

# Interpretation of Discordant Rifampicin Susceptibility Test Results Obtained Using GeneXpert vs Phenotypic Drug Susceptibility Testing

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**Background.** The 3-month difference in turnaround time between Xpert and conventional phenotypic drug susceptibility testing (pDST) causes patient treatment challenges when pDST rifampin (RIF) susceptibility results and earlier Xpert results disagree, resulting in unnecessary tuberculosis (TB) patient exposure to toxic second-line drugs. Here, the prevalence of discordant RIF susceptibility test results, specifically Xpert (resistant) vs pDST (susceptible) results, was determined.

**Methods.** Tuberculosis patients enrolled between January 2015 and June 2018 at Beijing Chest Hospital who consecutively tested positive for RIF resistance using Xpert then negative using pDST were studied. DNA sequences and minimal inhibitory concentration (MIC) results provided insights for understanding discordant results.

**Results.** Of 26 826 patients with suggestive TB symptoms undergoing Xpert MTB/RIF testing, 728 diagnosed as RIF-resistant were evaluated. Of these, 118 (16.2%) exhibiting Xpert RIF resistance and phenotypic RIF susceptibility yielded 104 successfully subcultured isolates; of these, 86 (82.7%) harbored *rpoB* gene RIF resistance-determining region mutations and 18 (17.3%) did not. The Leu511Pro (25.0%) and Leu533Pro (17.3%) mutations were most frequently associated with discordant RIF susceptibility test results. Of the 86 isolates with *rpoB* mutations, 42 (48.8%) with MICs  $\leq 1.0$  mg/L were assigned to the RIF-susceptible group, with Leu511Pro being the most common mutation observed. Isolates with a very low bacterial load were most frequently misdiagnosed as RIF-resistant by Xpert.

**Conclusions.** Approximately one-sixth of RIF-resistant TB isolates identified via Xpert yielded discordant pDST results due to questionable interpretation of specific “disputed” mutations. Thus, a diagnostic flowchart should be used to correctly interpret Xpert RIF resistance results to best guide patient treatment.

**Keywords.** tuberculosis; rifampin; resistance; discordance; *rpoB*

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB) complex, remains a major public health concern worldwide [1, 2]. The current epidemic of drug-resistant tuberculosis, especially multidrug-resistant/rifampin-resistant tuberculosis (MDR/RR-TB), is further impeding global TB control [1, 3]. According to World Health Organization (WHO) estimates, 0.5 million MDR/RR incident TB cases were identified in 2018 [1]. However, the fact that only 41% of these estimated cases had been reported in 2017 highlights the urgent need for accelerated access to susceptibility testing for RIF resistance to improve MDR/RR case detection [4].

Conventional drug susceptibility testing (DST) takes months to yield results, causing a diagnosis delay that itself is a risk factor that perpetuates transmission of drug-resistant TB in the community [5]. In addition, laboratories performing conventional DST require an extensive and sophisticated laboratory infrastructure and thus cannot routinely conduct testing outside of reference facilities [6]. Recently, GeneXpert MTB/RIF (Xpert, Cepheid, Sunnyvale, CA, USA), an integrated real-time polymerase chain reaction assay, was developed to simultaneously diagnose TB and detect RIF resistance via the detection of mutations within the RIF resistance-determining region (RRDR) of the *rpoB* gene [7–9]. Indeed, Xpert results are available within just 2 hours, as compared with the several months needed for completion of conventional DST [8]. Nevertheless, the great difference in turnaround time between Xpert and conventional DST has actually created a diagnostic dilemma, as RIF susceptibility results revealed at DST completion often conflict with earlier Xpert assay results showing RIF resistance. This discordance between results can impact patient care if second-line drug treatment is unnecessarily administered during the interim (up to 3 months) between Xpert and DST completion due

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to flawed Xpert results interpreted as RIF resistance [10]. Thus, a precise understanding of why discordant results arise is essential to prevent initiation of inappropriate anti-TB treatment regimens. Although several published studies have investigated the occurrence of discordant results [10, 11], most had limitations stemming from small sample sizes that may have introduced systematic bias into the results.

Here we carried out a retrospective study of a large sample of patients to investigate the prevalence of discordant RIF susceptibility results between Xpert and phenotypic DST (pDST). In addition, analyses of DNA sequence results and minimal inhibitory concentration (MIC) results were conducted to reveal factors involved in discordant RIF susceptibility results obtained using Xpert and pDST.

## METHODS

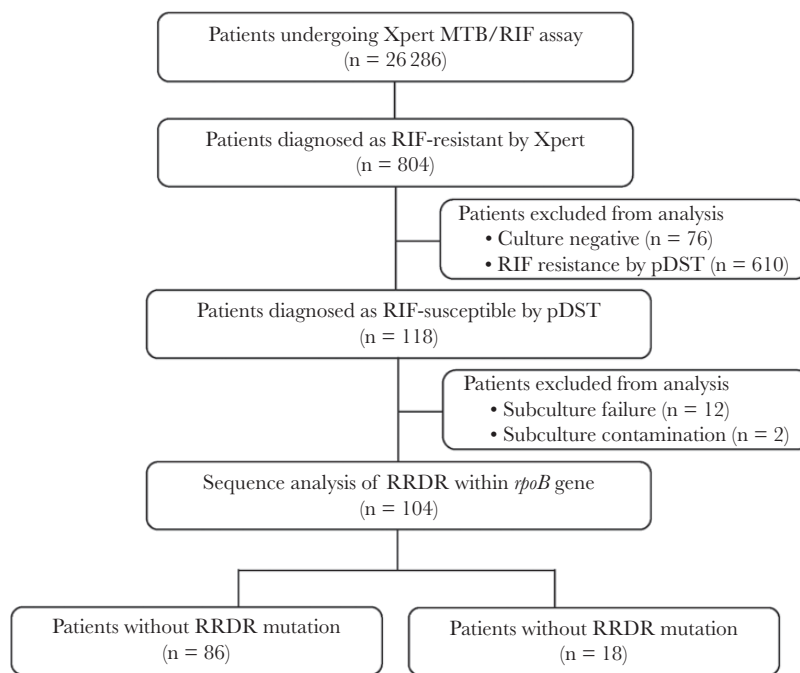
### Bacterial Isolates and Culture Condition

This study was conducted at the Beijing Chest Hospital, an affiliate of Capital Medical University, a 612-bed tertiary hospital providing health care for tuberculosis and chest disease patients. As a National Clinical Tuberculosis Center, it provides clinical services for Beijing residents, while also serving a large number of TB patients from Northern China, accounting for ~70% of all TB patients seeking care there. Patients who provided MTB isolates yielding discordant test results showing RIF resistance (Xpert) vs susceptibility (pDST) were enrolled in this study between January 2015 and June 2018 (Supplementary Figure 1); their medical records were the source of all discordant RIF

susceptibility results presented herein. Xpert MTB/RIF assays and pDST were performed by the National Clinical Laboratory on Tuberculosis following the manufacturers' instructions [8, 12]. For Xpert, G4 cartridges were used; for pDST, the commercial microdilution method was conducted to assess in vitro susceptibility using RIF concentrations 1.0, 2.0, 4.0, and 8.0 mg/L. The results were read after a 7- or 10-day incubation period depending on bacterial growth in the control well (Supplementary Table 1). As per the manufacturer's instructions approved by the Chinese Food and Drug Administration, MTB isolates exhibiting growth at 1.0 mg/L RIF were considered resistant according to the mycobacteria growth indicator tube (MGIT) method endorsed by the WHO [13]. All isolates were stored at -80°C in Middlebrook 7H9 medium supplemented with 10% oleic acid-albumin-dextrose-catalase complex (OADC; Becton Dickinson, Sparks, MD, USA) and 5% glycerol. Before determination of MIC values, isolates were cultured on Löwenstein-Jensen medium for 4 weeks.

