

Analysis of The Correlation Between inhA Gene Mutation and Resistance to Prothionamide in Drug-Resistant Mycobacterium Tuberculosis

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Abstract: *Objective:* To investigate the characteristics of katG and inhA gene mutations in multidrug-resistant tuberculosis (MDR-TB), pre-extensively drug-resistant tuberculosis (preXDR-TB), and their correlation with resistance to prothionamide (Pto). *Methods:* A total of 229 patients with MDR-TB and pre-XDR-TB diagnosed in the Eighth Affiliated Hospital of Xinjiang Medical University from January 2020 to February 2024 were selected to analyze the characteristics of katG and inhA mutations in MTB clinical isolates and their correlation with Pto resistance. *Results:* The mutation rate of katG (with or without inhA mutation) was 85.2%. The mutation rates in MDR-TB and pre-XDR-TB were 87.4% (125/143) and 81.4% (70/86), respectively. The mutation rate of inhA (including katG mutation) was 14.8% (34/229), which was 12.6% (18/143) and 18.6% (16/86) in MDR-TB and pre-XDR-MTB, respectively. There was no difference in mutation ($P > 0.05$). *Conclusion:* The total resistance rate to Pto in 229 strains was 8.7% (20/229), which was 8.4% (12/143) and 9.3% (8/86) in MDR-TB and pre-XDR-TB, respectively. Among the inhA mutant strains, 13 were resistant to the Pto phenotype, and the resistance rate was 65% (13/20). In MDR-TB and pre-XDR-TB strains resistant to Pto, inhA gene mutations occurred in 66.7% (6/9) and 63.6% (7/11), respectively. The resistance rates of MDR-MTB and pre-XDR-TB strains without inhA gene mutation to Pto were 2.4% (3/125) and 5.7% (4/70), respectively.

Keywords: Tuberculosis; Anti-multiple drug resistance; Prothionamide; Gene; Mutations

Online publication: April 24, 2024

1. Introduction

According to the 2023 World Health Organization (WHO) global tuberculosis (TB) report, it is estimated that the number of new cases in China will be 748,000 in 2022, accounting for 7.1% of the global incidence, ranking third among the top 30 high-burden countries, of which multidrug-resistant TB (MDR-TB/RR-TB) patients are 30,000^[1]. In the 2022 WHO guidelines for the treatment of drug-resistant TB, it is recommended that MDR-TB/RR-TB patients receive a long-term regimen that contains at least 4 possible effective drugs^[2]. However,

due to the poor accuracy of drug resistance detection, the heavy burden of second-line drugs, and the obvious intolerance of adverse reactions in some patients, it is difficult to select effective and well-tolerated drugs and develop an effective treatment plan. It is particularly evident in patients with quasi-extensively drug-resistant TB and extensively drug-resistant TB. Isoniazid (INH) is one of the core drugs in treating TB. The drug resistance mechanism is mainly related to KatG and inhA gene mutations. Prothionamide (Pto), as an analog of INH, has the same mechanism of action as isoniazid to inhibit the synthesis of TB mycolic acid. Studies have shown that inhA gene mutation was one of the mechanisms of resistance to Pto and one of the mechanisms of cross-resistance between the two [3]. Liu *et al* showed that the cross-resistance rate of INH and Pto reached 20% [4]. In this study, MDR-TB and XDR-TB clinical isolates containing isoniazid resistance were selected for gene mutation detection, and the correlation between inhA gene mutation and Pto resistance was analyzed, to provide a reference for clinicians in the selection of drugs for drug-resistant TB.

2. Information and methods

2.1. Subjects

A total of 229 patients diagnosed in the Eighth Affiliated Hospital of Xinjiang Medical University from January 2020 to February 2024 were selected as subjects. The clinical isolates of the subjects were cultured and identified as *Mycobacterium tuberculosis* complex, with the results of drug sensitivity test of isoniazid, rifampicin (RFP), fluoroquinolones, and prothionamide, and the results of katG and inhA mutation detection.

2.2. Bacterial culture and drug sensitivity test

The specimens were processed according to the Tuberculosis Laboratory Test Procedures. The BACTEC MGIT 960 automatic rapid mycobacterial culture was used to culture positive colonies using a mycobacterial drug sensitivity test kit (purchased from Zhuhai Yinke Company) for drug sensitivity tests and bacterial type identification. The critical concentrations were: INH 0.2 ug/mL, RFP 0.25 ug/mL, levofloxacin (Lfx) 1 ug/mL, and Pto 10 ug/mL. Growth at these concentrations was defined as resistance.

2.3. Gene mutation detection

Sputum was decontaminated by the N-acetyl-L-cysteine-sodium hydroxide method and DNA was extracted by centrifugation. The INH resistance gene mutation detection kit (purchased from Xiamen Zhishan Biotechnology Co., Ltd.) was used to analyze the fluorescence quantitative polymerase chain reaction (PCR) melting curve, and the gene mutation results were recorded.

2.4. Statistical analysis

Excel was used for data collection, and the SPSS 26 software was used for statistical analysis. Count data were expressed as %. The differences between groups were compared by the chi-squared (χ^2) test or Fisher's exact probability method. Results were considered statistically significant at $P < 0.05$.

3. Results

3.1. Drug sensitivity test results

The results of the phenotypic drug sensitivity test in 229 patients were 62.4% (143/229) and 37.6% (86/229) in MDR-TB and pre-XDR-TB strains, respectively. No XDR-TB-resistant strains were found.

