Jundishapur J Microbiol. 2019 November; 12(11):e95713. doi: 10.5812/jjm.95713.

Published online 2020 January 15. **Research Article**

Genotypic Characterization of *katG*, *inhA*, and *ahpC* in Isoniazid-Resistant *Mycobacterium tuberculosis* Clinical Isolates in Shanghai, China

Xingwei Cao [1], Qing Zhan [2], Yinjuan Guo [3, 4], Jinghui Yang [3], Yin Liu [3], Baoshan Wan [3], Xiaocui Wu [3], Qiaoshi Zhong [1], Yaping Hang [1], Yanhui Chen [1], Yanping Xiao [1], Fangyou Yu [3, 4] and Longhua Hu [1, *]

[1] Jiangxi Provincial Key Laboratory of Medicine, Clinical Laboratory of the Second Affiliated Hospital of Nanchang University, Nanchang, China
[2] Jiangxi Provincial Key Laboratory of Preventive Medicine, Nanchang University, Nanchang, China
[3] Department of Laboratory Medicine, Shanghai Pulmonary Hospital, School of Medicine, Tongji University, Shanghai China
[4] Shanghai Key Laboratory of Tuberculosis, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China

[*] *Corresponding author*: Jiangxi Provincial Key Laboratory of Medicine, Clinical Laboratory of the Second Affiliated Hospital, Nanchang University, Nanchang, China. Email: longhuahu@163.com

Received 2019 June 25; **Revised** 2019 December 26; **Accepted** 2019 December 29.

Abstract

Background: *Mycobacterium tuberculosis* is a pathogen that causes Tuberculosis and can invade various organs in infected patients. Its high morbidity and high mortality seriously threaten human health. In recent years, the continuous emergence of drug-resistant Tuberculosis bacteria has brought severe challenges to the prevention and control of Tuberculosis.
Objectives: This study aimed to characterize the most frequent mutations of the *katG*, *inhA*, and *ahpC* genes in isoniazid (INH)-resistant *M. tuberculosis* clinical isolates in Shanghai Pulmonary Hospital, China, and investigate the relationship between gene mutations and the minimum inhibitory concentrations (MICs) of INH against *M. tuberculosis*.
Methods: We collected 92 INH-resistant and 30 INH-susceptible clinical isolates of *M. tuberculosis*. The drug resistance profiles of *M. tuberculosis* clinical isolates against common anti-tuberculosis drugs were determined and sequencing analysis was performed.
Results: Of 92 INH-resistant strains, mutations in the *katG* and *inhA* genes were observed in 64 (69.6%) isolates and five (5.4%) isolates, respectively, and only had one (1.1%) strain both *katG* and *inhA* mutations. Among them, 62 (67.4%) strains carried a single mutation at codon 315 of the *katG* gene and a new mutation site was found in the *katG* gene of two strains. We detected a single mutation site at codon 271 and three simultaneous mutation sites at codons 315, 431, and 439. Only one (3.3%) of the 30 isoniazid-sensitive strains had the *katG* mutation. The *AhpC* mutation was detected in no experimental strains. The *KatG* Ser 315 Thr (AGC315ACC) mutation occurred in 53 (68.8%) out of 77 strains with high MICs ($\geq$ 1 g/mL) of isoniazid-resistant *M. tuberculosis* while five (33.3%) out of 15 strains with low MICs (less than 1 g/mL) had *katG* Ser 315 Thr (AGC315ACC) mutation.
Conclusions: Isoniazid-resistant strains were dominated by Ser315 $\rightarrow$ Thr (AGC $\rightarrow$ ACC) substitution, which seems to be associated with multidrug resistance and high-level resistance to INH. Multisite mutations are related to multidrug-resistant *M. tuberculosis* and the discovery of new mutation sites provides a new basis for the detection of drug-resistant *M. tuberculosis*.

