Author's response to reviews

Title: A first insight into the genotypic diversity of Mycobacterium tuberculosis from Rwanda

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Author's response to reviews: see over
RESPONSE TO REVIEWER’S COMMENTS

Reviewer's report
Title: A first insight into the genotypic diversity of Mycobacterium tuberculosis from Rwanda
Version: 3 Date: 2 October 2012
Reviewer: Matthew Bates

- Major Compulsory Revisions

The manuscript is much improved and mainly requires only spelling or grammar corrections as
detailed below. I have just one major reservation remaining which I think could be easily
addressed by the authors.

In the abstract the authors conclude:

“Mycobacterium tuberculosis is the most prevalent species of Mycobacterium tuberculosis
complex in Rwanda, and SIT 52 (T2) the predominant strain. Drug resistance was more
pronounced in retreatment cases but no significant difference was observed by HIV status in
relation to any spoligotypes”

And again at the end of the paper they conclude:

”Mycobacterium tuberculosis is the most prevalent species of Mycobacterium tuberculosis
complex in Rwanda, and SIT 52 (T2) the predominant strain. Drug resistance was more
pronounced in retreatment cases but no significant difference was observed by HIV status.”

So these are the two main findings?

Response: No, these are not two different findings; we regret the incomplete sentence at the end
of the main text. The last sentence at the end of the paper has been rewritten to match that in the
abstract section.

Whilst I have a minor issue with text being simply cut and paste and duplicated in different
sections, I would overlook this possibly justifiable repetition, if the results and analysis presented
actually supported the conclusion.

Response: We find the repetition justified as this is the take home message, hence appearing in
the conclusion of both the abstract and main text. Moreover, our p-value calculations by chi-
square (as now shown on page 9 of the revised manuscript) support the conclusion.

1) With respect to the HIV conclusion statement
When the word ‘significance’ is used, some statistical analysis has to have been undertaken to
show the ‘significance’ or lack thereof. I propose something like the following change to your
results (blue) and text to be replaced (green):
“In the sample analyzed, 69 patients (45.7%) were HIV sero-positive, 76 (50.3%) sero-negative, while 6 (4%) did not have test results hence their status unknown. Of the 69 sero-positive cases, 42 (60.9%) were TB retreatment cases while 52/76 (26.3%) of the sero-negative cases were retreatment. An analysis of the drug susceptibility pattern of isolates from the 69 HIV seropositive individuals showed that 31 had strains resistant to isoniazid, 32 to rifampicin while 30 (43.5%) were MDR isolates. Analysis of the two major spoligotypes and HIV sero-status of patients showed that among HIV positive patients, ??/?? (??%) had strains of the SIT 52 cluster, vs ??/?? (??%) HIV negative patients (p =?.???). Likewise, among HIV positive patients ??/?? (??%) had strains of the SIT 125 cluster, vs ??/?? (??%) in HIV negative patients (p = ?.???).strains of the SIT 52 cluster had 19/48 (39.6%) sero-positive individuals while 26/48 (54.2%) were sero-negative and the remaining three patients did not have test results. SIT 125 with 12 strains, on the other hand, had seven sero-positive and five sero-negative individuals. The sero-status of the patients carrying unclustered strains in the study is shown in Figure 1. There was no statistical relationship between HIV sero-status of the patients and any particular spoligotypes pattern.”

Response: We do not entirely agree with the reviewer. The format suggested above will distort the facts from our data. For example, if you write “Analysis of the two major spoligotypes and HIV sero-status of patients showed that among HIV positive patients, 19 /48 (54.2%) had strains of the SIT 52 cluster”, then it means to the best of our understanding, that there were 48 HIV positive patients which is not true (48 is the number of strains in the SIT 52 cluster). However, we have re-written the section as follows:

Analysis of the two major spoligotypes above (SIT 52 and SIT 152) vs. HIV sero-status of patients showed that 19 of the 48 SIT 52 strains (39.6%) were from HIV positive patients while 26/48 (54.2%) strains were from HIV negative patients (p =0.16). Likewise 7 of the 12 SIT 152 strains (58.3%) were isolated from HIV positive patients while 5/12 (41.7%) were from HIV negative patients (p = 0.45). There was no statistical relationship between HIV sero-status of the patients and any particular spoligotypes pattern. The sero-status of the patients carrying unclustered strains in the study is shown in Figure 1.

The reviewer is looking at the data using HIV status as the reference point while the authors are using strains diversity (in line with our manuscript title) as the reference point, then analysing categories of patients associated with each strain type. The corrected section is written in red text on page 9 of the revised manuscript.

I also think that Figure 1 should group strains by HIV, so that the reader can clearly see no pattern, in support of the conclusions.

Response: By the same argument above, (strain types and not HIV status as our reference point) we strongly believe that figure 1 communicates our intended message very well and all authors agree that we maintain it in the original form.

2) With respect to the MDR conclusion statement
Then with respect to the conclusion about MDR: “Drug resistance was more pronounced in retreatment cases”. Is the ‘pronouncement’ significant or not? Either way... the two proportions
need to be compared by chi-squared and p values stated. It is the following excerpt from the results which needs the p values adding to it:

“Among the retreatment cases, 48/94 (51.1%) were MDR, while 13/46 (28.3%) of the new cases were MDR (p = 0.0105). A summary of patient demographic characteristics and associated drug susceptibility pattern is shown in Table 1.” There is significantly more MDR in the retreatment cases (as you would expect). Suggest you replace ‘more pronounced’ with ‘significantly more’ wherever in the document you refer to this finding.

**Response:** We thank the reviewer for the guidance. The p-value has been calculated and inserted while the concluding statement for both the abstract and main text has been re-written as advised. All revisions are in red text.

- **Minor Essential Revisions**
  The rest of the document needs combing through to make sure it is confluent with the above changes... plus more careful attention to typos and grammar:

  In the abstract alone:
  1) “ability to transmit” change to “transmission”
  2) “Of 94 of the retreatment cases” change to “Of 94 retreatment cases”
  3) Small “p” for significance

  **Response:** All have been corrected.

  In the discussion, add an ‘s’ to ‘finding’ in the following statement. “Finding from the current study, however, are in agreement with the previous data from Uganda”

  **Response:** thank you, this has been corrected.

- **Discretionary Revisions**
  None

**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