### Minimal Inhibitory Concentration

MIC values were obtained using previously reported methods [12, 14]. Analytical-grade RIF powder was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Testing was performed in 96-well microtiter plates. Cell suspensions were adjusted to a cell turbidity value of 1.0 on the McFarland scale and then diluted 20-fold into Middlebrook 7H9 medium supplemented with OADC; 100 µL of the inoculum was pipetted into each well of plates containing 100 µL of 2-fold serial dilutions of drugs, for a final concentration range between 0.063 mg/L



**Figure 1.** Patient enrollment and analysis. Abbreviations: pDST, phenotypic drug susceptibility testing; RIF, rifampin; RRDR, rifampin resistance-determining region.

**Table 1. Mutations of MTB Isolates Within the RRDR of the *rpoB* Gene by Sanger Sequencing**

Mutation Type	No. of Isolates With Different Mutations (n = 104) (%)
Leu511Pro	22 (21.2)
Asp516Val	3 (2.9)
Asp516Tyr	7 (6.7)
Ser522Gln	1 (1.0)
Ser522Leu	1 (1.0)
His526Asn	4 (3.8)
His526Cys	3 (2.9)
His526Gly	1 (1.0)
His526Leu	10 (9.6)
His526Ser	1 (1.0)
Ser531Leu	5 (4.8)
Ser531Cys	1 (1.0)
Leu533Pro	18 (17.3)
Leu511Pro + Met515Ile	2 (1.9)
Leu511Pro + Ser509Arg	1 (1.0)
Leu511Pro + His526Gln	1 (1.0)
Asp516Gly + Ser522Leu	1 (1.0)
Asp516Gly + Asn518Asp	1 (1.0)
His526Asp + Glu541Gly	1 (1.0)
Gln517Gln	1 (1.0)
Heteroresistance	1 (1.0)
Wild-type	18 (17.3)

Abbreviations: MTB, *Mycobacterium tuberculosis*; RRDR, rifampin resistance-determining region.

\*Heteroresistance was defined as a heterogeneous population of tubercle bacilli harboring wild-type and mutant Asp516Asn according to the sequencing chromatograms.

and 64 mg/L. Plates were incubated at 37°C in an atmosphere of 5% CO<sub>2</sub> for 7 days. On day 7, 70 µL of freshly prepared Alamar blue solution was added to each well, and plates were incubated for 24 hours in the dark at 37°C. A color change from blue to pink indicated bacterial growth. MIC was defined as the lowest concentration of drug that prevented a color change. Each isolate was tested in triplicate with the same inoculum on the same day; reference MTB strain H37Rv (ATCC27294) was included in each test batch as a control.

#### DNA Amplification and Sequencing

The boiling method was performed using a previously reported method to extract crude genomic DNA from fresh bacterial colonies [12]. Heated (inactivated) bacterial suspensions were used as templates for DNA amplification. The 688-bp *rpoB* gene fragment (codons 426 to 656 according to a numbering system based on the *Escherichia coli* sequence annotation; or codons 345 to 575 according to a numbering system based on the *M. tuberculosis* sequence annotation) comprising the rifampin resistance-determining region was amplified using published primer sets *rpoB*-F (5'-TCAGACCACGATGACCGTTCC -3') and *rpoB*-R (5'-GTCCATGTAGTCCACCTCAGACG -3') [12]. Amplicons were sent to the Tsingke Company (Beijing, China) for DNA sequencing. DNA sequences were analyzed and

compared with the sequence of the MTB H37Rv strain using BioEdit software, version 7.1.11 (<https://bioedit.software.informer.com>). The MTB *rpoB* codon numbering scheme used here was based on the *Escherichia coli* numbering system.

#### Statistical Analysis

Categorical variables were summarized as percentages and then compared using the chi-square or Fisher exact test, as appropriate. A *P* value <.05 was considered statistically significant. All calculations were conducted using SPSS, version 20.0 (IBM Corp.).

#### Ethics Statement

Approval was obtained from the Ethics Committee of Beijing Chest Hospital, Capital Medical University. Because this study only included data obtained from clinical isolates and not from other recorded patient data, no individual patient consent was required.

