Reviewer's report

Title: Analysis of gene mutations associated with isoniazid, rifampicin and ethambutol resistance among M. tuberculosis isolates from Ethiopia

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Reviewer: Andrea von Groll

Reviewer's report:

General comments:
The authors present an important study using the commercial molecular method for detection of M. tuberculosis resistant. This commercial method have been widely studied for the detection of M. tuberculosis resistant to INH and RIF since it has been recommended by WHO to be introduced in reference laboratories in several countries. As noted by the authors, each population may have a genetic variation of strains of Mycobacterium tuberculosis that could interfere with the results of this method. Thus it is important to validate with strains of the local population before its implementation in the routine.

The methodology, results and analysis of results were conducted in a scientifically appropriate and following the standards of this type of study.

The results were in line with previous studies in other populations, but it did not present any new information in relation to previous studies. Thus, to enrich the manuscript, authors are encouraged to provide more information:

- Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

1. Whereas the authors performed the tests GenoType® MTBDRsl, because they only present the results for EMB and did not present the results to fluoroquinolones and aminoglicosides obtained from this test?

2. Have the authors any information on the genotypes of the MDR strains? Since all had the same mutations could have an epidemiological link.

3. Both the background and during the discussion the authors should review the sentence: “Studies have shown that mutations in the katG, inhA, kasA and ahpC genes were associated with INH resistance.”

Mutations in kasA and ahpC ... are no longer considered to be linked to INH resistance and there are other genes, such as ndh, which could be cited.

4. A important point that should be reassessed is the sentence present in the discussion: “Studies have also shown that 8% to 43% of INH resistance are defined as the low-level drug resistance mainly caused by the mutations in the promoter region of inhA gene, involving the -15, -16 and -8 locus [29]. The genoType MTBDRplus involves two wild-type probes (WT -15/-16 and WT -8) and four mutation-type probes, covering mutations of Cys15#Thr, Ala16#Gly,
Thr8#Cys and Thr8#Ala, for detection of INH low-level drug resistance. In this study, we have observed that the low-level drug-resistance proportion was 6%, close to the low limit of the reported range."

- In the paper 29 cited in this text, there is no information about the level of resistance and mutations involving the -15, -16 and -8 locus. I think that the authors should not affirm that “observed that the low-level drug-resistance proportion was 6%, close to the low limit of the reported range”, unless they have determined the minimal inhibitory concentration (MIC) to INH.

5. To enrich the manuscript, authors are encouraged to provide more information on the non-concordant strains for INH and EMB. It would be interesting to identify the mechanism of resistance by sequencing of the genes (whole katG, inhA promoter and gene, embCAB genes) to serve as subsides to improve molecular methods.

- Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Title:
1. The title should not have abbreviations: M. tuberculosis should be changed to Mycobacterium tuberculosis.

Abstract:
Review small details like:
2. the first M. tuberculosis should also be complete: Mycobacterium tuberculosis.
3. Standardize the test name (there is a space after GenoType in the first name and not in the second name): GenoType MTBDRplus GenoTypeMTBDRsl. The correct is GenoType® MTBDRplus and GenoType® MTBDRsl
4. Genes must be in italics: rpoB, inhA…
5. It would be interesting to insert the period of collection of isolates in the methodology
6. In the results: correct the position of the mutation in rpoB gene: “one at His531#Asp..”, the correct is His526#Asp

Text:
7. Genes must be in italics throughout the text: rpoB, inhA…
8. In the background, the author could add the percentage of MDR in Ethiopia.
9. The authors should add in the text, the country which was performed the study 31:
   “In 20% of the resistant isolates, mutation was detected only at the wild type probes, which is different from the previously reported gene mutation distribution, 37% at Ser531#Leu, 3% at His526#Asp and in 60% of the isolates, mutation was detected only at the wild type probes [31]”
10. Since Patients’ history of previous treatment was associated to resistance,
the authors could report in the discussion, the treatment regimen for TB and availability of these drugs in Ethiopia.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests