect to the spectrum of gene mutations that may participate in INH resistance, and provide tantalizing clues for the elucidation of the drug-resistance mechanisms that are employed by MTB.

Ethics Approval and Consent to Participate

This study used archived strains isolated from clinical samples, no human subjects and no identifiable human data were included. The authors obtained approval for this study and exemption from the requirement for written informed patient consent from the Ethics Committee of Chongqing Public Health Medical Center (Permit Number: 2022-093-01-KY). During the study, patient data confidentiality and compliance with the Declaration of Helsinki were followed.

Funding

This work was supported by the Chongqing medical scientific research project (Joint project of Chongqing Health Commission and Science and Technology Bureau) (2024MSXM120, 2024QNXM020, 2025QNXM052).

Disclosure

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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