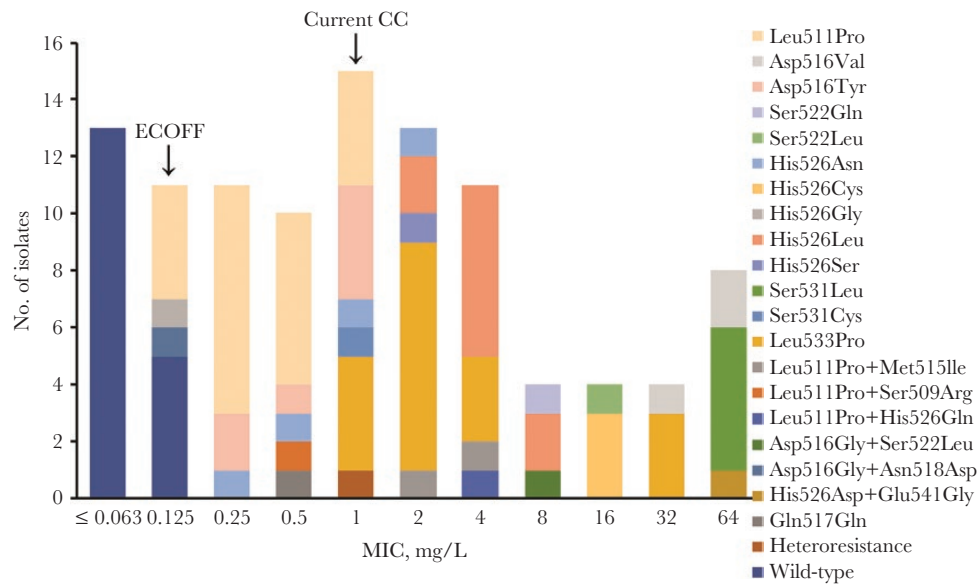
## RESULTS

#### Identification of *rpoB* Mutations

Between January 2015 and June 2018, a total of 26 826 patients with suggestive TB symptoms underwent Xpert MTB/RIF testing at Beijing Chest Hospital. Of these, 804 patients were diagnosed with RIF-resistant MTB by Xpert, and 76 (9.5%) patients were excluded due to culture negativity, resulting in our inability to perform phenotypic DST. Isolates from the remaining 728 patients were included in the analysis, of which 118 (16.2%) exhibited RIF resistance using Xpert and RIF susceptibility using phenotypic susceptibility testing; of these, 14 isolates were excluded from the analysis due to subculture failure (12 isolates) and subculture contamination (2 isolates), leaving 104 RIF-resistant isolates. Next, partial DNA fragment *rpoB* RRDR sequences of these 104 isolates were analyzed using Sanger sequencing. Subsequently, 86 (82.7%) were shown to harbor mutations within the *rpoB* RRDR sequence, while RRDR sequences of the remaining 18 (17.3%) isolates lacked mutations (Figure 1). Notably, Leu511Pro was the most frequently observed mutation associated with cases producing discordant RIF susceptibility test results, with 25.0% (n = 26) of isolates possessing this mutation; the second frequently observed mutation was Leu533Pro (n = 18, 17.3%), followed by His526Leu (n = 10, 9.6%) and Asp516Tyr (n = 7, 6.7%). Notably, 1 strain showed a synonymous mutation at codon 517 (CAG→CAA), leading to a false-positive result indicating resistance (Table 1).

#### MICs and *rpoB* Mutations

We further analyzed the distribution of MICs of isolates to search for associations with various *rpoB* mutations. As summarized in Figure 2, of 86 isolates with *rpoB* mutations, 42 (48.8%) had MICs ≤1.0 mg/L and thus were categorized into



**Figure 2.** Distribution of *Mycobacterium tuberculosis* isolates with different MICs grouped according to *rpoB* mutation profile. Abbreviations: CC, critical concentration; ECOFF, epidemiological cutoff value; MIC, minimal inhibitory concentration.

the RIF-susceptible group using the critical concentration endorsed by the manufacturer. Of note, the tentative epidemiological cutoff value (ECOFF) based on MICs of wild-type MTB isolates and H37Rv strains was 0.125 mg/L. When this tentative value was used to discriminate between RIF-resistant and RIF-susceptible MTB isolates, only 5 isolates (5.8%, 4 isolates with Leu511Pro and 1 isolate with His526Gly), rather than the previous 42 (48.8%) isolates, were considered susceptible to RIF.

