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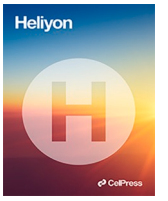

Research article

# Factors influencing false-positive results of rifampicin resistance detected by Xpert MTB/RIF: A retrospective study in Zhejiang, China

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ABSTRACT

*Objective:* This study aimed to explore the factors influencing false-positive results for rifampicin resistance (RIF-R) detected using Xpert MTB/RIF (Xpert).
*Methods:* This retrospective analysis included the clinical data of patients from September 2019 to February 2023. The chi-square and rank sum tests were used to compare differences in patient characteristics between the true-positive and false-positive groups. Logistic regression was used to analyze the factors influencing false positives in the detection of RIF-R by Xpert.
*Results:* A total of 384 patients were included. Logistic regression analysis revealed that, with mutation of probe E as the reference, mutations on probe A or C (OR = 72.68, $P < 0.001$), probe D (OR = 6.44, $P < 0.001$), and multiple probes (OR = 5.94, $P = 0.002$) were associated with false-positive results in Xpert detection of RIF-R. Taking probe delay ΔCt <4 as the reference, ΔCt (4–5.9) (OR = 13.54, $P < 0.001$), ΔCt (6–7.9) (OR = 48.08, $P < 0.001$) probe delays were associated with false positives in Xpert detection of RIF-R. When very low quantification is accompanied by a probe delay, the probability of false-positive RIF-R detection can reach 80 %.
*Conclusions:* Clinicians should consider factors such as probe mutation type, probe delay, and very low quantification accompanied by probe delay when interpreting Xpert results, which can reduce the misdiagnosis of tuberculosis drug resistance.

## 1. Introduction

Tuberculosis, caused by the *Mycobacterium tuberculosis* (MTB) complex, remains an important public health problem worldwide. According to the 2022 World Health Organization report, approximately 10.6 million people developed incident tuberculosis (TB) worldwide, and 484,000 people developed RIF-R TB in 2021. The prevalence of drug-resistant TB, particularly multidrug-resistant TB (MDR-TB), limits global TB control [1]. MDR-TB requires long-term second-line anti-tuberculosis drug treatment, which is costly and has apparent drug toxicity. Early diagnosis of drug-resistant TB is essential for preventing, controlling, and treating TB [2]. Traditional phenotypic drug susceptibility testing (pDST) requires months to obtain results, leading to delays in diagnosis and increasing the risk of continued spread of drug-resistant TB in the community [3]. In addition, conventional pDST requires extensive and complex infrastructure, making it impossible for ordinary laboratories to perform pDST [4]. With the development of molecular diagnostic technology, molecular detection technologies, such as line probe assays, drug resistance chips, and melting curves, are widely used to

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detect RIF-R; however, these molecular detection technologies require bacterial load and specialized reference laboratories. Some studies have shown that Xpert Ultra has significantly improved the sensitivity and false-positive detection rate of RIF-R [5,6], but this product has not been widely promoted in China. Xpert (Cepheid, Sunnyvale, CA, USA) plays an important role in RIF-R detection in China.

Xpert is a molecular detection technology based on seminested real-time fluorescence quantitative polymerase chain reaction (qPCR). This technology uses five molecular beacon-overlapping probes to simultaneously detect mutations in the RRDR gene (including codons 507–533). These probes are probes A (codons 507–511), B (codons 512–518), C (codons 518–523), D (codons 523–529), and E (codons 529–533), which simultaneously diagnose TB and RIF-R. Detecting RIF-R using the Xpert assay relies on the absence or the delay in binding of five probes spanning the 81 bp RIF-R determining region. Probe delay is quantified by a notable disparity in the PCR threshold cycle (Ct) value among the different probes ($\Delta Ct > 4$) [7–9]. Xpert has been rapidly promoted worldwide given its advantages of speed (approximately 2 h), accuracy, simultaneous detection of TB and RIF-R, and independence from laboratories. The widespread use of Xpert has greatly improved the diagnosis of MDR-TB [10,11]. However, due to certain factors, 9.00–17.31 % of patients record false positives in Xpert detection of RIF-R [12–14]. During the transition period (approximately 3 months) between the completion of Xpert and pDST, some patients are unnecessarily treated with second-line drugs due to false-positive RIF-R results following Xpert [15]. Therefore, exploring the factors contributing to false-positive RIF-R results detected by Xpert is important for reducing the occurrence of false drug resistance and preventing inappropriate TB treatment. Although several studies have analyzed the reasons for detection of false-positive RIF-R by Xpert, most of these studies had small sample sizes, included fewer influencing factors, and lacked pDST results. However, this requires further investigation.

This study was a retrospective study of patients admitted to our hospital to analyze the factors affecting false-positive RIF-R results detected by Xpert. These findings may provide a reference for clinical practice.