3.2. Drug-resistant gene mutations

The *katG* mutation rate (with or without *inhA* mutation) was 85.2%. The mutation rates in MDR-TB and pre-XDR-TB were 87.4% (125/143) and 81.4% (70/86), respectively. The *inhA* mutation rate (including *katG* mutation) was 14.8% (34/229), which was 12.6% (18/143) and 18.6% (16/86) in MDR-TB and pre-XDR-TB, respectively. There was no difference in mutation ($P > 0.05$).

3.3. Phenotypic resistance to Pto

The total resistance rate of 229 strains to Pto, MDR-TB, and pre-XDR-TB was 8.7% (20/229), 8.4% (12/143), and 9.3% (8/86), respectively. Among the *inhA* mutant strains, 13 were resistant to the Pto phenotype, and the resistance rate was 65% (13/20). In MDR-TB and pre-XDR-TB strains resistant to Pto, *inhA* gene mutations occurred in 66.7% (6/9) and 63.6% (7/11), respectively. The resistance rates of MDR-TB and pre-XDR-TB strains without *inhA* gene mutation to Pto were 2.4% (3/125) and 5.7% (4/70), respectively.

Table 1. Correlation between phenotypic drug sensitivity test results and drug resistance gene mutations of 229 clinical isolates of *Mycobacterium tuberculosis*

Type of strain Drug sensitivity test results	InhA gene mutation	KatG gene mutation
MDR-TB		
Pto sensitive	12	122
Pto resistance	6	3
Pre-XDR-TB		
Pto sensitive	9	66
Pto resistance	7	4

4. Discussions

Recently, the resistance rate of INH has gradually increased. According to the results of the fifth tuberculosis epidemiological sampling survey in China, the resistance rate of TB patients to INH is the highest at 28.6%, and the resistance rate to Pto is 12.9%, which was higher than the results of this study^[5]. The resistance of *Mycobacterium tuberculosis* to INH is related to a variety of genes. The most frequent mutations are *KatG* and *inhA* genes, accounting for 64% and 19% of INH-resistant strains, respectively^[6]. The results of this study showed that the *inhA* single mutation rate in MDR-TB and pre-XDR-TB was 12.6% and 18.6% respectively, which was similar to Seifert's data^[6]. Studies have shown that there were regional differences in the mutation rates of *katG* and *inhA* in INH-resistant *Mycobacterium tuberculosis* strains^[7]. The *inhA* mutation rate (with and without *katG* mutation) of *Mycobacterium tuberculosis* in different regions is different. According to Abate *et al.*, the *inhA* mutation rate in North Africa was 8.7%, while Jagielski *et al.* showed that the *inhA* mutation rate in INH-resistant *Mycobacterium tuberculosis* strains in Poland was 4%, in Southeast Asia, the Philippines was 22%, much higher than in North Africa and Eastern Europe^[8,9]. In China, the rate in Beijing is 15.9%^[7]. Song *et al.* showed that the *inhA* single mutation rates of MDR-TB, Pre-XDR-TB, and XDR-TB strains were 18.0%, 15.4%, and 5.9%, respectively^[7]. The *inhA* gene mutation of *Mycobacterium tuberculosis* strains is related to the low concentration of INH resistance. Only those infected with *Mycobacterium tuberculosis* strains with *inhA* mutation are considered for high-dose INH treatment. The results of this study showed that with the increase of drug resistance (from MDR-TB to Pre-XDR-TB), the change of *inhA* single

mutant strain was not obvious, which was different from the results of Song and Katiyar^[7,11]. Therefore, the author does not recommend the use of high-dose INH for either patient as they might not benefit from it. The total resistance rate of 229 strains to Pto, MDR-TB, and pre-XDR-TB was 8.7%(20/229), 8.4%(12/143), and 9.3%(8/86), respectively^[7,11]. However, no double gene mutation was found in this study, which may be related to the small number of specimens analyzed in this study.

The *inhA* gene mutation of *Mycobacterium tuberculosis* strains is the molecular basis of cross-resistance to INH and Pto. It has been reported that 8%–43% and 33%–65% of INH-resistant strains and Pto-resistant clinical isolates have *inhA* gene mutations, respectively^[12]. In South Africa, some studies have suggested that the resistance to ethamsylate was mainly caused by *inhA* mutation, and *inhA* mutation can be used as a molecular marker to predict the resistance of *Mycobacterium tuberculosis* to ethamsylate^[11]. In this study, the resistance rate of MDR-TB to Pto was gradually increased from MDR-TB to Pre-XDR-TB *inhA* mutant, but it was lower than the findings of Song *et al.*^[7]. Most *inhA* mutant strains are still sensitive to Pto. The author speculates that the *inhA* mutation in MDR-TB strains may not be the main mechanism of Pto resistance, which is similar to the results of Song *et al.* It is suggested that Pto should be considered when the effective drug composition scheme cannot be selected for MDR-TB patients.

5. Conclusion

The INH resistance of *Mycobacterium tuberculosis* in China is mainly due to the *katG* gene mutation. There was no significant difference in the *inhA* mutation rate between MDR-TB and preXDR-TB patients. High-dose INH may not benefit from conventional treatment in patients with drug-resistant TB. *InhA* mutation may not be the main reason for Pto resistance. It is recommended to improve other drug sensitivity tests and develop individualized treatment plans. Nonetheless, this study still has limitations and shortcomings. First of all, this study used a commercial drug-resistant gene mutation detection kit in gene detection, only reporting the results of *katG* and *inhA* mutations, with no specific description of *katG* and *inhA* gene mutation sites and *inhA* gene regulatory region or coding region mutations. Second, the number of research objects is limited, hence the results may be biased.

Funding

Study on the Correlation Between *inhA* Gene Mutation of Multidrug-Resistant MTB and Resistance to Protionamide (Project number: 2022013)

Disclosure statement

The authors declare no conflict of interest.

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