The most frequently detected mutation in this group was Leu511Pro (22/42, 52.4%), followed by 6 other mutations, including His526Asn, Asp516Tyr, 2 dual mutations (Leu511Pro plus Ser509Arg and Asp516Gly plus Asn518Asp), and 1 mutation detected in a heteroresistant culture (wild-type and Asp516Asn). In addition, we found 6 missense mutation types among isolates with MICs between 2 mg/L and 4 mg/L, including Ser522Gln, His526Leu, Leu533Pro, and 2 dual mutations (Leu511Pro plus His526Gln and Asp516Gly plus Ser522Leu). Interestingly, 20 (23.3%) isolates harboring mutations that conferred high-level RIF resistance were detected in this work (Supplementary Table 2).

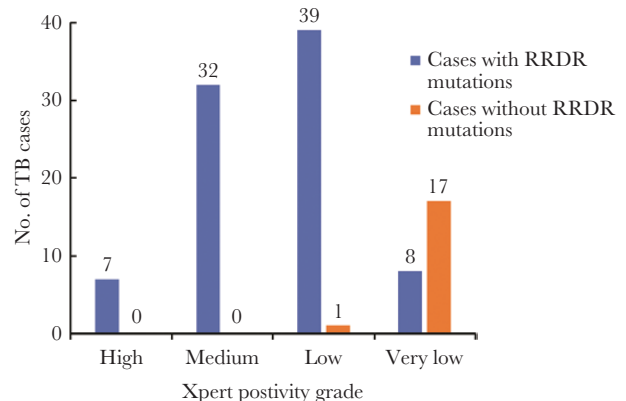
#### Cases Without RRDR Mutation and MTB Bacterial Load

Next, cases with and without RRDR mutations were grouped according to bacterial load. As shown in Figure 3, the numbers of cases with high, medium, low, and very low Xpert positivity grades were 7 (6.7%), 32 (30.8%), 40 (38.5%), and 25 (24.0%), respectively. Notably, among the 18 cases without RRDR mutations, 17 (94.4%) belonged to the very low positivity grade. Moreover, after excluding cases with mutations that had Xpert positivity grades above very low, the remaining cases falling within the very low group were more likely than not to be

misdiagnosed as RIF-resistant due to Xpert detection of *rpoB* mutations that did not confer resistance (odds ratio, 165.75; 95% CI, 19.42–1414.47).

## DISCUSSION

Widespread use of the Xpert MTB/RIF assay has revolutionized the diagnosis and management of RIF-resistant TB [15]. However, discordant Xpert and pDST results have greatly impeded development of effective anti-TB treatment regimens [10]. In this study, we attempted to investigate this puzzling dilemma through study of a large sample of retrospectively recruited patients undergoing Xpert testing in China. Our data demonstrated that approximately one-fifth of isolates with



**Figure 3.** Distribution of cases with or without the RRDR mutation grouped according to Xpert positivity grade. Abbreviations: RRDR, rifampin resistance-determining region; TB, tuberculosis.



discordant results lacked *rpoB* mutations, a result mainly observed in specimens with a very low bacterial load. Notably, similar results have been reported by several research groups that were also attributed to low bacterial load [16–19]. The occurrence of false-positive results arising from DNA sequence diversity may be due to unequal efficacy of Xpert probe binding to diverse target sequences [20], an effect that may be more pronounced in samples with low bacterial load. Such a scenario would have important implications with respect to the clinical interpretation of Xpert assay RIF resistance results. On the one hand, the universal >4-cycle difference in Ct values between probes for interpreting RIF resistance should be redefined by classifying results for clinical samples according to initial bacterial load, especially for samples with a very low bacterial load. On the other hand, the Xpert MTB/RIF has been recommended to diagnose TB in children, HIV-infected individuals, and extrapulmonary TB patients, samples of which have extremely low bacterial loads that often fall below detection limits of conventional methods [17, 21]. Due to the fact that a high proportion of very low bacterial load results can be expected, increased rates of false RIF resistance results would also be expected that would lead to inappropriate treatment of these populations with toxic and unnecessary second-line drugs. Considering that Xpert is endorsed over other tests due to its superior performance in detecting drug-resistant MTB in samples with low bacterial load, such as samples from pediatric, extrapulmonary, and HIV-coinfected pulmonary TB patients, more attention should be paid to understand the increased risk for false-positive RIF resistance results when evaluating these cases [17].