Keywords: *Mycobacterium tuberculosis*, INH-Resistant, *katG*, *inhA*, *ahpC*, Mutation, MICs

1. Background

Mycobacterium tuberculosis is a pathogen that causes tuberculosis. It can invade all organs of infected patients, but lung infection is most common. Tuberculosis is a chronic respiratory infection. Before the application of anti-tuberculosis drugs, tuberculosis was known as the "white plague" and its high morbidity and high mortality seriously threatened human health. The application of anti-tuberculosis drugs has effectively controlled tuberculosis and has been promising in curing tuberculosis. The emergence of Drug-resistant (DR) and Multidrug-resistant (MDR) strains has led to complications and difficulty in the treatment of tuberculosis (1). Early detection of these strains before the initiation of treatment is important for implementing an effective treatment regimen to prevent the spread of these strains, thus avoiding the further spread of DR tuberculosis. China is one of the 14 countries in the list of three high-burden countries for tuberculosis, tuberculosis/HIV, and MDR tuberculosis as identified by the World Health Organization (WHO) during 2016 - 2020 (2).

Multidrug-resistant tuberculosis is defined as resistance to at least two first-line drugs rifampicin (RIF) and isoniazid (INH). *Mycobacterium tuberculosis* resistance is mainly caused by drug activating enzymes or drug target mutations (3). Until today, INH is still the cornerstone of modern tuberculosis chemotherapy. However, *M. tuberculosis* is becoming more resistant to INH (4). A variety of genes are involved in *M. tuberculosis* resistance to INH. It is reported that frequent mutations primarily focus on the *katG* and *inhA* regulatory regions while very few mutations occur in the *ahpC* gene (5). *KatG* has a length of 2223 bp and synthesizes a catalase-peroxidase enzyme, which is involved in the synthesis of mycolic acid (6) while *inhA* is involved in the synthesis of fatty acids (7).

Tuberculosis is a chronic disease with a long treatment cycle. Before anti-infective treatment, the sensitivity of antibacterial drugs should be clear, which is the key to successful treatment. Molecular biology is a common method to detect drug resistance genes of *M. tuberculosis*. To understand the drug resistance characteristics of *M. tuberculosis* in Shanghai and its main drug resistance mechanism, this study used the clinical isolates of *M. tuberculosis* in Shanghai as experimental strains.

2. Objectives

The study determined the frequency of mutations in three specific resistance genes (*katG*, *inhA*, and *ahpC*) in *M. tuberculosis* strains and assessed the correlation between the minimum inhibitory concentration (MIC) of antibacterial drugs and different mutation sites.

3. Methods

3.1. Clinical Isolates

We collected 92 INH-resistant and 30 INH-susceptible clinical isolates of *M. tuberculosis* from the library of strains in Shanghai Pulmonary Hospital. All 122 strains of *M. tuberculosis* were isolated from patients with pulmonary tuberculosis during the period between January and June 2018. Each strain corresponded to a single patient. We identified all strains as *M. tuberculosis* using the mycobacteria growth indicator tube (MGIT) liquid culture method (BD Biosciences, New Jersey, USA) and MPT-64 antigen detection. The 16SrRNA sequence was then amplified to confirm the previous identification results. All strains were cultured on Lowenstein-Jensen (LJ) and MGIT liquid media for subsequent experiments.

3.2. Phenotypic Drug Susceptibility Testing

Cultures on the LJ medium were collected and tested for drug susceptibility to first-line drugs, RIF, ethambutol (EMB), and INH, as well as second-line drugs, ofloxacin (LFX), moxifloxacin (MOX), amikacin (AMK), kanamycin (KAN), ethionamide (ETH), and cycloserine (CYC). Drug susceptibility testing was performed using the broth microdilution method and Roch broth containing OADC (oleic albumin dextrose catalase) supplement (Thermo, USA). The critical concentrations of these drugs were as follows: 1 μg/mL for RIF, 5.0 μg/mL for EMB, 0.2 μg/mL for INH, 2.0 μg/mL for OFX, 0.5 μg/mL for MXF, 4.0 μg/mL for AMK, 5.0 μg/mL for KAN, 5.0 μg/mL for ETH, and 25.0 μg/mL for CYC. If MICs of INH were > 0.2 μg/mL, the strain was considered resistant according to the WHO recommendations. Pan-susceptible *M. tuberculosis* strain H37Rv (ATCC27294) was used as a reference.

3.3. Mycobacterium tuberculosis DNA Isolation

The frozen isolates were subcultured on the LJ medium for four weeks. The crushed bacterial colonies were recovered in 200 μL of 1 $\times$ TE (Tris-EDTA) buffer and boiled at 100°C for 10 minutes to inactivate bacteria and release the mycobacterial DNA. The recovered DNA was immediately used for PCR amplification stored at -20°C until use. The *M. tuberculosis* reference strain H37Rv was used as the control.