## 2. Methods

### 2.1. Study design

We retrospectively analyzed the clinical data of patients hospitalized at Hangzhou Red Cross Hospital between September 2019 and February 2023, and all patient samples were tested using Xpert. Basic patient characteristics, laboratory test indicators, and clinical information were collected from electronic medical records. Chronic kidney disease was defined as a disease causing progressive loss of kidney function. Chronic cardiovascular disease was defined as a condition requiring long-term use of heart medications. Chronic liver disease was defined as a disease resulting in progressive destruction and regeneration of the liver parenchyma. The study protocol strictly complied with the requirements of the Declaration of Helsinki of the World Medical Association. Members of our research project teams are committed to maintaining the confidentiality of all patient information collected from electronic medical records.

### 2.2. Patient selection

The inclusion criteria were as follows: (1) detailed basic population characteristics and detailed clinical information; (2) underwent Xpert testing and had valid RIF-R results and detailed information; and (3) confirmed positive by MTB culture and pDST results. The exclusion criteria were as follows: (1) samples with negative, RIF sensitive (RIF-S), or invalid Xpert test results and (2) negative MTB culture or lack of pDST results. The study outcomes were as follows: initial Xpert and pDST results positive for RIF-R were defined as the "true-positive" group. The "false-positive" group included patients who were RIF-R on initial Xpert and RIF-S on pDST. The first Xpert test was selected if the patient underwent several Xpert tests during hospitalization.

### 2.3. MTB culture and pDST

The operating procedures in the "Standardized Operation and Network Construction of Tuberculosis Laboratories" were followed. The processed specimen (0.5 mL) was added to a mycobacteria growth indicator tube (MGIT) for liquid culture. Subsequently, the MGIT was placed in the Bactec MGIT 960 liquid culture system for culture. The culture-positive specimens were further tested for drug susceptibility. The Bactec MGIT 960 system drug susceptibility test involved culturing MTB isolated from tuberculosis patients in vitro in the presence of a known concentration of the test drug (RIF concentration: 1.0 μg/ml). The growth status was observed and compared with a control tube without any drug. If both the control and drug culture tubes exhibited growth, the bacterial strain was considered resistant to the drug. In contrast, if growth was observed only in the control tube, the bacterial strain was considered sensitive to the drug.

### 2.4. Xpert MTB/RIF

One millilitre of sputum or other specimens was added to a pretreatment tube with a screw cap. Then, 2 mL of specimen volume and SR treatment solution (sodium hydroxide, isopropyl alcohol) was shaken 10–20 times and allowed to stand at room temperature for 15 min. After standing for 5–10 min, the sample was vigorously shaken 10–20 times to ensure complete homogenization. The reaction box was opened, and 2 mL of the processed sample was slowly added to the reaction box. After that, the reaction box was placed in the detection module. The instrument automatically detects and reports the results of MTB and RIF-R after 2 h. The detection of RIF-R is based on the absence or delay in the binding of the five probes. ΔCt is the difference between the first (early Ct) and the last (late Ct)

MTB-specific beacon (ΔCt) and is the basis for detecting RIF-R [15].

### 2.5. Fluorescence PCR melting curve

An MTB RIF-R mutation detection kit (Xiamen Zhishan Biotechnology Company; http://www.zsandx.com) was used according to the fluorescent PCR melting curve method. When the melting temperature (Tm) of the sample was consistent with the Tm of the positive control (within 1 °C), the sample was considered wild type (sensitive). When the Tm of the sample was >2 °C of the Tm of the positive control, the sample was considered mutant (resistant) (Supplementary Fig. 1). Fluorescence PCR melting curve technology was used to determine whether the detected gene had mutated, as indicated by differences in the Tm of single nucleotides. Strong congruence between Xpert results and fluorescent PCR melting curves indicates that RIF-R stemmed from mutations rather than from other mechanisms.

### 2.6. Statistical analysis

For measurement data with a skewed distribution, the median and upper and lower quartiles (Q1, Q3) were used to describe the central tendency and dispersion of the data. The Mann–Whitney *U* test was used to compare groups. The enumeration data were described by the number of cases (n) and constituent ratio (%), and the $\chi 2$ test or Fisher's exact probability test was used. Factors influencing false-positive RIF-R results were analyzed using logistic regression (Enter's method). SPSS 26.0 software was used, all tests were two-sided, and $P < 0.05$ indicated significance.

## 3. Results

Between September 2019 and February 2023, 21,998 Xpert assays were performed at our hospital. The following 21,534 Xpert tests were excluded: 14,456 negative tests, 6821 RIF-S tests, 51 invalid tests, and 206 tests without pDST results. Finally, 464 RIF-R tests were included in the study, including 384 initial tests and 74 repeated tests (68 were repeated once, and six were repeated twice). Finally, 384 patients who underwent initial testing were included. According to Xpert, 299 tests indicated RIF-R, and 85 tests indicated RIF-S. The false-positive rate for Xpert was 22.14 % (85/384) (Fig. 1).

