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False-positive rifampicin resistance on Xpert® MTB/RIF: case report and clinical implications

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SUMMARY

The World Health Organization had endorsed Xpert® MTB/RIF (Xpert) as the initial diagnostic for multidrug-resistant tuberculosis (TB) or TB suspects co-infected with the human immunodeficiency virus. We investigated an unexpected case of rifampicin (RMP) resistance on Xpert using repeat Xpert, smear microscopy, MTBDR*plus* assay, culture, drug susceptibility testing, spoligotyping and *rpo*B gene sequencing. A false-positive result was most likely, given the wild type *rpo*B gene sequence and exclusion of both mixed infection and mixture of drug-susceptible and drug-resistant populations. When decentralising Xpert, test performance characteristics need to be understood by health care workers and methods of confirmation of RMP resistance need to be accessible.

Keywords

tuberculosis; MDR-TB; assay performance; false-positive rifampicin resistance

MULTIDRUG-RESISTANT tuberculosis (MDR-TB) threatens global TB control. The World Health Organization (WHO) has endorsed the Xpert® MTB/RIF test (Xpert; Cepheid, Inc, Sunnyvale, CA, USA) as the initial diagnostic for those at risk of MDR-TB or human immunodeficiency virus (HIV) associated TB.[1] Rapid diagnosis of rifampicin (RMP) resistance could reduce the morbidity, mortality and transmission of drug-resistant TB.

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The first large clinical Xpert validation study reported 100% specificity for the detection of RMP resistance after resolution of discordances by *rpo*B genotyping.[2] In a subsequent multicentre study, the specificity for RMP resistance was found to be lower (98.3%).[3]

We report a comprehensive investigation of an unexpected case of RMP resistance on Xpert and discuss the implications for patient management.

CASE REPORT

In April 2010, a 49-year-old HIV-infected (CD4 count 169 cells/mm^3) male presented to a primary care clinic in Johannesburg, South Africa, with a 6-week history of cough. He had no TB treatment history, but had recently moved from Msinga, KwaZulu-Natal, where an outbreak of extensively drug-resistant TB had occurred in 2006.

An Xpert assay was positive for RMP-resistant *Mycobacterium tuberculosis*, with ΔCt Max exceeding 3.5 cycles for probe B (first generation software). A repeat Xpert assay indicated RMP-susceptible *M. tuberculosis* complex (MTC). Anti-tuberculosis treatment and antiretroviral treatment were initiated while awaiting confirmatory results. The patient successfully completed 6 months of first-line anti-tuberculosis treatment.

The discrepancy in the above results led to a full investigation, for which the patient gave informed consent. Smear microscopy was negative or scanty for acid-fast bacilli, except for induced sputum (Table 1). Cultures (BACTEC Mycobacteria Growth Indicator Tube 960, BD, Sparks, MD, USA), GenoType® MTBDR*plus* (Hain LifeScience GmbH, Nehren, Germany) and Xpert assays were positive for MTC. No assays indicated technical errors. The first and third Xpert assays indicated RMP resistance, based on a delay in probe B (Ct max 4.9 and 4.1); the second Xpert was RMP-susceptible. MTBDR*plus*, performed directly on decontaminated sputum to avoid RMP-susceptible strain overgrowth during culture, showed RMP-susceptible MTC for all three specimens. Phenotypic drug susceptibility testing (indirect proportion method on 7H10 media containing 1.0 μg/ml RMP) also demonstrated RMP susceptibility. DNA sequencing confirmed wild type *rpo*B sequences in all cultures.[4] Spoligotyping demonstrated an identical spoligotyping pattern (ST4) for all three cultures.[5]

An administrative error was considered unlikely, as RMP resistance on Xpert was detected on independent specimens collected on 2 different days. All three cultures shared the same spoligotype pattern (Table 2), and no drug-resistant TB patient was diagnosed or treated at the clinic at that time. Mixed infection with multiple *M. tuberculosis* strains was unlikely, as spoligotyping demonstrated the absence of a background hybridising pattern and an identical pattern (ST4) in all three isolates. Heteroresistance, i.e., mixed infection with resistant and susceptible populations of the same *M. tuberculosis* strain, was unlikely, as no growth was observed in any of the RMP-containing plates of isolates 1 and 2 (isolate 3 was contaminated), and careful examination of the DNA sequence chromatogram failed to identify underlying peaks.

DISCUSSION

A false-positive result was the most likely cause of the observed discrepancies, given the wild type *rpo*B gene sequence, exclusion of mixed infection with multiple *M. tuberculosis* strains and exclusion of a mixture of drug-susceptible and drug-resistant populations. Despite these comprehensive investigations, heteroresistance could not be confidently excluded as unprocessed sputum or cartridge amplicons were not sequenced. Others have reported false-positive RMP results;[3,6,7] Marlowe et al. also identified a specimen that was repeatedly RMP-resistant on Xpert but susceptible on phenotypic DST and *rpo*B

sequencing.[6] In contrast, Theron et al. identified six RMP-resistant cases on Xpert, five of which were susceptible on phenotypic DST, although five were genotypically resistant by sequencing and/or MTBDR*plus*.[7] The complexity of these investigations demonstrates the difficulty in confidently distinguishing false-positive from true-positive RMP-resistant results, particularly in clinical practice.