3.4. PCR Amplification and Sequencing of katG, inhA, and ahpC

The amplification of drug-resistant genes included an amplicon of 820 bp for the *katG* gene amplified using the forward primer (5′CGGCGATGAGCGTTACAGC3′) and reverse primer (5′TCGTTGACCTCCCACCCGACTTG3′). For the *inhA* gene, an amplicon of 650 bp was amplified using the forward primer (5′AGGTCGCCGGGGTGGTCAGC3′) and reverse primer (5′ATGTTGATCAGGGTCTGC3′). For the *ahpC* gene, an amplicon of 730 bp was amplified using the forward primer (5'GCAACCAAATGCATTGTCCG3') and reverse primer (5'GAGCTTTTCTATACTCATTG3').

The PCR mixture was prepared in a volume of 50 μL as follows: 25 μL 2 $\times$ PCR Mixture (Sangon, Shanghai, China), 2 μL of DNA template, and 2 μL of DNA template. The reaction was carried out in a thermal cycler, as follows: 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, primer annealing (*katG* at 61°C, *inhA* at 63°C, and *ahpC* at 53°C) for 30 seconds, extension at 72°C for one minute, and final elongation at 72°C for 10 minutes. The PCR products were separated by 1% agarose gel electrophoresis. A positive product of the target gene fragment was sent to the company (Tsingke, China) for sequencing. Resulting sequences were compared with wild-type sequences of *M. tuberculosis* H37Rv using Bio-Edit software (version 7.0.9).

3.5. Statistical Analysis

All statistical analyses were performed using SPSS (V 20.0; SPSS Inc.). Data were compared by the χ^2 test. For all analyses, a P value of < 0.05 was considered statistically significant.

4. Results

4.1. Drug Resistance Profiles of Isoniazid-Resistant Mycobacterium tuberculosis Clinical Isolates

Based on drug susceptibility testing results, 73 (79.3%) isolates were resistant to RIF and 30 (32.6%) were resistant to EMB. There were 73 (79.3%) MDR isolates and 19 (20.7%) non-MDR INH-resistant isolates. The drug susceptibility testing results of 92 INH-resistant isolates against six second-line anti-tuberculosis drugs showed that 39 (42.4%) isolates were resistant to LFX and MOX, 17 (18.5%) isolates to AMK, 19 (20.7%) isolates to KAN, 12 (13.0%) isolates to ETH and eight (8.7%) isolates were resistant to CYC (Table 1). Among 92 INH-resistant clinical isolates, 15 strains were found to have MICs of < 1 μg/mL and 77 had MICs of ≥ 1 μg/mL (Table 2).

Table 1. Drug Resistance Patterns of 92 Isoniazid-resistant *Mycobacterium tuberculosis* Clinical Isolates from Shanghai Pulmonary Hospital in China

Drug	No. (%)
RIF	73 (79.3)
EMB	30 (32.6)
LFX	39 (42.4)
MOX	39 (42.4)
AMK	17 (18.5)
KAN	19 (20.7)
ETH	12 (13.0)
CYC	8 (8.7)
Total	92
MDR	73 (79.3)
non-MDR INH-resistant	19 (20.7)

Abbreviations: AMK, amikacin; CYC, ethionamide; EMB, ethambutol; ETH, ethionamide; KAN, kanamycin; MDR: rifampicin and isoniazid-resistant; MOX, moxifloxacin; non-MDR INH-resistant: non-multidrug resistant but INH-resistant; OFX, levofloxacin; RIF, rifampicin.

4.2. Mutations in the katG, inhA, and ahpC Genes

Out of 92 INH-resistant clinical isolates, 64 (69.6%) isolates had an amino acid substitution in *katG*, with a vast majority carrying Ser to Thr (AGC $\rightarrow$ ACC) substitution at codon 315. The mutation at codon 315 prevailed in the *katG* gene with three mutations, including Ser315 $\rightarrow$ Thr (AGC $\rightarrow$ ACC) (63.0%), Ser315 $\rightarrow$ Asn (AGC $\rightarrow$ ACC) (2.2%), and Ser315 $\rightarrow$ Thr (AGC $\rightarrow$ ACA) (2.2%). Among these isolates, one (1.1%) isolate showed simultaneous mutations at codons 315, 431, and 439 (1.1%). Furthermore, a single mutation at codon 271 was observed in one (1.1%) isolate. Mutations in the *inhA* regulatory region were observed in five (5.4%) INH-resistant clinical isolates and a single mutation at codons 3, 21, and 94 was observed in one (1.1%) isolate, three (3.3%) isolates, and one (1.1%) isolate, respectively (Table 2).

