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


*Department of Epidemiology, University of North Carolina, Chapel Hill, North Carolina, USA; †Right to Care, Johannesburg, ‡Clinical Microbiology and Infectious Diseases, University of Witwatersrand, Johannesburg, §National Health Laboratory Services, Johannesburg, *Department of Molecular Medicine and Haematology, University of the Witwatersrand, Johannesburg, †Department of Molecular and Cellular Biology, Division of Molecular Biology and Human Genetics, Faculty of Health Sciences, Stellenbosch University, Cape Town, South Africa

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, MTBDRPlus assay, culture, drug susceptibility testing, spoligotyping and rpoB gene sequencing. A false-positive result was most likely, given the wild type rpoB 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.

KEY WORDS: 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. Rapid diagnosis of rifampicin (RMP) resistance could reduce the morbidity, mortality and transmission of drug-resistant TB.

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

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/mm³) 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® MTBDRplus (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. MTBDRplus, 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 rpoB sequences in all cultures. Spoligotyping demonstrated an identical spoligotyping 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 rpoB

Table 1 Description of laboratory investigations performed

<table>
<thead>
<tr>
<th>Date</th>
<th>Smear microscopy result</th>
<th>Xpert® MTB/RIF</th>
<th>Liquid culture</th>
<th>Line-probe assay directly on sputum</th>
<th>Culture on 7H10 media with 1.0 µg/ml RMP</th>
<th>Spoligotype</th>
<th>Gene sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>Negative for AFB</td>
<td>Not performed</td>
<td>Not performed</td>
<td>NA</td>
<td>Not performed</td>
<td>RMP-susceptible</td>
<td>ST4</td>
</tr>
<tr>
<td>Day 9</td>
<td>Scanty positive</td>
<td>Not performed</td>
<td>Contaminated</td>
<td>Positive for M. tuberculosis after 19 days</td>
<td>Positive for M. tuberculosis, RMP- and INH-susceptible</td>
<td>RMP-susceptible</td>
<td>ST4</td>
</tr>
<tr>
<td>Day 11</td>
<td>Scanty positive</td>
<td>M. tuberculosis present, no RMP resistance detected</td>
<td>Positive for M. tuberculosis after 14 days</td>
<td>Positive for M. tuberculosis after 17 days</td>
<td>Contaminated</td>
<td>ST4</td>
<td>wt for inhA promoter, rpoB and katG gene</td>
</tr>
<tr>
<td>Day 16</td>
<td>Positive +</td>
<td>RMP-resistant M. tuberculosis</td>
<td>Not performed</td>
<td>Not performed</td>
<td>Not performed</td>
<td>RMP-susceptible</td>
<td>ST4</td>
</tr>
</tbody>
</table>

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

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

2GenoType MTBDRplus assay (Hain LifeScience GmbH, Nehren, Germany).

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

Table 2 Investigation of main hypothesis (false-positive RMP resistance) and alternative hypotheses

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

*MTBDRplus assay (Hain LifeScience GmbH, Nehren, Germany).

RMP = rifampicin; RRDR = RMP resistance-determining region.
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;\textsuperscript{3,6,7} Marlowe et al. also identified a specimen that was repeatedly RMP-resistant on Xpert but susceptible on phenotypic DST and *rpoB* sequencing.\textsuperscript{8} 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\textsuperscript{plus}.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{plus} assay will lead to a median delay between initial diagnosis and availability of results at the clinic of 40 days,\textsuperscript{3} while phenotypic DST will result in even longer delays.\textsuperscript{2} 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,\textsuperscript{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\textsuperscript{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.

Acknowledgements

The authors thank J Basset, N Beylis, G Coetzee, N Gous, A Jordaan, I Sanne, W Stevens and L Streicher for their contributions. This work was supported by the National Institutes of Health (ICOHRTA AIDS/TB U2RTW007370) and the United States Agency for International Development (President’s Emergency Plan for AIDS Relief in a grant to Right to Care [674-A-00-08-00007-00] and to Reproductive Health Research Unit).

References

L’Organisation Mondiale de la Santé a adopté Xpert® MTB/RIF (Xpert) comme outil initial de diagnostic des sujets suspects de tuberculose à germes multirésistants ou de tuberculose associée au VIH. Nous avons investigué un cas inattendu de résistance à la rifampicine lors de l’Xpert, en utilisant une répétition de l’Xpert, l’examen microscopique des frottis, le test MTBDRplus, la culture, les tests de sensibilité aux médicaments, le spoligotypage et le séquençage du gène rpoB. Il est le plus probable que le résultat soit faussement positif vu le type sauvage de la séquence du gène rpoB et vu l’exclusion tant d’une infection mixte que d’une population mixte de germes sensibles et résistants aux médicaments. Si l’on devait décentraliser l’Xpert, les caractéristiques de performance du test devraient être comprises par les travailleurs des soins de santé et la confirmation de la résistance à la rifampicine devrait être accessible.

La Organización Mundial de la Salud aprobó la prueba Xpert® MTB/RIF (Xpert) en el diagnóstico inicial de las personas con presunción de TB multidrogorresistente (MDR) o de TB asociada con la infección por el virus de la inmunodeficiencia humana (VIH). En el presente artículo se investigó un caso inesperado de resistencia a rifampicina, con el uso de la prueba Xpert: se practicó una repetición de la prueba Xpert, una baciloscopia, la prueba MTBDRplus, el cultivo, las pruebas de sensibilidad a los medicamentos, el espoligotipado y la secuenciación del gen rpoB. Muy probablemente se trató de un resultado positivo falso, dada la secuencia natural del gen rpoB y la exclusión de una infección mixta y de la presencia de poblaciones de micobacterias sensibles y resistentes. Cuando se descentraliza el diagnóstico de la TB resistente a los medicamentos, es importante que los profesionales de salud comprendan las características del mecanismo de la prueba y es preciso contar con un método de confirmación de la resistencia a rifampicina.