Reviewer's report

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

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Reviewer: Matthew Bates

Reviewer's report:

- Major Compulsory Revisions

1. Abstract, results: If the speciation and typing is the main objective and purpose of the paper, then I think these findings should come at the end of this section. Was the MDR data generated by the study or simply taken from the National Reference Laboratory records? I suspect the latter as no mention of culture DST in methods. If so source of DST data needs to be stated. Also... the high levels of MDR are quite striking... especially the 30.4% in new treatment cases. The study design needs to explain the rationale for what cultures were analysed. Were they chosen at random, if so how? If they were not randomly chosen then by what criteria were they selected? In the discussion you state “This, to the best of our knowledge, is the first report describing the species and strain diversity of M. tuberculosis complex isolates from TB patients in Rwanda” This suggests you 151 isolates are randomly representative of the population of strains in Rwanda. Based on the MDR rates I suspect this is not true. The study design needs to be more explicit in how isolates were selected and the discussion needs to acknowledge this limitation.

2. Introduction: you state “Currently, the only data available on MTC in Rwanda focuses on drug resistance studies, and less is known about the prevalent species and strains, and how these relate with host demographic characteristics as well as drug resistance of the strains.”41% of your samples are MDR, which shows a massive bias towards drug resistance. What is the current data you refer to? Not referenced. If there are any studies with species or typing data from Rwanda then these need to be presented in the introduction and your data needs to be compared with them in the discussion. If data is un-published then suggest discussion with Rwanda NTP to include it in this paper, or at least refer to their internal reports and documentation.

3. Materials and Methods, Ethical Considerations: Was this a prospective or a retrospective study? No mention of recruiting and consenting patients in abstract. Maybe consent and ethics approval was through a linked prospective study? What were the inclusion and exclusion criteria of the consenting study and what were the sites that the patients were recruited from?

4. Materials and methods, study setting: If it was a prospective study then how many sites were there, how many patients were recruited from each site and what was the rationale for choice of sites and sample number from each site – presume inclusion criteria is ‘suspicion of TB’ – this must be defined.
5. Results, HIV sero-status: What about it? Suggest throughout resulting summarizing key finding in section headings. Is there a link between HIV status and spoligotype or not? I think you are trying to answer this question but your answer is not clear?

6. Conclusion: If no significant difference by HIV status is one of your key findings then you need to show these stats on table 1 and bring this finding to the fore in the discussion.

7. What are the limitations of your study and what is the next question arising from your findings? What is the next study you want to do?

- Minor Essential Revisions

8. Abstract, Background: “of” instead of “f”
9. Abstract, Setting: put “National Reference Laboratory” here and remove from design to reduce repetition
10. Abstract, Objective: Remove “technique so as”.
11. Abstract, Design: Where these 151 cultures from sputum? Please specify.
12. Abstract, Results: Give figures/percentages for the “predominance” of T2 and SIT 52.
13. Materials and Methods, Study Setting: Remove “26,338km2” and replace with population density, if indeed the purpose is to illustrate that Rwanda has quite a high population density compared to other countries in the region... which is of relevance to TB.
14. Materials and Methods, DNA extraction: Your statement “Only thermolysates with enough harvest were subjected to DNA extraction using standard protocols [18], while the rest were used directly for PCR in subsequent analyses” does not explain the rationale for this approach. Also method of DNA extraction should be briefly stated although reference is supplied... sonication? QIAGEN extraction kit?
15. Results, demographics: Give IQR for age... as more descriptive than range and helps one get an ideal of the age distribution. Was the difference in median ages in men and women statistically significant? Mann Whitney U. In less you analyse your speciation and typing results by age then this break down is probably not required. What about TB treatment history and HIV status? Just describe what you have shown in table 1... which could do with some percentages as well as raw data to make it easier to interpret.
16. Results, Table 1: Susceptible to what? Maybe just put “Non-MDR”. What does “not interpretable” mean? Give percentages out of each row. Put non-MDR (including the three mono-resistant isolates) and MDR side by side (so 70 vs 64). The mono-resistance probably doesn’t need ot be tabulated –just stated in body text. Get rid of “not interpretable” and just explain in footer and in body text why 17 isolates were excluded.
17. Whenever you give a percentage give the fraction from which it is derived and vice versa eg. Results, spoligotyping, “revealed that 35/48 strains in SIT52
were retreatment cases while 11 of the 12 cases in SIT125 were retreatment” – also... is the second “retreatment” supposed to read “new treatment”?