Univariate analysis of demographic, clinical and TB diagnosis related characteristics among false positives in Xpert detection of RIF-R.

When comparing true-positive and false-positive RIF-R tests, the basic and clinical characteristics did not differ significantly between the two groups. The mutation probes and probe delays differed significantly between the two groups (all *P* values < 0.05) (Table 1).

### 3.1. Univariate and multivariate analysis of false positives in the detection of RIF-R by Xpert

The multivariate analysis included factors that were significant in the univariate analysis. Using the mutation of probe E as a reference, probes A and C (OR = 72.68, $P < 0.001$), probe D (OR = 6.44, $P < 0.001$), and multiple probes (OR = 5.94, $P = 0.002$) were risk factors for false-positive RIF-R detection, but probe B was not a risk factor. With probe delay ΔCt <4 as a reference, ΔCT (4–5.9) (OR = 13.54, $P < 0.001$) and ΔCt (6–7.9) (OR = 48.08, $P < 0.001$) probe delays were risk factors for false-positive RIF-R detection by Xpert, but ΔCt ≥8 was not a risk factor (OR = 0.95, $P = 0.947$) (Table 2).

Analysis of probe delay distribution and false positive incidence rate in different quantitative groups.

Among the 384 tests, 32 were probe-delayed samples. Probe delay was classified into high (n = 1), medium (n = 14), low (n = 11),

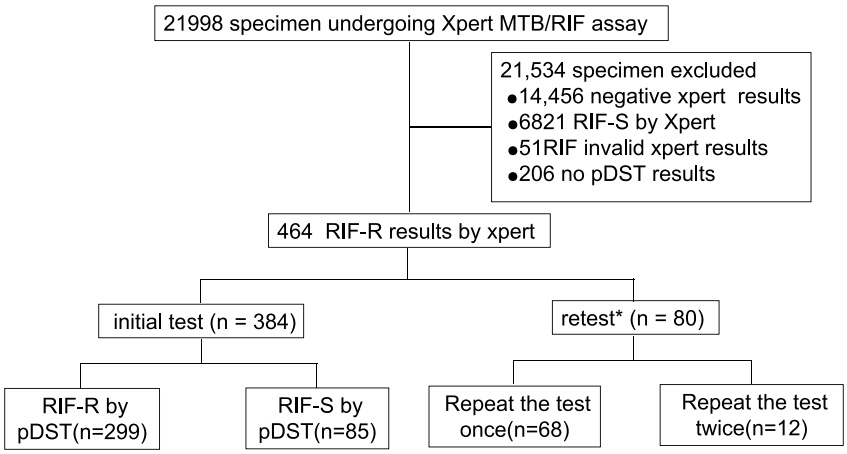


**Fig. 1.** Patient enrollment and analysis.

**Table 1**
Univariate analysis of demographic, clinical and TB diagnosis related characteristics among false positives in Xpert detection of RIF-R from Zhejiang, China, N = 384.

| characteristics | True positivegroup | False positive group | Z/χ2-value | *P*-value |
|---|---|---|---|---|
| | N = 299 | N = 85 | | |
| Age | 39.00(28.00,59.00) | 45.00(29.00,60.00) | −0.33 | 0.741 |
| gender | 100(33.44 %) | 23(27.06 %) | 1.24 | 0.266 |
| Resistance probe | | | 118.03 | <0.001 |
| Probe A | 5(1.67 %) | 33(38.82 %) | | |
| Probe B | 20(6.69 %) | 5(5.88 %) | | |
| Probe C | 2(0.67 %) | 0(0.00 %) | | |
| Probe D | 45(15.05 %) | 19(22.35 %) | | |
| Probe E | 213(71.24 %) | 22(25.88 %) | | |
| Multiple probes | 14(4.68 %) | 6(7.06 %) | | |
| Specimen Type | | | 1.14 | 0.566 |
| sputum | 108(36.12 %) | 29(34.12 %) | | |
| BALF | 161(53.85 %) | 44(51.76 %) | | |
| others | 30(10.03 %) | 12(14.12 %) | | |
| ΔCT in probe delay | | | 26.70 | <0.001 |
| <4 | 283(94.65 %) | 69(81.18 %) | | |
| 4–5.9 | 5(1.67 %) | 7(8.24 %) | | |
| 6–7.9 | 2(0.67 %) | 7(8.24 %) | | |
| ≥8 | 9(3.01 %) | 2(2.35 %) | | |
| Xpert Quantitative result | | | 4.28 | 0.233 |
| High | 44(14.72 %) | 8(9.41 %) | | |
| Medium | 114(38.13 %) | 31(36.47 %) | | |
| Low | 96(32.11 %) | 26(30.59 %) | | |
| Very low | 45(15.05 %) | 20(23.53 %) | | |
| Types of tuberculosis | | | 0.46 | 0.794 |
| pulmonary | 255(85.28 %) | 72(84.71 %) | | |
| extrapulmonary | 18(6.02 %) | 4(4.71 %) | | |
| both | 26(8.70 %) | 9(10.59 %) | | |
| TB treatment history | 144(%) | 54(%) | 6.26 | 0.12 |
| Bacillary load of sputum | | | 6.04 | 0.110[a] |
| negative | 135(45.15 %) | 43(50.59 %) | | |
| Low(scanty) | 8(2.68 %) | 4(4.71 %) | | |
| High(1+to4+) | 127(42.47 %) | 25(29.41 %) | | |
| unknown | 29(9.70 %) | 13(15.29 %) | | |
| HIV status | 1(%) | 0(%) | 0.808 | 0.688 |
| Diabetes | 46(15.38 %) | 14(16.47 %) | 0.06 | 0.808 |
| Chronic liver | 43(14.38 %) | 10(11.76 %) | 0.38 | 0.537 |
| Chronic nephrosis | 14(46.82 %) | 4(47.06 %) | 0.00 | 0.993 |
| hypertension | 31(10.37 %) | 11(12.94 %) | 0.045 | 0.502 |
| autoimmune disease | 7(2.34 %) | 1(1.18 %) | 0.44 | 0.507 |
| Malignancy | 11(3.68 %) | 1(1.18 %) | 1.37 | 0.242 |
| Cardiovascular diseases | 8(2.68 %) | 1(1.18 %) | 0.65 | 0.420 |

