Reviewer's report

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

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Reviewer: Prasit Palittapongarnpim

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Minor Essential Revisions

Specific comments

Abstract
Page 2 Line 2: “f” should be “of”.
Page 2 Line 23: “different” should be “difference”. Please correct the P value in the parenthesis.

Materials and Methods
Page 4, Line 25
Study Design: The fact that not all samples were genotyped indicate a possible bias for sample collection. More description of the selection criterion, “the highest ZN score”, is required.

Page 4, Culture and drug susceptibility testing
Explicit description on how the bacterial isolates were identified as MTC is needed.

Page 4 analysis and spoligotyping.
The target of analysis is the 16S rRNA gene not the 16S rRNA itself. Since the gene is present in every bacterium and very conserved, more details, such as primer sequences or exact positions of primers, need to be given so that the readers can evaluate the specificity of the primers.

Results
Page 8 Line12: This sentence seems self-conflicting. A Euro-American lineage should not be M. africanum.
Page 8 Line 18 and 20: should be “isoniazid”.

Page 8 Last paragraph: This paragraph should include a description on whether there are any statistical significant associations between any genotypes and drug resistance patterns.

Page 9 Last paragraph: There should also be a description on whether there are any statistical significant associations between any genotypes and HIV-serostatus of the patients.

X Discussion
Page 10 Paragraph 2 The authors reason that high genetic diversity of isolates in Rwanda was due to high level of transborder influx of strains due to human migration. However, at the same time, they seem to cite data indicating that the predominant strain in Uganda does not appear frequently in Rwanda. This discrepancy need to be explained. It may also be useful to indicate how many strains identified in Uganda were also found in Rwanda.

Page 10 Paragraph 3 There are a few precautions that need to be taken to conclude the high transmission rate in Rwanda. First, spoligotyping is known to have relatively low discriminating power compared to other popular genotyping methods. Isolates in the same spoligocluster are usually differentiable by IS6110 RFLP or VNTR typing or SNP typing. Second, the period of sample collections was short, just for 7 months. It is unlikely that two patients with the same genotypes in this study are the results of direct transmission events during the study period. All these facts should be discussed.

Page 11 Line 11-13: This sentence cannot be accurate. The gene coding for 16S-rRNA cannot be deleted as M. tuberculosis is known to contain a single copy of the gene. Its deletion would render the bacterium completely unviable. The absence of amplification product of 16S rRNA gene must be explained by another reason. In reverse the ability to amplify the 16S rRNA gene may not be considered as evidence in species identification of M. tuberculosis because the gene region is very conserved. Some sets of primers can actually amplify a vast range of bacterial species. The information regarding the primer sequences should be provided in the manuscript.

Level of interest: An article whose findings are important to those with closely related research interests

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'