Nearly half of cases with nonsynonymous mutations in RRDR were categorized into the RIF-susceptible group using the critical concentration of 1.0 mg/L. The specific “disputed” mutations lie at the heart of the discordance between Xpert and pDST RIF susceptibility results. For example, as shown in previous reports [10, 11, 22, 23], isolates carrying the amino substitutions Leu511Pro, Asp516Tyr, His526Asn, or His526Gly exhibited only slightly increased RIF MICs compared with wild-type amino acid sequences. Recently, the WHO has deemed that any mutation (excluding silent mutations) identified in the RRDR of the *rpoB* gene is known or assumed to be associated with RIF resistance [13]. Thus we speculate that the laboratory errors associated with the high critical RIF concentration breakpoint for scoring RIF resistance may be the major explanation for this discordance.

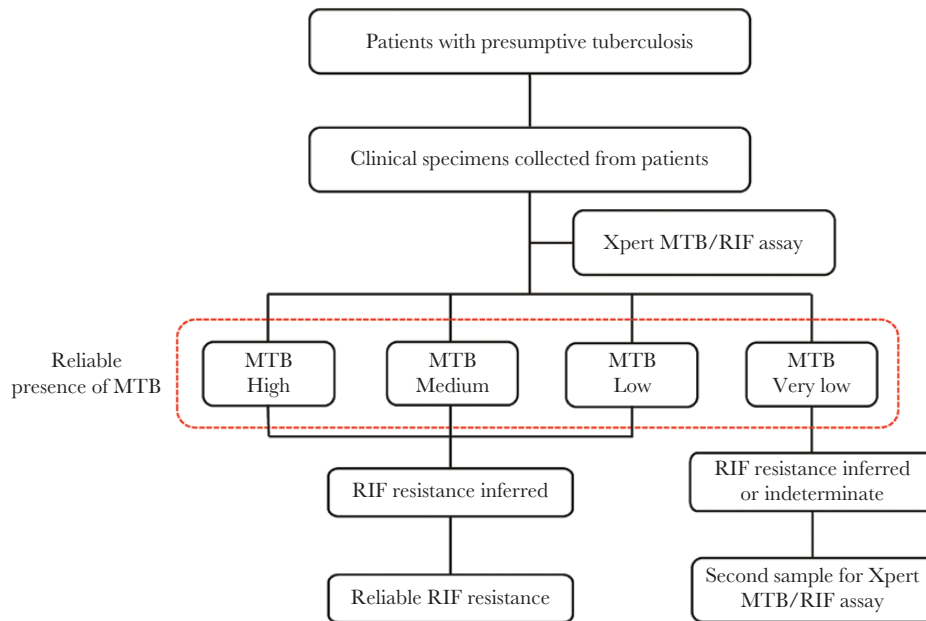
Another possible explanation of why samples with nonsynonymous mutations in RRDR were misidentified as susceptible is the inappropriately high cutoff values for broth-based DST methods. In line with our results, a recent study by Gonzalo and colleagues found that rifampicin resistance was missed by the MGIT system and commercial microtiter plate [24]. We also noted that the lowered critical concentration of

0.125 mg/L could boost the sensitivity of resistance detection and improve concordance between the *rpoB* genotype and phenotype. Even so, a small number of isolates with Leu511Pro and His526Asn substitutions still would not be detected using the lower breakpoint due to overlap between mutated and nonmutated strains. This diagnostic dilemma highlights a critical need to retrospectively investigate the clinical response of RIF-treated patients harboring MTB with these “disputed” mutations in order to reassess the definition of MTB RIF resistance.