**Note:** Values are numbers (%) or medians (interquartile ranges); Multiple probes, More than one probe.

**Table 2**
Univariate and multivariate analysis of false positives in Xpert detection of RIF-R from Zhejiang, China.

| Characteristics | Univariate analyses | | Multivariable analyses | |
|---|---|---|---|---|
| | OR(95 % CI) | P-value | adjusted OR(95 % CI) | *P*-value |
| Resistance probe | | | | |
| Probe E | 1 (ref) | 1 (ref) | 1 (ref) | 1 (ref) |
| Probe A or C | 45.64(18.08–115.24) | <0.001 | 72.68(27.19–194.27) | <0.001 |
| Probe B | 2.42(0.83–7.08) | 0.107 | 2.17(0.58–8.07) | 0.249 |
| Probe D | 4.09(2.04–8.17) | <0.001 | 6.44(2.98–13.94) | <0.001 |
| Multiple probes | 4.15(1.45–11.88) | 0.008 | 5.94(1.92–18.36) | 0.002 |
| ΔCT in probe delay | | | | |
| <4 | 1 (ref) | 1 (ref) | 1 (ref) | 1 (ref) |
| 4–5.9 | 5.74(1.77–18.64) | 0.004 | 13.54(3.60–50.88) | <0.001 |
| 6–7.9 | 14.36(2.92–70.63) | 0.001 | 48.08(9.11–253.68) | <0.001 |
| ≥8 | 0.91(0.19–4.31) | 0.907 | 0.95(0.18–4.96) | 0.947 |

Multiple probes, More than one probe.

and very low (n = 6) quantification groups. Using pDST as a reference, the false-positive rates of RIF-R in each group were 0.00 %, 42.86 %, 45.45 %, and 80.00 %, respectively (Table 3).

### 3.2. Analysis of inconsistent initial and retest Xpert results among 74 patients

Among the 384 patients in whom Xpert detected RIF-R, 74 underwent Xpert retesting. Among these, 72 patients had consistent initial and retest Xpert results, and two had inconsistent initial and retest results, yielding an inconsistency rate of 2.70 % (2/74). The false-positive rate between initial and repeated testing in the very-low quantitative group was 9.09 % (2/22). Both patients showed very low quantification and probe delays (Table 4).

### 3.3. Comparison of Xpert and melting curve tests in 258 patients

Among the 384 patients, 258 underwent melting curve resistance detection, among which 256 RIF-R detection results from the melting curve test were consistent with those from the initial Xpert test. The results of the two tests were inconsistent, with an inconsistency rate of 0.78 % (2/258), including one test in the low group (missing D probe) and one test in the very-low group (probe delay) (Table 4).