No mutation was observed in the *ahpC* gene. Only had one (1.1%) isolate mutations in both *katG* and *inhA*. Of 30 INH-susceptible clinical isolates, only had one (3.3%) isolate an amino acid substitution Ser315 $\rightarrow$ Thr (AGC $\rightarrow$ ACC) in *katG* ($P < 0.05$) (Table 3). However, 30 INH-susceptible isolates showed no mutation in the *inhA* and *ahpC* genes. Out of 77 high-MICs (MIC ≥ 1 μg/mL) INH-resistant clinical isolates, 53 (68.8%) isolates were associated with Ser315 $\rightarrow$ Thr (AGC $\rightarrow$ ACC) substitution while only five (33.3%) isolates had this mutation out of 15 isolates with low MICs (MIC < 1 μg/mL) ($P < 0.05$) (Table 3). In addition, the frequency of Ser315 $\rightarrow$ Thr (AGC $\rightarrow$ ACC) substitution in the *katG* gene was more in MDR isolates (67.1%) than in non-MDR INH-resistant isolates (47.4%) ($P < 0.05$) (Table 3).

5. Discussion

Anti-tuberculosis drug resistance poses a major threat to human health. It is usually caused by a change in the drug target due to a mutation in the chromosomal gene of *M. tuberculosis*. Information on the genetic diversity of *M. tuberculosis* plays an important role in controlling tuberculosis (8). This can help us monitor the disease, determine the origin and spread of pathogens in the area, and effectively prevent and control the disease. *Mycobacterium tuberculosis* resistance to INH is associated with mutations in several genes. Isoniazid is known to cause the exclusive lethal action to *M. tuberculosis* cells because of the pathogen's own catalase-peroxidase (*katG*) enzyme that converts INH to a very reactive radical. Isoniazid is a prodrug and its activation in a cell is performed by catalase-peroxidase, coded by the *katG* gene in *M. tuberculosis* (9). Mutations in the *inhA* regulatory region are known to induce overexpression of *inhA* and promote INH resistance by increasing the number of target molecules (7, 10). Mutations in the *katG* and *inhA* genes are most clinically relevant and determine resistance in most clinical isolates (11, 12).

In our study, 64 (69.6%) isolates had an amino acid substitution in *katG*. The prevalence of mutations in *katG* varies strongly among different regions of the world. The mutation rate was 98% in Russia (13), 66.7% in Finland (14),

Table 2. Prevalence and Mutation Profile in the *katG*, *inhA* and *ahpC* Genes in Isoniazid-resistant *Mycobacterium tuberculosis* Clinical Isolates in Shanghai

Gene	Condon	Nucleotide Replacement	Amino Acid Replacement	No. (%)	MICs (μg/mL) (No.)
katG	No mutation			28 (30.4)	< 1 (10), ≥ 1 (18)
	315	AGC → ACC	Ser315 → Thr	58 (63.0)	< 1 (5), ≥ 1 (53)
		AGC → AAC	Ser315 → Asn	2 (2.2)	1 (1), > 4 (1)
		AGC → ACA	Ser315 → Thr	2 (2.2)	4 (1), 2 (1)
	271	ACT → GCT	Thr271 → Ala	1 (1.1)	> 4 (1)
	315 + 431 + 439	AGC → ACC + GTC → GCC + CAG → GAG	Ser315 → Thy + Val431 → Ala + Gln439 → Glt	1 (1.1)	> 4 (1)
inhA	No mutation			87 (94.6)	
	3	GGA → GGC	Synonymous mutation	1 (1.1)	0.5 (1)
	21	ATC → ACC	Ile → Thr	3 (3.3)	1 (1), 2 (2)
	94	TCG → GCG	Ser → Ala	1 (1.1)	0.5(1)
ahpC	No mutation			92	
Total				92	