In view of our findings and previous experience, we have generated a revised flowchart for use in diagnosing TB patients based on Xpert MTB/RIF assay results (Figure 4). A positive Xpert result for MTB reflects detection of MTB in clinical specimens regardless of bacterial load. However, for specimens with very low positivity grade, RIF resistance results obtained using Xpert are unreliable. In such cases, collection of a second sample for culturing to higher bacterial load may produce more reliable RIF susceptibility results, as cultured specimens may attain a higher positivity grade.

This study has several obvious limitations. First, original cycle threshold (Ct) values for probes used in the Xpert assay were not included here in view of the fact that only interpretations of RIF susceptibility results, not of raw amplification plots, are normally reported during routine practice. Second, despite being approved by the Chinese FDA, a nonstandardized phenotypic DST method was used to determine RIF susceptibility. Our primary results indicated that the use of critical concentrations endorsed by the WHO for MGIT was not appropriate to determine RIF susceptibility for the microdilution method, as systematic differences may exist. However, we included a limited number of wild-type strains, thus limiting the definition of ECOFFs according to EUCAST principles [25]. Third, 1 strain with the Gln517Gln mutation had an elevated MIC compared with the ECOFF, suggesting that it harbors another mutation missed by Xpert (ie, either because it is outside of the *rpoB* region interrogated or because its frequency is below the limit of detection of Xpert). Unfortunately, the amplicons of the partial-length rather than full-length *rpoB* gene sequence were analyzed, which hampers the interpretation of this observation. Fourth, although the Xpert MTB/RIF Ultra assay is expected to have greater sensitivity than the existing MTB/RIF assay, the newer assay has not yet been approved by the Chinese FDA. Therefore, it may be impossible to assess the performance of Xpert Ultra in specimens with low bacterial load. Finally, this study failed to include RIF-resistant cases detected using pDST that had been missed via Xpert. Thus, we could not address potential systematic bias contributed by the pDST methodology.

In conclusion, our data demonstrate that approximately one-sixth of pDST results obtained from cases initially deemed RIF-resistant via Xpert were discordant for RIF resistance in this study. Notably, specific “disputed” mutations with questionable impact on RIF susceptibility were the primary reason for



**Figure 4.** Diagnostic flowchart of RIF-resistant cases based on Xpert MTB/RIF assay results. “RIF resistance inferred” represents the cases with 1 or more undeveloped wild-type probes in the rifampin resistance–determining region but undetermined mutation types in the corresponding region. Abbreviations: MTB, *Mycobacterium tuberculosis*; RIF, rifampin.

discordance in the results of the 2 tests. Meanwhile, cases with very low bacterial load were more likely to be misdiagnosed with RIF resistance by Xpert. Collectively, these results were used to generate a diagnostic flow chart that should be useful for guiding TB patient treatment by emphasizing correct clinical interpretation of Xpert RIF resistance results.

### Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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**Potential conflicts of interest.** All authors: no reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