## 4. Discussion

The Xpert test has greatly shortened the diagnosis time of TB [16] and has been widely used in many countries with a high TB burden since being recognized by the World Health Organization. However, when molecular testing identifies controversial mutations in the rpoB gene, these often conflict with the pDST results, hampering the development of effective anti-TB treatment regimens [15]. Different studies have reached slightly different conclusions regarding the factors influencing false positives for RIF-R, as detected by Xpert. False positives are believed to be related to factors such as the detection of silent mutations in the rpoB gene, delayed binding of the probe, very low bacterial load, and controversial resistance site mutations [17–19]. However, many studies have limitations. For example, Berhanu et al. reported that inconsistent RIF susceptibility results were associated with Xpert probe B and delayed probe binding; however, line probe testing, phenotypic drug susceptibility testing, and repeated Xpert tests were selected as confirmatory tests, and the study lacked unified confirmation standards [13]. Qi et al. reported that more than half of single-probe A deletion isolates had inconsistent RIF molecular phenotype results; however, their sample size was relatively small (only 63 patients), and the findings may not apply to large populations [20]. In 2016, Ocheretina et al. investigated 22 TB patients, revealing that the Xpert test was more likely to produce false-positive results for RIF-R in clinical samples with extremely low bacterial loads [19]. Subsequently, studies from Beijing, China, and Rwanda have confirmed this finding [21,22]. In contrast, Berhanu et al., using “medium” load as a reference, revealed similar levels of discordance as observed in “very low” samples, using Xpert testing (IRR = 1.22, 95 % CI 0.4–3.70) [13]. In summary, several studies on very low bacterial loads used different reference standards, and the conclusions remain controversial. In 2020, a prospective study from Rwanda included a large population-based sample, revealing that 86 % of patients with very low bacterial loads were incorrectly diagnosed with RIF-R TB [21]. However, this study had several limitations. First, the analysis did not include comorbidities other than HIV infection [21]. In addition, this study confirmed that only a small number of patients had RIF-S (57 cases), and it was impossible to conduct a detailed study of the probe factors [21]. Finally, this study was conducted only in Rwanda, which is not representative of other regions [21]. Our study retrospectively analyzed the clinical data of 384 inpatients at the Hangzhou Red Cross Hospital from September 2019 to February 2023. Our study comprehensively collected information on the general characteristics of the patient population, laboratory test results, and clinical characteristics. In addition, our study uniformly used pDST as the gold standard for determining false positives of RIF-R using Xpert. We hope our findings may help verify and supplement the factors that influence false positives in the Xpert detection of RIF-R.

In our study, we report a false-positive rate of 22.14 % for RIF-R detected by Xpert among 384 patients. This rate is significantly lower than that reported in Rwanda, which reported a false-positive rate of 47.00 % (57/121) [21]. There are apparent differences in the false-positive rates between studies in different regions, which may be related to various factors. The prevalence of drug-resistant strains varies geographically. For example, in areas where the prevalence of drug resistance is low, high false-positive rates of RIF-R detected by Xpert have been reported. In contrast, in areas with a high prevalence of drug resistance, low false-positive rates have been reported. The distribution and frequency of mutation sites in the rpoB gene also differ geographically. Rajendran et al. showed that the

**Table 3**
Analysis of probe delay distribution and false positive incidence rate in different quantitative groups in Xpert detection of RIF-R from Zhejiang, China.

| Xpert Quantitative groups | Total Specimen(N) | probe delay(N) | Xpert result vs pDST | |
|---|---|---|---|---|
| | | | R–R[a] | R–S* |
| High | 52 | 1 | 1 | 0 |
| Medium | 145 | 14 | 8 | 6 |
| Low | 122 | 11 | 6 | 5 |
| Very low | 65 | 6 | 1 | 5 |

Comparison of probe delay-related Xpert RIF-R results with pDST.
[a] Xpert result show RIF-R, pDST show RIF-R; * Xpert result show RIF-R, pDST show RIF-S.

**Table 4**
Comparison of Xpert initial detection with repeated detection or melting curve among different quantitative groups in Xpert detection of RIF-R from Zhejiang, China.

| | | Xpert Quantitative groups | | | |
|---|---|---|---|---|---|
| | | High | Medium | Low | Very low |
| **initial test VS retest** | R–R[a] | 6 | 30 | 16 | 20 |
| | R–S[b] | 0 | 0 | 0 | 2[b] |
| **initial test of Xpert** | R–R[c] | 40 | 111 | 78 | 27 |
| **VS melting curve** | R–S[d] | 0 | 0 | 1[e] | 1★ |

[a] Both initial and retest of Xpert test show RIF-R;[b] Initial test show RIF-R, retest how RIF-S.
[b] The initial resistance characteristics is very low Quantitative concomitant probe delay.
[c] Both initial test of Xpert and melting curve show RIF-R.
[d] initial test of Xpert show RIF-R, melting curve show RIF-S.
[e] The resistance is cased by probe D mutation;★ The resistant is caused by probe delay.

distribution of drug-resistant probes in the Chennai area was E, D, A, B, and C, and in Mizoram, Northeast India, the distribution of probes was A, E, D, B, and C [23,24]. In our study, the distribution of drug-resistant probes was E, D, B, A, and C. These findings highlight the importance of considering the prevalence of drug-resistant strains, mutation site distribution, and frequency differences in different regions for the rational application of Xpert detection technology.

