

LETTER TO THE EDITOR

Prevalence and causes of rifampicin-resistance genotypic/phenotypic discrepancy detected on Xpert MTB/RIF in Singapore

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Dear Editor,

The Xpert *Mycobacterium tuberculosis*/rifampicin (MTB/RIF) (Cepheid, Sunnyvale, CA, US) has been pivotal in tuberculosis (TB) diagnostics, enabling the rapid detection of both TB and RIF resistance. Xpert, a nucleic acid amplification test (NAAT), is recommended by both the World Health Organization (WHO)¹ and Singapore's clinical management guidelines² as a frontline diagnostic tool for TB and RIF resistance. With a high specificity rate (98%) in detecting RIF resistance, Xpert has a positive predictive value (PPV) exceeding 90% in settings where the prevalence of RIF resistance is above 15%.³ However, in Singapore, a country with medium incidence of TB and low prevalence of RIF resistance (<1% among individuals born in Singapore),⁴ the PPV of detecting RIF resistance via Xpert is substantially lower.³ Phenotypic-genotypic discrepant RIF resistance has been reported to result from technical errors, false-positive results in paucibacillary samples,⁵ silent mutations⁶ or disputed mutations where minimal inhibitory concentrations (MICs) fall below critical concentrations in phenotypic drug susceptibility testing (pDST) systems.⁷ We conducted a quality assessment of sputum samples which tested positive for TB and RIF resistance using point-of-care Xpert at the Singapore National Tuberculosis Care Centre (NTBCC) to determine the prevalence and causes of false-positive RIF resistance.

There were 15,427 sputum specimens subjected to Xpert point-of-care testing at NTBCC between December 2018 and June 2022. Of these, 1786 (11.6%) tested positive for MTB with 36/1786 (2%) showing RIF resistance. These 36 samples belonged to 31 unique cases, 29 of which had both pDST and whole genome sequencing (WGS) results available. Two cases failed to isolate MTB on culture and were excluded. Among the 29 cases, 12 (41.4%) had Xpert RIF-resistant/phenotypic-susceptible results, while 17 (58.6%) were confirmed RIF-resistant by both pDST and WGS (Table 1).

We identified 3 main reasons for genotypic-phenotypic discrepancies in RIF resistance detected by Xpert. The first and most common reason for discrepancy was false-positive RIF resistance due to very low bacterial load. This aligns with the understanding that in paucibacillary samples, extended polymerase chain reaction cycles may exaggerate probe signal variability, leading to false-positive results. A significant proportion of discrepancies in our cohort at 9/12 (75%), involved cases with a very low load (cycle threshold [Ct] >28) and no *rpoB* mutation detected on WGS. Five of these cases had separate samples that did not show RIF resistance and were started on first-line treatment. Three cases were initially treated with second-line treatment but were switched to first-line treatment once pDST and WGS confirmed RIF-susceptibility with no *rpoB* mutation. One left the country before treatment initiation. Seven of the 9 cases involved individuals born in Singapore or Malaysia—both low-incidence regions for RIF resistance. For patients presenting with a very low bacterial load and low clinical suspicion of RIF resistance, repeat testing on a separate sputum specimen may be warranted to avoid unnecessary treatment with second-line anti-TB drugs. It is anticipated that the Xpert Ultra (Cepheid, Sunnyvale, CA, US) assay will reduce the likelihood of false-positive RIF resistance results in very low-load samples.^{3,8} However, its utility in similar low-incidence settings remains to be thoroughly evaluated.

The second reason for genotypic-phenotypic discrepancies was silent mutations, detected in 2/12 (16.7%) cases. One case had the *rpoB* T444T mutation (absent probe C on Xpert), diagnosed during contact tracing around an index case with this silent mutation. This case was treated with first-line TB treatment. The second case had mutation *rpoB* c.1278C>T (absent probe A on Xpert; the GenoType MTBDRplus test, also known as the Hain test [Hain Lifescience, Nehren, Germany] showed

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Table 1. Characteristics of rifampicin (RIF)-resistant cases detected by Xpert.