Table 3. Comparison and Statistics of the Results of *katG* Gene Sequencing Among the Three Groups

Phenotypic DST	Ser315 → Thr (AGC → ACC) Substitution in *katG*		Total
	Mutation	No Mutation	
INH-susceptible	1	29	30
INH-resistant	58	34	92
Total	59	63	112
Non-MDR INH- resistant	9	10	19
MDR	49	24	73
Total	58	34	92
Lower MICs	5	10	15
High MICs	53	24	77
Total	58	34	92

60% in South Africa (15) and other areas of China (16), and 46% in Switzerland (17). Our results were similar to results from Finland, South Africa, and other areas of China, but lower than the result from Russia and higher than the result from Switzerland. The differences in clinical medication habits in different countries and limitations of selected experimental strains may be the main reasons for the inconsistent incidence of *katG* gene mutations. Of particular interest, a vast majority of isolates showed a Ser to Thr (AGC → ACC) substitution at codon 315 (63.0%). This is consistent with the results reported previously (18, 19). We also detected mutations at codons 315, 431, and 439 of *katG* in one isolate, meanwhile, the Thr271 → Ala (ACT → GCT) substitution was detected in another isolate.

These results have not been previously reported. We detected two strains with a high level of resistance to INH (MIC > 4 μg/mL). Therefore, their possible participation in the process of resistance to INH needs to be further explored. Mutations in the *inhA* regulatory region were observed in five (5.4%) INH-resistant isolates, which was lower than the result recorded in Kazakhstan (20). We only detected a single mutation at codon 3 in one (1.1%) isolate, codon 21 in three (3.3%) isolates, and codon 94 in one (1.1%) isolate and their amino acid substitutions were synonymous mutation (GGA → GGC), Ile21 → Thr (ATC → ACC), and Ser94 → Ala (TCG → GCG), respectively. It is worth noting that we did not detect the common frequent mutation (C-15T) in the *inhA* gene while this mutation was reported in phenotypically resistant isolates in many countries such as Myanmar, the Kyrgyz Republic, and Ecuador (18, 19, 21). It might be related to regional differences. In our study, no mutation was observed in the *ahpC* gene, which might be related to the sample size of this study that needs to be expanded for further studies. Only have one (1.1%) isolate mu-

tations in both *katG* and *inhA*. This double mutation also occurred with lower frequency in our study that in studies from the Kyrgyz Republic and Kazakhstan (19, 20).

We also observed a strong correlation between the MICs of INH and the mutation of Ser315 → Thr (AGC → ACC) in our isolates. Out of 77 high MIC (MIC ≥ 1 μg/mL) INH-resistant clinical isolates, 53 (68.8%) isolates were associated with Ser315 → Thr (AGC → ACC) substitution while only had five (33.3%) isolates this mutation out of 15 isolates with low MICs (MIC < 1 μg/mL). These findings are in agreement with previous studies (22). In addition, the frequency of Ser315 → Thr (AGC → ACC) substitution in the *katG* gene was more in MDR isolates (67.1%) than in non-MDR INH-resistant isolates (47.4%). Therefore, the most common mutation was Ser315 → Thr (AGC → ACC) (63.0%) in the isolates of our study, and most of them showed high-level resistance to INH (MIC ≥ 1 μg/mL). These results indicate the Ser315 → Thr (AGC → ACC) substitution was likely associated with MDR and high-level resistance to INH in our study.

5.1. Conclusions

In conclusion, our results further enhance our understanding of the molecular mechanisms involved in INH resistance. By detecting frequent mutation sites in the *katG* and *inhA* genes and discovering new mutation sites, we provided a basis for further study of the mechanism of INH resistance and rapid detection of DR-Tuberculosis.

Footnotes

Authors' Contribution: Xingwei Cao, Qing Zhan, Yinjuan Guo, Jinghui Yang, Yin Liu, Baoshan Wan, Xiaocui Wu, Qiaoshi Zhong, and Yanping Xiao designed the study, analyzed and interpreted the data for the work; Xingwei Cao, Fangyou Yu, and Longhua Hu drafted the work and revised it critically for important intellectual content. Jinghui Yang, Yin Liu, Baoshan Wan, Yanping Xiao, Yanhui Chen, and Xiaocui Wu participated in the experimental design and data analysis. Longhua Hu and Fangyou Yu agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All authors read and approved the final manuscript.