### References

- World Health Organization. Global Tuberculosis Report 2019. Geneva: World Health Organization; 2019.
- Wang L, Zhang H, Ruan Y, et al. Tuberculosis prevalence in China, 1990–2010; a longitudinal analysis of national survey data. *Lancet* 2014; 383:2057–64.
- Abubakar I, Zignol M, Falzon D, et al. Drug-resistant tuberculosis: time for visionary political leadership. *Lancet Infect Dis* 2013; 13:529–39.
- Weyer K, Mirzayev F, Migliori GB, et al. Rapid molecular TB diagnosis: evidence, policy making and global implementation of Xpert MTB/RIF. *Eur Respir J* 2013; 42:252–71.
- Kim SJ. Drug-susceptibility testing in tuberculosis: methods and reliability of results. *Eur Respir J* 2005; 25:564–9.
- Wilson ML. Recent advances in the laboratory detection of *Mycobacterium tuberculosis* complex and drug resistance. *Clin Infect Dis* 2011; 52:1350–5.
- Helb D, Jones M, Story E, et al. Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *J Clin Microbiol* 2010; 48:229–37.
- Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med* 2010; 363:1005–15.
- World Health Organization. Xpert MTB/RIF Implementation Manual. Technical and Operational “How-To”: Practical Considerations. Geneva: World Health Organization; 2020.
- Miotto P, Cabibbe AM, Borroni E, Degano M, Cirillo DM. 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* 2018; 56:e01599–17.
- Hu P, Zhang H, Fleming J, et al. Retrospective analysis of false-positive and disputed rifampin resistance Xpert MTB/RIF assay results in clinical samples from a referral hospital in Hunan, China. *J Clin Microbiol* 2019; 57:e01707–18.
- Huo F, Luo J, Shi J, et al. A 10-year comparative analysis shows that increasing prevalence of rifampin-resistant *Mycobacterium tuberculosis* in China is associated with the transmission of strains harboring compensatory mutations. *Antimicrob Agents Chemother* 2018; 62:e02303–17.
- World Health Organization. Technical Manual for Drug Susceptibility Testing of Medicines Used in the Treatment of Tuberculosis. Geneva: World Health Organization; 2018.
- Pang Y, Lu J, Wang Y, Song Y, Wang S, Zhao Y. Study of the rifampin monoresistance mechanism in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother* 2013; 57:893–900.
- McNerney R, Cunningham J, Hepple P, Zumla A. New tuberculosis diagnostics and rollout. *Int J Infect Dis* 2015; 32:81–6.
- Ocheretina O, Byrt E, Mabou MM, et al. False-positive rifampin resistant results with Xpert MTB/RIF version 4 assay in clinical samples with a low bacterial load. *Diagn Microbiol Infect Dis* 2016; 85:53–5.
- Sanker P, Kottuthodi RP, Ambika AP, et al. Predictable repeatability issues with GeneXpert-Xpert MTB/RIF (version 4) derived rifampicin resistant tuberculosis results from South India: appreciating the limits of a technological marvel! *Biomed Biotechnol Res J* 2017; 1:76–80.
- Ajvani K, Naik S, Kazi M, et al. Interpreting very low *Mycobacterium tuberculosis* detected on Xpert *Mycobacterium tuberculosis*/rifampicin. *Lung India* 2019; 36:555–7.

19. Berhanu RH, Schnippel K, Kularatne R, et al. Discordant rifampicin susceptibility results are associated with Xpert® MTB/RIF probe B and probe binding delay. *Int J Tuberc Lung Dis* **2019**; 23:358–62.
20. Van Rie A, Mellet K, John MA, et al. False-positive rifampicin resistance on Xpert® MTB/RIF: case report and clinical implications. *Int J Tuberc Lung Dis* **2012**; 16:206–8.
21. World Health Organization. Automated Real-time Nucleic Acid Amplification Technology for Rapid and Simultaneous Detection of Tuberculosis and Rifampicin Resistance: Xpert MTB/RIF System: Policy Statement. Geneva: World Health Organization; **2011**.
22. Berrada ZL, Lin SY, Rodwell TC, et al. Rifabutin and rifampin resistance levels and associated rpoB mutations in clinical isolates of *Mycobacterium tuberculosis* complex. *Diagn Microbiol Infect Dis* **2016**; 85:177–81.
23. Van Deun A, Barrera L, Bastian I, et al. *Mycobacterium tuberculosis* strains with highly discordant rifampin susceptibility test results. *J Clin Microbiol* **2009**; 47:3501–6.
24. Gonzalo X, Claxton P, Brown T, et al. True rifampicin resistance missed by the MGIT: prevalence of this pheno/genotype in the UK and Ireland after 18 month surveillance. *Clin Microbiol Infect* **2017**; 23:260–3.
25. Kahlmeter G. The 2014 Garrod Lecture: EUCAST - are we heading towards international agreement? *J Antimicrob Chemother* **2015**; 70:2427–39.