This study showed that probe mutation type and probe delay are independent factors affecting false positives in the Xpert detection of RIF-R. Worldwide, the most frequent mutation conferring RIF-R occurs within the RRDR at rpoB codon 531, which specifically binds to the Xpert probe E [25]. Therefore, we selected the probe E mutation as the reference for multivariate analysis. We found that probes A or C, probe D, and multiple probes are risk factors for false-positive RIF-R detection by Xpert. This finding is different from that of Berhanu et al., who reported that, compared with probe E, probe B resistance was a risk factor for false positivity (IRR 5.67, 95 % CI 1.95–16.45) [13]. In the Rwanda study, probes E or B were selected as references. When resistance was detected by other probes (A, C, D), the risk of false positives increased (OR = 8.6, 95 % CI 1.5–49.1). However, different studies have reported different conclusions. These conclusions may be related to regional mutation differences. Unfortunately, we lacked detailed sequencing information and could not analyze mutation site information. We hope that more in-depth studies will reveal the mechanisms underlying these mutation differences.

Probe delay is a widely discussed factor, and product companies have long noted that probe delays can lead to false positives in RIF-R. The Ct used to determine RIF-R increased from 3.5 to 5 in 2010 and then adjusted to 4 in the G4 assay [10]. Nonetheless, probe delay was still an important factor that led to false-positive RIF-R results in our study. Considering that probe delay is a recognized influencing factor, we adjusted the probe delay data to understand its impact on false positives for RIF-R in more detail. Using probe delay $\Delta Ct < 4$ as a reference, $\Delta CT$ (4–5.9) and $\Delta Ct$ (6–7.9) probe delays were risk factors for false positives in the Xpert detection of RIF-R, but $\Delta Ct \geq 8$ was not. Berhanu showed that high inconsistency rates mainly occur when the probe has a delayed $\Delta Ct$ (4–4.9) relative to $\Delta Ct \geq 5$ [13]. In contrast, our study showed that $\Delta Ct$ (6–7.9) had the greatest impact on false positives for RIF-R. These findings suggest that RIF-R false positives may be related to probe delays within a certain range and that not all delays are factors affecting the occurrence of false positives. Research has shown that probe delay is distributed in high, medium, low, and very-low quantification groups. Notably, only one instance of probe delay occurred in the high-quantification group. We also found that probe delay in the very-low quantification group resulted in a false-positive rate of 80.00 % for RIF-R, significantly greater than that in the other groups. We speculate that very-low quantification accompanied by probe delays may increase the occurrence of false positives for RIF-R, which needs to be confirmed in further studies.

Research in Rwanda shows that the Xpert test has a low positive predictive value (53 %) for RIF-R in samples with very low bacterial loads and recommends repeat testing of very low samples [21]. Our study revealed that among the 74 patients who underwent Xpert retests, 72 had initial results consistent with the retests, while two did not. Interestingly, these two patients had very low concomitant probe delays. Therefore, these findings suggest that if we encounter a sample with very low quantification, we need to pay special attention to the occurrence of probe delays. If there are probe delays, we recommend repeated testing. If there are no probe delays, repeat testing cannot be economically justified. Except for tests with probe delays, the repeat testing results in the very-low group did not differ from those of the initial testing.

The fluorescence PCR melting curve is an analytical technique in which melting curves of different shapes are generated based on the different melting temperatures of single nucleotides. To obtain information on drug resistance, this technology detects whether a target sequence contains mutations based on changes in the melting point of the target sequence [26]. Of the 384 patients in this study, 258 underwent melting curve resistance testing. The initial Xpert test results for 256 tests were consistent with the melting curve test results, and only two tests revealed inconsistent results. One sample had a mutation in probe D, and the other test had a very low concomitant probe delay. Our findings suggest that there was no difference between the initial Xpert detection and melting curve results except for the one test with a probe delay among the very low samples. Therefore, we believe that when Xpert detects very few samples with RIF-R, the results are credible if there is no probe delay.

This study has several limitations. First, when studying the relationship between RIF-R and resistance sites, we used only melting curve detection methods and lacked gene sequencing data. Therefore, we could not accurately determine whether the false-positive results of RIF-R were caused by silent or doubtful mutations, limiting our in-depth understanding of the false-positive mechanism

of RIF-R. The lack of genetic sequencing information also prevented us from clarifying the mechanism of probe delay and whether the probe delay phenomenon was related to a very low bacterial load. Second, although we included many comorbidities in our study, there was a lack of HIV cases among the patients. Therefore, we could not determine whether HIV status affected the occurrence of false positive RIF-R. Finally, our study was retrospective, preventing us from conducting further analyses of differential samples over time.

## 5. Conclusion

Overall, our results show that the main factors affecting false positives for RIF-R include probe type and probe delay. Compared with probe E mutations, probe mutation types other than probe B mutations were regarded as relevant factors in the occurrence of false positives. The occurrence of false positives in RIF-R is affected only if a probe delay occurs in a specific segment. Although a very low load alone is not an independent factor affecting false positives for RIF-R, when it occurs simultaneously with a probe delay, the probability of false positives increases significantly.