Conflict of Interests: The authors have no competing interests to declare.

Ethical Approval: The study was approved by the responsible Institutional Ethics Committee of the Shanghai Pulmonary Hospital Affiliated to School of Medicine, Tongji University.

Funding/Support: This study was supported by a grant from the Jiangxi Provincial Department of Science and Technology, China (20181BBG70030) and the National Natural Science Foundation of China (81672078).

References

1. Zenteno-Cuevas R, Cuevas-Cordoba B, Parissi-Crivelli A. rpoB, katG and inhA mutations in multi-drug resistant strains of Mycobacterium tuberculosis clinical isolates from southeast Mexico. *Enferm Infecc Microbiol Clin*. 2019;**37**(5):307–13. doi: 10.1016/j.eimc.2018.09.002. [PubMed: 30316618].
2. Lan Y, Li Y, Chen L, Zhang J, Zhang H. Drug resistance profiles and trends in drug-resistant tuberculosis at a major hospital in Guizhou province of China. *Infect Drug Resist*. 2019;**12**:211–9. doi: 10.2147/IDR.S188538. [PubMed: 30666136]. [PubMed Central: PMC6330984].
3. Liu J, Shi W, Zhang S, Hao X, Maslov DA, Shur KV, et al. Mutations in Efflux pump Rv1258c (Tap) cause resistance to pyrazinamide, isoniazid, and streptomycin in M. tuberculosis. *Front Microbiol*. 2019;**10**:216. doi: 10.3389/fmicb.2019.00216. [PubMed: 30837962]. [PubMed Central: PMC6389670].
4. Vilcheze C, Jacobs WR Jr. The isoniazid paradigm of killing, resistance, and persistence in Mycobacterium tuberculosis. *J Mol Biol*. 2019;**431**(18):3450–61. doi: 10.1016/j.jmb.2019.02.016. [PubMed: 30797860]. [PubMed Central: PMC6703971].
5. Seifert M, Catanzaro D, Catanzaro A, Rodwell TC. Genetic mutations associated with isoniazid resistance in Mycobacterium tuberculosis: A systematic review. *PLoS One*. 2015;**10**(3). e0119628. doi: 10.1371/journal.pone.0119628. [PubMed: 25799046]. [PubMed Central: PMC4370653].
6. Jagielski T, Grzeszczuk M, Kaminski M, Roeske K, Napiorkowska A, Stachowiak R, et al. Identification and analysis of mutations in the katG gene in multidrug-resistant Mycobacterium tuberculosis clinical isolates. *Pneumonol Alergol Pol*. 2013;**81**(4):298–307. [PubMed: 23744165].
7. Zhang Y, Yew WW. Mechanisms of drug resistance in Mycobacterium tuberculosis: Update 2015. *Int J Tuberc Lung Dis*. 2015;**19**(11):1276–89. doi: 10.5588/ijtld.15.0389. [PubMed: 26467578].
8. Papaventsis D, Casali N, Kontsevaya I, Drobniewski F, Cirillo DM, Nikolayevskyy V. Whole genome sequencing of Mycobacterium tuberculosis for detection of drug resistance: A systematic review. *Clin Microbiol Infect*. 2017;**23**(2):61–8. doi: 10.1016/j.cmi.2016.09.008. [PubMed: 27665704].
9. Reyes YIA, Janairo GC, Franco FC Jr. Theoretical insights on the binding of isoniazid to the active site residues of Mycobacterium tuberculosis catalase-peroxidase. *Tuberculosis (Edinb)*. 2019;**114**:61–8. doi: 10.1016/j.tube.2018.11.005. [PubMed: 30711159].
10. Xia Y, Zhou Y, Carter DS, McNeil MB, Choi W, Halladay J, et al. Discovery of a cofactor-independent inhibitor of Mycobacterium tuberculosis InhA. *Life Sci Alliance*. 2018;**1**(3). e201800025. doi: 10.26508/lsa.201800025. [PubMed: 30456352]. [PubMed Central: PMC6238539].
11. Kelley CL, Rouse DA, Morris SL. Analysis of ahpC gene mutations in isoniazid-resistant clinical isolates of Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 1997;**41**(9):2057–8. [PubMed: 9303417]. [PubMed Central: PMC164068].
12. Lee AS, Teo AS, Wong SY. Novel mutations in ndh in isoniazid-resistant Mycobacterium tuberculosis isolates. *Antimicrob Agents Chemother*. 2001;**45**(7):2157–9. doi: 10.1128/AAC.45.7.2157-2159.2001. [PubMed: 11408244]. [PubMed Central: PMC90621].
13. Mokrousov I, Narvskaya O, Otten T, Limeschenko E, Steklova L, Vyshnevskiy B. High prevalence of KatG Ser315Thr substitution among isoniazid-resistant Mycobacterium tuberculosis clinical isolates from northwestern Russia, 1996 to 2001. *Antimicrob Agents Chemother*. 2002;**46**(5):1417–24. doi: 10.1128/aac.46.5.1417-1424.2002. [PubMed: 11959577]. [PubMed Central: PMC127151].

