Molecular Diagnosis of Rifampin-Monoresistant Tuberculosis in Indian Patients: Problems with a Discordance Analysis

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We read the recent paper by Rufai et al. with interest (1). The authors tested a subset of samples containing Mycobacterium tuberculosis previously tested by the Hain line probe assay (LPA) with the Xpert MTB/RIF assay to identify discordant results between these two test methods. The authors’ results favored LPA over Xpert MTB/RIF in sensitivity for rifampin (RIF) resistance detection. These results vary considerably from ones that we reported in several previous studies as well as the results of a recent Cochrane review, which show approximately 95% sensitivity for RIF resistance detection. Several aspects of the authors’ study design raise concerns. First, instead of prospectively testing all 405 samples against a gold standard drug susceptibility test (DST) and then examining the performance of LPA and Xpert MTB/RIF in comparison to this standard, the authors instead tested each sample with LPA alone. LPA results were then used to select two sets of samples for confirmatory testing by Xpert MTB/RIF, one set which was RIF monoresistant and one set which was susceptible to both isoniazid (INH) and RIF. Failing to test all samples with both methods and to judge the results against phenotypic DST, the gold standard, introduced a strong bias in favor of LPA. As designed, the study would identify isolates that were defined as RIF resistant by LPA but missed by Xpert MTB/RIF; however, it would not detect isolates that were defined as RIF resistant by Xpert MTB/RIF but missed by LPA. Thus, the study highlights problems with the Xpert MTB/RIF assay, but it was not designed to reveal all of the false-negative results that could result from LPA testing. Second, it is not clear why the authors excluded testing the 72 samples associated with multidrug resistance. As presented, the results are valid only for an unusual subset of isolates which test RIF monoresistant by LPA. We also noted that this study included an unexpectedly high percentage of specimens with uncommon RIF resistance mutations. This observation raises additional questions such as the patient population from which these samples were drawn and whether all samples came from the same center, which may represent clonal spread. These are important questions for which we have no data. This study highlighted a specific Xpert MTB/RIF defect in detecting mutations at position 533 (CCG mutations, in particular). Our unpublished data suggest that this assay actually detects these specific mutations quite well. However, it should be noted that the Xpert assay is not designed to pick up mixtures of RIF-resistant and RIF-susceptible infections, and this is a particular problem with mutations such as 533 CCG. We wonder whether the test samples originated from a population with a high incidence of RIF-monoresistant mixed infections. Again, there are no data on this key point. In summary, the apparent peculiarities of the patient population from which study samples were obtained and the strong potential for bias inherent in comparing Xpert results against known positive LPA samples raise considerable questions as to the generalizability of the results to tuberculosis patients in India and elsewhere.

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