## Ethical statement

This study was retrospective. Patients were not identified during data collection, and personal privacy and commercial interests were not involved. The Ethics Committee of the Hangzhou Red Cross Hospital approved this study, with an exemption from the informed consent requirement (Ethical Application Ref:2023YS108).

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## CRediT authorship contribution statement

**Guihua Wu:** Writing – original draft, Funding acquisition. **Jing Wang:** Investigation. **Xiaoqun Xu:** Investigation. **Hui Wei:** Investigation. **Long Cai:** Resources, Project administration. **Libin Liu:** Writing – review & editing, Funding acquisition, Data curation.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

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## References

[1] World Health Organization, Global Tuberculosis Report 2022, World Health Organization, 2022. https://www.who.int/tb/publications/global_report/en/.
[2] G. van Cutsem, P. Isaakidis, J. Farley, E. Nardell, G. Volchenkov, H. Cox, Infection control for drug-resistant tuberculosis: early diagnosis and treatment is the key, Clin. Infect. Dis. S238-S243 (2016), https://doi.org/10.1093/cid/ciw012.
[3] S.J. Kim, Drug-susceptibility testing in tuberculosis: methods and reliability of results, Eur. Respir. J. 25 (2005) 564–569, https://doi.org/10.1183/09031936.05.00111304.
[4] M.L. Wilson, Recent advances in the laboratory detection of Mycobacterium tuberculosis complex and drug resistance, Clin. Infect. Dis. 52 (2011) 1350–1355, https://doi.org/10.1093/cid/cir146.
[5] S. Chakravorty, A.M. Simmons, M. Rowneki, H. Parmar, Y. Cao, J. Ryan, et al., The new Xpert MTB/RIF Ultra: improving detection of Mycobacterium tuberculosis and resistance to rifampin in an assay suitable for point-of-care testing, mBio 8 (17) (2017) e00812.
[6] S.E. Dorman, S.G. Schumacher, D. Alland, P. Nabeta, D.T. Armstrong, B. King, et al., Xpert MTB/RIF Ultra for detection of Mycobacterium tuberculosis and rifampicin resistance: a prospective multicentre diagnostic accuracy study, Lancet Infect. Dis. 18 (2018) 76–84, https://doi.org/10.1016/S1473-3099(17)30691-6.
[7] D. Helb, M. Jones, E. Story, C. Boehme, E. Wallace, K. Ho, et al., Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology, J. Clin. Microbiol. 48 (2010) 229–237, https://doi.org/10.1128/JCM.01463-09.
[8] C.C. Boehme, P. Nabeta, D. Hillemann, M.P. Nicol, S. Shenai, F. Krapp, et al., Rapid molecular detection of tuberculosis and rifampin resistance, N. Engl. J. Med. 363 (2010) 1005–1015, https://doi.org/10.1056/NEJMoa0907847.