14. Hamze M, Ismail MB, Rahmo AK, Dabboussi F. Pyrosequencing for rapid detection of tuberculosis resistance to Rifampicin and Isoniazid in Syrian and Lebanese clinical isolates. *Int J Mycobacteriol*. 2015;**4**(3):228–32. doi: 10.1016/j.ijmyco.2015.05.007. [PubMed: 27649870].
15. Haas WH, Schilke K, Brand J, Amthor B, Weyer K, Fourie PB, et al. Molecular analysis of katG gene mutations in strains of Mycobacterium tuberculosis complex from Africa. *Antimicrob Agents Chemother*. 1997;**41**(7):1601–3. [PubMed: 9210694]. [PubMed Central: PMC163968].
16. Zhang M, Yue J, Yang YP, Zhang HM, Lei JQ, Jin RL, et al. Detection of mutations associated with isoniazid resistance in Mycobacterium tuberculosis isolates from China. *J Clin Microbiol*. 2005;**43**(11):5477–82. doi: 10.1128/JCM.43.11.5477-5482.2005. [PubMed: 16272473]. [PubMed Central: PMC1287806].
17. Fenner L, Egger M, Bodmer T, Altpeter E, Zwahlen M, Jaton K, et al. Effect of mutation and genetic background on drug resistance in Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2012;**56**(6):3047–53. doi: 10.1128/AAC.06460-11. [PubMed: 22470121]. [PubMed Central: PMC3370767].
18. Aye KS, Nakajima C, Yamaguchi T, Win MM, Shwe MM, Win AA, et al. Genotypic characterization of multi-drug-resistant Mycobacterium tuberculosis isolates in Myanmar. *J Infect Chemother*. 2016;**22**(3):174–9. doi: 10.1016/j.jiac.2015.12.009. [PubMed: 26806152].
19. Isakova J, Sovkhozova N, Vinnikov D, Goncharova Z, Talaibekova E, Aldasheva N, et al. Mutations of rpoB, katG, inhA and ahp genes in rifampicin and isoniazid-resistant Mycobacterium tuberculosis in Kyrgyz Republic. *BMC Microbiol*. 2018;**18**(1):22. doi: 10.1186/s12866-018-1168-x. [PubMed: 29566660]. [PubMed Central: PMC5863813].
20. Kozhamkulov U, Akhmetova A, Rakhimova S, Belova E, Alenova A, Bismilda V, et al. Molecular characterization of rifampicin- and isoniazid-resistant Mycobacterium tuberculosis strains isolated in Kazakhstan. *Japan J Infect Dis*. 2011;**64**(3):253–5. [PubMed: 21617314].
21. Franco-Sotomayor G, Garzon-Chavez D, Leon-Benitez M, de Waard JH, Garcia-Bereguiain MA. A First insight into the katG and rpoB gene mutations of multidrug-resistant Mycobacterium tuberculosis strains from ecuador. *Microb Drug Resist*. 2019;**25**(4):524–7. doi: 10.1089/mdr.2018.0203. [PubMed: 30513056].
22. van Soolingen D, de Haas PE, van Doorn HR, Kuijper E, Rinder H, Borgdorff MW. Mutations at amino acid position 315 of the katG gene are associated with high-level resistance to isoniazid, other drug resistance, and successful transmission of Mycobacterium tuberculosis in the Netherlands. *J Infect Dis*. 2000;**182**(6):1788–90. doi: 10.1086/317598. [PubMed: 11069256].