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: Pontus Jureen

Reviewer’s report:

The manuscript (denoted as MS from hereon) is performed on Ethiopian strains. Its essence is to evaluate the HAIN kits for detection of RMP, INH and EMB resistance by using the phenotypic BacT/ALERT system and thereafter discuss such methods suitability for in this particular tuberculosis population.

Local studies like this is important to report as the authors correctly discuss that methods that specifically detects certain mutations may show different specificity and/or sensitivity depending on the geographical setting. Thus the target population that the kit was developed for may not resemble the global situation, especially in various local tuberculosis populations.

General comments:

The MS is in a premature state and should preferably be carefully revised in regards to several aspects:

- To the language, there are several grammatical and typographic errors as well as misspellings.
- Data should be chosen to either be presented in the running text or in the tables. As it is now the majority of the information in the results part is also given in the tables.
- MS should preferably be shortened as much of the information is repeatedly given i.e. in introduction, results and discussion. This is true for both information quoted from the current literature as well as the data reported in the present MS, but not of curse the introduction for the latter.
- Throughout the MS, preferably use the HAIN kit denominations as much as possible, this instead of using the specific mutations. The HAIN testing is an indirect testing which do not give the sequence, for sequence analysis the application of DNA sequencing would be needed.
- Genes are denoted in italic whereas the protein is denoted as normal text. This should be corrected throughout the MS.
- Mutations are commonly written as Ser531Leu, not Ser531#Leu. This should be corrected throughout the MS.
- Please insert numbers for each line, it is difficult to comment upon a MS without these numbers.
- In terms of drug resistance; preferable use “susceptible strains” and not
Abstract:
It is not necessary to include “%” when total and fractions are given as well.
Please use either or.

Background:
WHO data can be updated, there are several more recent reports since 2006.
Pg3, last sentence: “the development of “ should be deleted as these genes are
indeed involved in resistance and not only involved in the development of
resistance.
Pg3, last sentence: What is the sens. / spec. and speed based on? Any
reference? Any particular method in mind? i.e. in house methods, HAIN,
Cepheid?
Pg 4 an example of misspelling; aminoglycosides and not aminoglicosides.
Please revise the MS for misspellings.
Pg 4. DNA does not need to be explained, hardly PCR either.
Pg 4. First paragraph last sentence. If not all, so nearly all RMP-R strains do
contain mutations in rpoB.

Methods:
First paragraph: How were the strains selected? Any exclusion or inclusion
criteria? Were there any consideration of excluding clonal isolates / resampling of
the same patients? What laboratory steps were performed before culture and
HAIN?
Second paragraph: It is of less interest to quote the HAIN manual. Instead try to
focus and describe what was not done according to the manual or what was
chosen in the optional steps of the HIAN procedures. Was the DNA extracted
from cultures or clinical samples? Which PCR program was chosen, the
extended (for clinical samples) or the shorter (for cultures). Was the DNA
concentration measured? What brand and model was the water bath? This could
be of interest for readers as it open up opportunities for alternative apparatus
than the standard Twincubator. The usage of water bath (instead of Twincubator)
could also briefly be dwelled upon in the discussion part.
Third paragraph: I strongly suggest that the authors should read a book or so in
basic genetics. A promotor (not promoter) region is a non coding region, thus for
these regions only the nucleotides ATCG are valid. In this context the translation
into amino acids T = Thr, A = Ala is completely wrong. This should be revised
throughout the MS.
Fourth paragraph: The DST testing concentrations (breakpoints) are indeed odd.
WHO recommends the following concentrations: BACTEC 460 INH 0,1 mg/l,
RMP 2 mg/l, EMB 2,5 mg/l and for MGIT 960 INH 0,1 mg/l, RMP 1 mg/l, EMB 5
mg/l. Using other breakpoints will likely skew the results and conclusions, I will
come back to this later on. What is the rational for using these concentrations? Is
there any international breakpoint recommendations for BacT/ALERT that can be
referred to? For the present study this is an essential standpoint information and a reference (no 23) in the German language is not advisable as this is only comprehensible for a minority of the research society (I wonder if even all authors for this MS can read and understand reference no 23?).

Results:

The demographic paragraph and table 1; It is unclear how these contributes to the MS? These parts should either be deleted or integrated into the MS. If integrated, the demographic data should be combined and discussed with the main scope of the present study, i.e. the HAIN results. If this is not possible or there is no added value, then the demographic part should preferably be excluded.

Second paragraph, second sentence; were there no isolates with both katG and inhA alterations?

Second paragraph, last sentence; a phenotypic and a genotypic method can have “concordant etc” results but not “similar” results, revise this throughout the MS.

Discussion.

The first two sentences are exactly the same, word by word, as what is found in the introduction. I do believe that the authors can put more effort in writing a manuscript than this. If there are any more by me unnoted things like this, please rewrite! It will be for your own benefit as the future readers surely will interpret this as sloppiness.

2nd paragraph; There is a meta study of the HAIN test by Bawanga F that preferably should be considered to be discussed, especially when discussing the sens. and spec. for this method.

pg 12 first sentence. The interpretation of the results have been extrapolated in an incorrect way. The materials in the present study are not from all Ethiopia, only the Gondor region. Also, there is no evidence that this material is representing the Gondor region. A better description of the material (in the M&M part) may convince the reader that the present material is representative for Gondor.

Pg 12 end of second paragraph. Why is the presence of low level INH resistance marker (inhA) indeed low? Does the author think this is a local variation? Or do the authors think this has something to do with that they phenotypically test the strains at a 10 fold higher INH concentration than recommended for other broth based methods?

Pg 12, 3rd line from end. Write: …in the region of codon 507-533.

Pg 12, last sentence, This describes the HAIN kit and thus belongs to M&M

Pg 13, ref 31 and the discussion with the comparison of the Ethiopian mutation frequencies. This reference (no 31) is unique in its way of describing mutations and their frequencies among clinical isolates. This comparison leads to skewed conclusions. The material presented in the present MS do reflect well what is found globally, although there is an exception, the high frequency (20%) of
RMP-R isolates with no mutations detected by HAIN, in spite being only 3 isolates this would be more relevant to discuss.

Pg 13. Once again, do the authors think their results would have looked differently if a higher EMB concentration was tested for? i.e. a concentration that more resembles what is by WHO recommended for other broth based methods. If a higher breakpoint for EMB was used, wouldn´t more strains be defined as susceptible? Would that increase sensitivity?

**Level of interest:** An article of limited interest

**Quality of written English:** Needs some language corrections before being published

**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