[9] A. Alemu, M. Tadesse, G. Seid, H. Mollalign, K. Eshetu, W. Sinshaw, et al., Does Xpert® MTB/RIF assay give rifampicin resistance results without identified mutation? Review of cases from Addis Ababa, Ethiopia, BMC Infect. Dis. 20 (2020) 87, https://doi.org/10.1186/s12879-020-4817-2.
[10] K.R. Steingart, I. Schiller, D.J. Horne, M. Pai, C.C. Boehme, N. Dendukuri, Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults, Cochrane Database Syst. Rev. 21 (2014) CD009593, https://doi.org/10.1002/14651858.CD009593.pub3.
[11] H. Albert, R.R. Nathavitharana, C. Isaacs, M. Pai, C.M. Denkinger, C.C. Boehme, Development, roll-out and impact of Xpert MTB/RIF for tuberculosis: what lessons have we learnt and how can we do better? Eur. Respir. J. 48 (2016) 516–525, https://doi.org/10.1183/13993003.00543-2016.
[12] M. Kohli, I. Schiller, N. Dendukuri, K. Dheda, C.M. Denkinger, S.G. Schumacher, et al., Xpert(®) MTB/RIF assay for extrapulmonary tuberculosis and rifampicin resistance, Cochrane Database Syst. Rev. 8 (2018) CD012768, https://doi.org/10.1002/14651858.CD012768.pub2.
[13] R.H. Berhanu, K. Schnippel, R. Kularatne, C. Firnhaber, K.R. Jacobson, C.R. Horsburgh, et al., Discordant rifampicin susceptibility results are associated with Xpert(®) MTB/RIF probe B and probe binding delay, Int. J. Tuberc. Lung. Dis. 23 (2019) 358–362, https://doi.org/10.5588/ijtld.16.0837.
[14] F. Huo, Y. Ma, R. Liu, L. Ma, S. Li, G. Jiang, et al., Interpretation of discordant rifampicin susceptibility test results obtained using GeneXpert vs phenotypic drug susceptibility testing, open. Forum, Infect. Dis. 7 (2020), https://doi.org/10.1093/ofid/ofaa279 ofaa279.
[15] P. Miotto, A.M. Cabibbe, E. Borroni, M. Degano, D.M. Cirillo, Role of disputed mutations in the rpoB gene in interpretation of automated liquid MGIT culture results for rifampin susceptibility testing of Mycobacterium tuberculosis, J. Clin. Microbiol. 56 (1617) (2018) e01599.
[16] S.D. Lawn, P. Mwaba, M. Bates, A. Piatek, H. Alexander, B.J. Marais, et al., Advances in tuberculosis diagnostics: the Xpert MTB/RIF assay and future prospects for a point-of-care test, Lancet Infect. Dis. 13 (2013) 349–361, https://doi.org/10.1016/S1473-3099(13)70008-2.
[17] A. Van Rie, K. Mellet, M.-A. John, L. Scott, L. Page-Shipp, H. Dansey, et al., False-positive RIF-R on Xpert® MTB/RIF: case report and clinical implications, Int. J. Tuberc. Lung. Dis. 16 (2012) 206–208, https://doi.org/10.5588/ijtld.11.0395.
[18] V. Mathys, M van de Vyvere, E de Droogh, K. Soetaert, G. Groenen, False-positive RIF-R on Xpert® MTB/RIF caused by a silent mutation in the rpoB gene, Int. J. Tuberc. Lung. Dis. 18 (2014) 1255–1257, https://doi.org/10.5588/ijtld.14.0297.
[19] O. Ocheretina, E. Byrt, M.-M. Mabou, G. Royal-Mardi, Y.-M. Merveille, V. Rouzier, et al., False-positive RIF-R results with Xpert MTB/RIF version 4 assay in clinical samples with a low bacterial load, Diagn. Microbiol. Infect. Dis. 85 (2016) 53–55.
[20] Q. Nie, D. Sun, M. Zhu, S. Tu, N. Chen, H. Chen, et al., Phenotypic drug susceptibility characterization and clinical outcomes of tuberculosis strains with A-probe mutation by GeneXpert MTB/RIF, BMC Infect. Dis. 23 (2023) 832, https://doi.org/10.1186/s12879-023-08509-0.
[21] J.C.S. Ngabonziza, T. Decroo, P. Migambi, Y.M. Habimana, A.V. Deun, C.J. Meehan, et al., Prevalence and drivers of false-positive RIF-R Xpert MTB/RIF results: a prospective observational study in Rwanda, Lancet. Microbe. 1 (2020) e74–e83, https://doi.org/10.1016/S2666-5247(20)30007-0.
[22] L. Qin, F. Huo, W. Ren, Y. Shang, C. Yao, X. Zhang, et al., Dependence of Xpert MTB/RIF accuracy for detecting rifampin resistance in bronchoalveolar lavage fluid on bacterial load: a retrospective study in Beijing, China, Infect. Drug Resist. 14 (2021) 2429–2435, https://doi.org/10.2147/IDR.S307488.eCollection2021.
[23] P. Rajendran, M.P. Kumar, K. Thiruvengadam, P. Sreenivasan, T. Veeraraghavan, R. Ramalingam, et al., Characterization of probes associated with RIF-R in M. tuberculosis detected by GenXpert from a national reference laboratory at Chennai, Tuberculosis 133 (2022) 102182, https://doi.org/10.1016/j.tube.2022.102182.
[24] C.V. Sailo, R. Lalremruata, Z. Sanga, V. Fela, F. Kharkongor, Z. Chhakchhua, et al., Distribution and frequency of common mutations in rpoB gene of Mycobacterium tuberculosis detected by Xpert MTB/RIF and identification of residential areas of rifampicin resistant-TB cases: a first retrospective study from Mizoram, Northeast India, J. Clin. Tuberc. Other. Mycobact. Dis 29 (2022) 100342, https://doi.org/10.1016/j.jctube.2022.100342.
[25] W. Jing, Y. Pang, Z. Zong, J. Wang, R. Guo, F. Huo, et al., Rifabutin resistance associated with double mutations in rpoB gene in Mycobacterium tuberculosis isolates, Front. Microbiol. 8 (2017) 1768, https://doi.org/10.3389/fmicb.2017.01768.eCollection2017.
[26] C.N. Gundry, J.G. Vandersteen, G.H. Reed, R.J. Pryor, J. Chen, C.T. Wittwer, Amplicon melting analysis with labeled primers: a closed-tube method for differentiating homozygotes and heterozygotes, Clin. Chem. 49 (2003) 396–406, https://doi.org/10.1373/49.3.396.