18. Discussion, paragraph one: Is there previous data from Rwanda or not? It’s eluded to elsewhere as you state previous studies have focussed on MDR... which I feel is also the focus of your study. In this paragraph state what your key findings are and then discuss them in more detail in the paragraphs that follow. Discuss speciation results first, then typing... so same order as in results. The second paragraph of the discussion contains just one reference. This section si supposed to compare your findings with that found elsewhere... select 3 or 4 key papers which give a solid picture of the spoligotypes found in other setting and compare your findings with them.

19. Discussion, third paragraph: You state “The 17 clusters identified in this study comprised 76.2% (115/151) of the sample, signalling a high transmission rate within the population” – why does this signal a high transmission rate? In the following sentence add an ‘s’ to ‘rate’. Still on transmission rates: you suggest the link between HIV and TB transmission is traced back to one study in Kenya? This statement should simply refer to a review or a couple of seminal studies in which this was shown. In your results section on the 17 clusters, you make no mention of HIV status, yet you are discussing it?

- Discretionary Revisions

20. Abstract, Background: – “spread”... consider re-phrasing using “transmission”


22. Abstract, Results: I don’t think MDR definition needs to be in abstract – suggest put in methods

23. Abstract results: suggest condensing “Among the 151 isolates, 64 (42.4%) were multidrug resistant (MDR, resistant to both isoniazid and rifampicin) while two were resistant to rifampicin and one was resistant to isoniazid. Additionally, 94 of the 151 isolates were retreatment cases, of which 48 (51.1%) were MDR cases. Of the 46 newly presenting cases, 14 (30.4%) were MDR” to “Among the 151 isolates, 64 (42.4%) were multidrug resistant (MDR) with 3 cases on mono-resistance. Of 94 of the retreatment cases, 48 (51.1%) were MDR and of 46 newly presenting cases 14 (30.4%) were MDR”

24. Introduction: The first sentence of the introduction is clumsy and needs to be rephrased. MTC is very nicely defined in the abstract. This detailed definition could be removed from abstract (where the detail is possibly not necessary, as MTC is a well known concept, and used to start introduction.

25. Methods, Ethical considerations: The statement “Clinical isolates and patient data were treated anonymously. Laboratory codes were used for all strains and patient data throughout the study, with no possibility to identify the patients except investigators only” could be removed. This is all standard procedure.

26. Not clear why laboratory analysis had to be done in Uganda? Always good to use local facilities and build capacity locally where possible.
27. Materials and methods, study setting: move “Sample processing, confirmatory microscopy as well as culture and susceptibility testing were performed here. Colonies were harvested in 400μl of sterile Tris-EDTA (TE) buffer, heat inactivated at 80°C for two hours and then shipped to the Department of Medical Microbiology at the College of Health Sciences, Makerere University, for identification and typing” to sample processing section. Sample transport and decontamination can all be in one section.

28. Materials and methods: suggest combine study setting and study design sections as these are intrinsically linked and would reduce word count and repetition. Then have a separate section on ‘patient recruitment’, with all the HIV VCT info.

29. Materials and Methods, RD analyses and spoligotyping: this section is not clearly explanatory to a non-TB diagnostics specialist.

30. Results, demograpahics: statements like “The demographic data of the patients were such that..” are wordy and unnecessary. Just say “59.6% (90/151) of isolates were from male patients”. You then don’t need to state the opposite figure for female patients as this is self explanatory.

31. Results, Figure 1: Not sure what Hlv status adds to this figure? What do you want to show? State what you want to show with respect to HIV and spoligotype in body text.

32. Results, spoligotyping: “Analysis of drug resistance in the major clusters revealed that SIT 52 (T2) with 48 strains had 34/65 (52.3%) of the total isoniazid resistant strains in the sample, 35/66 (53%) of the rifampicin resistant strains and 34/64 (51.3%) of the MDR isolates while SIT 125 (T2) with 12 strains had all eight isoniazid resistant strains being rifampicin resistant, hence MDR”. – this is a long sentence and is hard to follow. Suggest fragment?

33. Discussion: no need to state that Uganda neighbours Rwanda

34. Discussion: discuss speciation first, then typing.

35. Discussion: MDR section: again you need to explain your recruitment better for the reader to understand your findings with respect to MDR-TB in Rwanda as a whole.

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

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