Reviewer’s report

Title: A commercial line probe assay for the rapid detection of rifampicin resistance in Mycobacterium tuberculosis: a systematic review and meta-analysis

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Reviewer: Marcel A Behr

Reviewer’s report:

The authors have presented a systematic review that indicates that the commercial line probe assay for rapid detection of rifampin resistance appears to have very good operating characteristics. Therefore, what remains to be determined is whether this assay should be used in clinical settings, for which there appears to be no data at present. It is therefore unclear what take-home message can be derived from this work, other than that further studies are needed.

There are two points that I think might bear greater consideration, both to understand the existing data, and to position the potential utility of this test.

Major issues:

1) Bacterial variability. Since different strains in different countries have been associated with different rpoB mutations, I think it is important to determine to what degree the primary studies accounted for this issue. For instance, if one applied a single test for a single rpoB polymorphism during the W strain outbreak in New York, one might easily achieve very high sensitivities for predicting phenotypic rifampin resistance. But does this really represent a denominator or > 200 when you are essentially re-sampling the exact same clone out of > 200 patients affected by the same strain? It would be instructive to learn how many different strains have been tested by this method in order to get a sense of whether this tool will apply equally in different epidemiologic settings.

2) Laboratory issues, including cost. While the paper uses “samples” as the unit of analysis, it should be noted that microbiology labs are supposed to receive 2-3 samples per patients at the time of diagnosis, and then further samples to test for culture conversion (or failure, as the case may be). It would be useful to describe whether the studies tested different samples from the same patient, to look for concordance, or whether the studies simply chose one sample per patient, which is likely the case in these sample-based studies. The next issue is whether one can really estimate the specificity of a test from such non-representative studies. In terms of applying this test on clinical samples, it must be noted that for every 100 sputum samples coming to a lab, only 10 (at most) will be positive for M. tuberculosis. Then, of these 10 positive for M. tuberculosis, it would be remarkable if as many as 1 would be rifampin resistant. Therefore, applying this test directly to sputum would require well over 100 tests for every positive result. To correctly determine the specificity in regular laboratory conditions, it would be important to apply this test on such a ratio of samples, not on a series where 2/3 are resistant. Likewise, if this test were to be used on positive cultures, it might be important to reflect on the costs, based on the estimate provided of $116 per sample. For a laboratory with 10,000 sputum samples per year, there might be 500 positive cultures in a year, representing something like 150-200 distinct patients (multiple samples from a patient). Of these patients, one can then determine the cost based on different prevalence estimates, e.g. 2% rifampin-resistance would yield 3 patients per year. Testing these 500 positive cultures would cost ~$50,000 per year, to advance the detection of rifampin resistance in 3 patients by the interval between a LIPA and a phenotypic result (1-2 weeks?). Perhaps this issue is therefore relevant not
just in certain regions of the world but in most labs that have a budget to defend.

Minor points.

Page 4. there are 3 references provided to state that 95% of isolates have mutations in a 81-bp hot-spot. First, the literature on this is quite dense, perhaps it would be better to cite a review on this, rather than primary references. Second, one of the references pertains to E. coli. Third, it might be worth checking the literature for reports of rifampin resistance that do not have mutations in this hot-spot.

Methods: What does resolved by discussion mean?

Methods: By a minimum of 10 samples, do the authors require that the samples come from different patients, and is there any requirement that the samples have different genotypes (to avoid repeat testing of the same clone)?

Discussion: What is meant by regions of the world most in need of rapid detection of RIF-resistant TB? Is the US such a region, based on the experience of the 1990’s?

Discussion: “This prevalence of 67% may different from the prevalence seen in …many settings”. I am not aware of any clinical setting where 2/3 of M. tuberculosis isolates are rifampin resistant. I think it would be better to provide a reference or two on the prevalence that one observes in studies in relevant settings, where bacteria were isolates and routinely tested, and then state more definitively that this prevalence is not consistent with routine clinical practice.

Discussion: In terms of additional studies, do the authors advocate consecutive selection of patients or consecutive selection of sputum samples? The latter is clearly more conducive to routine laboratory work-flow, so if they suggest the former, it might be useful to determine the rationale. Is it worth including a point of demonstrating the operating characteristics across a broad panel of genotypically different strains?

What next?: Accept after minor essential revisions

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests: 'I declare that I have no competing interests'