In response to reports of false-positive RMP-resistant results, the manufacturer performed a root cause analysis, which identified the bead manufacturing scale-up and annealing temperature requirements of probe B as potential causes. Solutions include improved bead reconstitution, a software change and adjustment of probe B to increase robustness.[8] The revised assay is being evaluated. While fewer false-positive results can be expected following assay improvements, an almost perfect (close to 100%) assay specificity will be required before high positive predictive values are achieved in TB suspects in HIV endemic, low MDR-TB prevalence areas.[1,9]

The assay performance has important implications for patient management. The WHO recommends a confirmatory DST in patients with RMP resistance on Xpert. Use of the MTBDR*plus* assay will lead to a median delay between initial diagnosis and availability of results at the clinic of 40 days,[3] while phenotypic DST will result in even longer delays.[3] These delays give rise to the clinical dilemma of which regimen to start. The WHO recommends MDR-TB treatment in patients diagnosed with RMP resistance on Xpert, but Xpert can be performed at a clinic or microscopy centre, a setting that rarely has access to second-line drugs. Should health care workers start first-line treatment, or should they defer starting any treatment while awaiting the patient's arrival at the MDR-TB treatment centre? Starting first-line drugs could pose the risk of amplification of resistance to ethambutol or pyrazinamide,[10] limiting future treatment options, while not starting any drugs poses infection control issues and increased risk of death. In patients with low pretest probability living in an area with poor access to MDR-TB treatment, clinicians may reserve the currently limited MDR-TB treatment capacity for those with confirmed MDR-TB or try to balance the risks and benefits of different regimens based on risk assessment for MDR-TB, type of confirmatory test available ('rapid' MTBDR*plus* or slower phenotypic DST), ease of access to MDR-TB treatment, financial burden of referral for MDR-TB treatment (transport cost and loss of employment during hospitalisation), risk of transmission to vulnerable individuals (young children or HIV-positive relatives), patient's HIV status, risk of death while awaiting confirmatory results, risk of toxicity from second-line anti-tuberculosis drugs, and risk of amplification of drug resistance.

In conclusion, this report highlights the need for health care workers' understanding of assay performance characteristics when decentralising the diagnosis of drug-resistant TB. These issues should not, however, diminish enthusiasm for the Xpert assay.

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Table 1

Description of laboratory investigations performed

Date*	Smear microscopy result†	Xpert® MTB/RIF	Liquid culture	Line-probe assay directly on sputum‡	Culture on 7H10 media with 1.0 μg/ml RMP	Spoligotype	Gene sequence
Day 0	Negative for AFB	Not performed	Not performed	NA			
Day 1	Scanty positive	Not performed	Contaminated	Not performed			
Day 9	Scanty positive	RMP-resistant *M. tuberculosis*	Positive for *M. tuberculosis* after 19 days	Positive for *M. tuberculosis*, RMP- and INH-susceptible	RMP-susceptible	ST4	wt for *inh*A promoter, *rpo*B and *kat*G gene
Day 10	Positive++ (induced sputum)	Not performed	Positive for *M. tuberculosis* after 14 days	Positive for *M. tuberculosis*, RMP- and INH-susceptible	RMP-susceptible	ST4	wt for *inh*A promoter, *rpo*B and *kat*G gene
Day 11	Scanty positive	*M. tuberculosis* present, no RMP resistance detected	Positive for *M. tuberculosis* after 17 days	Positive for *M. tuberculosis*, RMP- and INH-susceptible	Contaminated	ST4	wt for *inh*A promoter, *rpo*B and *kat*G gene
Day 16	Positive+	RMP-resistant *M. tuberculosis*	Not performed	Not performed			

*Number of days represents time since presentation to clinic with symptoms of TB; first-line treatment was initiated on Day 9.

†Classification of smear microscopy: scanty positive = 1 AFB/100 immersion fields; positive + = 10–99 AFB/100 immersion fields; positive ++ = >100 AFB/100 immersion fields.

‡GenoType MTBDR*plus* assay (Hain LifeScience, GmbH, Nehren, Germany).

RMP = rifampicin; AFB = acid-fast bacilli; NA = not applicable; INH = isoniazid; wt = wild type.

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Table 2

Investigation of main hypothesis (false-positive RMP resistance) and alternative hypotheses

Hypothesis	Investigation	Result	Conclusion
Main hypothesis False-positive RMP resistance result according to the Xpert® MTB/RIF assay	Line-probe assay * Gene sequencing	RMP-susceptible *M. tuberculosis* complex Wild-type sequence for RRDR of the *rpoB* gene on three samples	False-positive RMP resistance likely
Alternative hypotheses Administrative error	Collection and on-site processing of 3 samples on 3 different days Spoligotyping of three samples to confirm same strain of *M. tuberculosis*	2 of 3 samples RMP-resistant, one RMP-susceptible Same pattern (ST4) for all three cultures	No administrative error
Infection with multiple strains	Spoligotyping of 3 positive cultures	No evidence of multiple strains	Infection with multiple strains unlikely
Mixed population of susceptible and resistant strains (heteroresistance)	Growth on 7H10 media containing 1.0 μg/ml RMP	No growth on any of the plates	Heteroresistance unlikely

* MTBDR*plus* assay (Hain LifeScience GmbH, Nehren, Germany).

RMP = rifampicin; RRDR = RMP resistance-determining region.