	True RIF resistance	Genotypic-phenotypic discrepant RIF resistance		
		False-positive RIF resistance	Silent mutation	Disputed mutation
Total, n=29	17 (58.6%)	9 (31%)	2 (6.9%)	1 (3.4%)
Load category ^a				
High (Ct value <16)	1 (5.9%)	0	0	0
Medium (Ct value 16–22)	3 (17.6%)	0	1 (50%)	1 (100%)
Low (Ct value 22–28)	6 (35.3%)	0	1 (50%)	0
Very low (Ct value >28)	7 (41.2%)	9 (100%)	0	0
Country of birth: Singapore/Malaysia	7 (41.2%)	7 (77.8%)	2 (100%)	1 (100%)
History of prior tuberculosis treatment	1 (5.9%)	1 (11.1%)	0	0
<i>rpoB</i> mutation on whole genome sequencing, (no.)	S450L (10) H445Y (4) H445D (2) N438- (1)	No mutations (9)	T444T (1) c.1278C>T (1)	L452P (1)

Ct: cycle threshold

Values are expressed as no. (%) unless otherwise indicated.

^a Load category based on Xpert (*Mycobacterium tuberculosis*/rifampicin) semi-quantitative measure.

faint wild type band 1 but absent mutant band). This case was initiated on second-line TB treatment but switched to first-line treatment after confirmation of the silent mutation on WGS. Silent mutations, which do not alter the amino acid sequence of the encoded protein, can result in a false-positive signal on Xpert despite the absence of phenotypic resistance, which can lead to possible overtreatment with second-line drugs.

The final reason was a disputed mutation (in this case the *rpoB* L452P mutation) which was identified in 1/12 (8.3%) case. Disputed mutations confer low-level RIF resistance, with MICs that fall below the critical concentration used in pDST systems. WHO has reported 7 disputed *rpoB* mutations, which account for 12% of RIF resistance based on surveillance data.⁹ Cases with disputed mutations are at high risk of treatment failure and relapse, if treated with first-line TB drugs,¹⁰ highlighting the limitations of pDST in accurately reflecting the clinical implications of certain mutations. In such cases, WGS plays a crucial role in the precise characterisation of *rpoB* mutations to guide treatment decisions.

The findings of this study have significant implications for TB control in settings like Singapore, where the prevalence of RIF-resistant TB among

the local population is low. Given the public health risks of RIF-resistant TB, it is recommended that all cases with RIF resistance detected on rapid genotypic testing be referred to NTBCC for further evaluation and initiation of second-line treatment while awaiting confirmatory tests, such as pDST and WGS, which have long turnaround times. The high rate of false-positive RIF resistance detected by Xpert in very low bacterial load specimens suggests that relying on a single diagnostic test may lead to unnecessary second-line treatment. One practical approach is to repeat the test on a new sample, though the same issue may persist if the bacterial load remains very low. Newer NAATs like Xpert Ultra and BD MAX MDR-TB (Becton, Dickinson and Company, NJ, US), which have improved sensitivity, may help mitigate this problem. The Hain test, which performed well on smear-positive samples and provided results more rapidly than the pDST, can be helpful. By detecting specific *rpoB* mutations, the Hain test can confirm or refute Xpert results, helping to guide early treatment decisions. While Hain tests are not as widely available or rapid as Xpert, their utility lies in providing additional layers of confirmation.

In conclusion, our study highlights the causes of genotypic-phenotypic discrepancies in RIF

resistance in Singapore. A substantial proportion was due to false-positive results in very low-load samples among patients born in Singapore or Malaysia, suggesting a need for cautious interpretation of Xpert RIF-resistant results in very low-load samples. Complementary diagnostic techniques, such as pDST and genotypic tests (Hain and WGS), are essential for confirming resistance and guiding appropriate treatment decisions.

Declaration

The authors declare they have no affiliations or financial involvement with any commercial organisation with a direct financial interest in the subject or materials discussed in the manuscript.

Ethics statement

This study was done as part of an internal audit and quality assessment and was granted exemption for review from National Healthcare Group Domain Specific Review Board (NHG DSRB Ref: 2023/00061).

Keywords: drug resistance, infectious diseases, molecular diagnostics, respiratory medicine, tuberculosis

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