Author’s response to reviews

Title: Genetic analyses revealed Mycobacterium tuberculosis as the causative agent of TB in the southern ecological zones of Cameroon

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Author’s response to reviews: see over
Title: Genetic analyses revealed Mycobacterium tuberculosis as the causative agent of TB in the southern ecological zones of Cameroon

Version: 2 Date: 21 January 2013

Reviewer: Sebastien Gagneux

1) There was no genotypic clustering based on the MIRU data; yet, the authors argue that there is a lot of "ongoing transmission" of TB in Cameroon. This is likely to be the case in reality, but the MIRU data reported in this paper do not support this conclusion! Most likely, this is because the samples included are a convenience sample (i.e. this is not a population-based study!). This limitation needs to be addressed and discussed in the manuscript.

Answer 1

We agree that this was not a population based study, but the spoligotyping results showed some clusters see figures 1, 3, 5. However the MIRU-VNTR further resolved the clusters into distinct genotypes, showing some differences between the isolates. This has been discussed in the revised text

2) The authors refer to a “correlation” between phenotypic drug resistance profiles and strain genotype as determined by spoligotyping. I don’t see any correlation in these data, much less any which might be statistically supported. Hence, this section should be deleted from abstract, results and discussion.

Answer 2

We have checked the statistics and agree with you that there is no significant difference in the correlation study between the spoligotype profile and the resistance profile of the isolates in the two ecological zones. We have corrected this in the Results and the Discussion.

3) There are many grammatical errors, typos and style issues throughout the paper. Please have the manuscript checked by someone with appropriate English writing skills.

Answer 3

The text has been edited to improve on the English.
4) In the results section, make to appropriately differentiate between “strain/isolate” and the “spoligotypes”. E.g. on page 1 of Results, it states” the 17 spoligotypes were compared with those contained….And it was found that 61 (!) of them (of what?) were already described…”’. This is very confusing and should be clarified. Please refer “to the spoligotypes of 61 strains were compared” or something similar. Similar sections in the Results suffer from the same problem.

**Answer 4**

“The 17 Spoligotypes (mean spoligotype profile) were compared with those contained in the international spoligotyping database (SpolDB4)\(^\text{11}\). And it was found that 61 isolate were already described in SpolDB4 while 10 were new or unique”. The apparent confusion between strain and isolate has been eliminated by defining the terms in the text.

5) The authors argue that based on their data, MIRU-VNTR was “100% sensitive and specific” fro MTBC isolates. How do they know this given they have not tested any MTBC-negative samples (incluzding non-MTBC mycobacteria)? Minor Essential Revisions:

**Answer 5**

We did not test any MTBC negative sample and non MTBC Mycobacteria. This sentence have been canceled

6) The introduction refers to “10 million cases” and “3million deaths” causes by TB yearly. According to the latest WHO report (2012) these numbers are ~9 million and 1.4 million, respectively. (…and please add the relecant reference!)

**Answer 6**

This was a typographical error. The correct reference WHO, 2011 has been inserted in the text.

7) A reference is missing in the introduction when referring to the study performed “30 years ago”.

**Answer 7**

The reference has been added:

8) Be consistent when reporting % values (e.g. use just on decimal) across the manuscript (including tables):

Answer 8

We have corrected all the data entries to one decimal except the depicting allelic diversity in Table 3 which were generated automatically using a delicate software.

9) When reporting the MIRU-VNTR data, there is no need to give so many details by region as none of the strains are clustered in any of the regions.

Answer 9

We don’t think that enough details have been given regarding MIRU-VNTR data. We summarized the result of MIRU-VNTR of the two ecological zones and noticed the allelic diversity result which is very important in the combination with spoligotyping.

10) The authors found a low prevalence of *M. africanum*, particularly compared to the older literature. Can they speculate about why *M. africanum* in Cameroon might be so rare by now?

Answer 10

We found a decrease of *M. africanum* compared to the older literature and as we said the factors that contribute to the reduction of this species have not been unraveled. We suggested the application of the DOTs strategy the use of different method during the identification of the MTBC species.
1. The title mentions about "ecological" selection of samples. But the manuscript does not analyse the results accordingly. The ecological distinction of the three selected region and a comparative analysis of the distribution of strains as per the ecological source of the same would make the relevant study.

Answer 1

Our study covers two ecological zones: the savana mosaic zone (West and North-West regions) and the tropical rainforest zone (Central region). We have compared the two ecological zones in Table 4. These changes have been effected in the revised manuscript.

2. The spoligotype and MIRU profile of *M. africanum* isolates should be given in a small table form to highlight the findings.

Answer 2

Since this can be seen in supplementary document there is no need to draw another table.

In page 9 it is mentioned that drug susceptibility of MDR was performed but the percentage profile has been given for "monoresistance"? This is confusing and needs to be clarified and corrected.

Answer 3

In a recent study at the same sites we found both monoresistance and MDR (Assam et al., 2011). This has been cited in the present manuscript.

4. Discussion is very superficial. The merits of spoligotyping and MIRU is well established. Authors should shorten this part.

Answer 4

We have rewritten the Discussion.

5. Comparative analysis of the findings in the light of regional distribution of the Isolates/molecular typing would add to the value of the manuscript.

Answer 5
We have made a comparative analysis of the 2 ecological zones in table 4 as recommended and found some differences, which are described in the revised text.

Minor comments:
The manuscript needs extensive correction of English language. At places the meaning conveyed is distorted because of this problem.

The text has been edited to improve on the English.