Silent Mutation in *rpoB* Detected from Clinical Samples with Rifampin-Susceptible *Mycobacterium tuberculosis*

We read with great interest the note of Alonso et al. (1) concerning the existence of a silent mutation in *rpoB* in *Mycobacterium tuberculosis* strains. In this note, the authors describe a phenomenon already observed by several study groups. The misassignment of phenotypic resistance due to the results of molecular tests, whenever there is an absence of hybridization with wild-type probes, is a matter of great relevance.

Our experience with the Xpert system in clinical samples, which we have published in part elsewhere (3), corroborates the results obtained by Alonso et al. (1) in clinical strains. In fact, in our series of almost 150 specimens retrospectively studied and detected as positive for the *Mycobacterium tuberculosis* complex with Xpert MTB/RIF, the system also reported a “Rif resistance detected” result in eight cases, six of which showed a lack of hybridization with probe E and two a lack of hybridization with probe B. The isolates corresponding to these samples had already been analyzed for susceptibility testing using the Bactec 460 radiometric method. Of the eight isolates defined as resistant by Xpert MTB/RIF, two were phenotypically susceptible to rifampin (those with the probe B failure). Sequencing confirmed that these two isolates (1.4%) had a silent TTC (Phe)-to-TTT (Phe) shift (the same replacement found in the isolates included in the study by Alonso et al. [1]).

In our opinion, these data highlight the importance of correct interpretation of results of rifampin resistance when working with molecular tests, since this drug is the cornerstone of antituberculosis treatment (6). Even though therapeutic alternatives to the use of rifampin are available, the noninclusion of this drug, based on a misleading molecular test result suggesting resistance, may have undesirable clinical and epidemiological consequences—all the more so when, as in our study, the detection is made directly in clinical specimens (2, 3), since this means that rifampin is excluded from the very beginning of the drug therapy.

We would also like to comment on the conclusion of Alonso et al. (1) regarding the need for confirmation of the results for drug resistance obtained by the molecular tests, especially when the detection of mutations (and therefore the assumption of resistance) is based on the “absence of hybridization

with the wild-type probe” pattern. Even though these methods are a very useful preliminary measure, phenotypic susceptibility testing is still the gold standard for determining the real drug resistance of the clinical isolates (4, 5). We also stress that specifications and reports of the results provided by the commercial molecular systems should be precise and rigorous and should not automatically correlate genetic mutation with phenotypic resistance.

REFERENCES

1. Alonso, M., et al. 2011. Isolation of *Mycobacterium tuberculosis* strains with a silent mutation in *rpoB* leading to potential misassignment of resistance category. *J. Clin. Microbiol.* **49**:2688–2690.
2. Boehme, C. C., et al. 2011. Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. *Lancet* **377**: 1495–1505.
3. Moure, R., et al. 2011. Rapid detection of *Mycobacterium tuberculosis* complex and rifampin resistance in smear-negative clinical samples by use of an integrated real-time PCR method. *J. Clin. Microbiol.* **49**:1137–1139.
4. WHO. 2010. Multidrug and extensively drug-resistant TB (M/XDR-TB). 2010 Global report on surveillance and response. World Health Organization, Geneva, Switzerland.
5. WHO. 2011. Rapid implementation of the Xpert MTB/RIF diagnostic test. Technical and operational ‘how to.’ Practical considerations. World Health Organization, Geneva, Switzerland.
6. WHO. 2010. Treatment of tuberculosis guidelines, 4th ed. World Health Organization, Geneva, Switzerland.

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