Author's response to reviews

Title: Long-term dominance of Mycobacterium tuberculosis Uganda family in peri-urban Kampala-Uganda is not associated with cavitary disease.

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Author's response to reviews: see over
To the Journal editorial office,
BMC Infectious Diseases Journal

Dear Sir/Madam,

RESPONSE TO REVIEWERS COMMENTS

Enclosed for your consideration is the revised version of the original article “Long-term dominance of *Mycobacterium tuberculosis* Uganda family in peri-urban Kampala-Uganda is not associated with cavitary disease”.

We have also attached the point by point responses to the reviewers comments.

We thank you for your consideration.

Sincerely,

Moses L. Joloba,
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Response to reviewer 1

Dear Andrew,

Thank you for accepting to review our manuscript that we submitted to BMC infectious diseases and for the positive comments. We have looked at the comments and we have provided point by point responses as shown below.

A. Major revisions

1: The isolates collected formed part of two separate studies, as described in the Methods section. The first study was performed in peri-urban Kampala; however the location of the second study is not explicitly stated (but is presumably also in Kampala) - could this be added.

Yes, it is true both studies were carried out in the same location (peri-urban Kampala). We have clarified this. See line 95-96.

2: Only smear positive patients were recruited as index cases - presumably from patients accessing routine health services in Kampala. This could possibly lead to a biased sample collection: if the non-Uganda strains are less frequently associated with cavitation, then it is possible that they may be a more common cause of smear negative disease and thus missed by only including smear positive index cases (with a possible over-representation of cavitary disease in the index cases) Could inclusion of all patients (smear positive and smear negative) possibly have increased the chance of finding an association between cavitation and the Uganda family? Could the authors comment on this possibility?

Household contacts were screened by smear and culture – so presumably some of these contacts were smear negative but culture positive. What proportion of the final patients analyzed were index cases versus contacts; and what proportion were smear positive versus smear negative? What is the normal proportion of smear negative disease seen in Kampala (if that is known)?

I. Could inclusion of all patients (smear positive and smear negative) possibly have increased the chance of finding an association between cavitation and the Uganda family? Could the authors comment on this possibility?

Yes, we agree by recruiting only index cases (smear positive TB patients only) we could introduce a selection bias, however in this particular study in addition we had isolates from the contacts (N=189) included and of these (151/189) 80 % were smear negative. Furthermore, this category of patients (contacts) did not show an association between cavitation and the Uganda family (p = 0.79). Hence, we have included this information in the discussion. See line 236-240. Although, we still acknowledge that this was a limitation of the study as we mentioned in the limitation section. See line 287-289

II. Household contacts were screened by smear and culture – so presumably some of these contacts were smear negative but culture positive.

True, a total of 189 household contacts were screened by smear and culture, of these 80 % (151/189) were smear negative.

III. What proportion of the final patients analyzed were index cases versus contacts; and what proportion were smear positive versus smear negative?

The data set used for the final analysis shows 84 % (1019/1213) as index cases, 14 % (170/1213) as contact cases while 2 % (24/1213) were of unknown category. As regards smear status; 15 % (183/1213) of isolates in this study were from smear negative TB patients.
IV. What is the normal proportion of smear negative disease seen in Kampala (if that is known)? The proportion of smear negative TB in Kampala is between 10-20% (National TB and leprosy control program report, 2011).

3: How was culture performed (solid or liquid), and were the culture protocols at the two labs (NTRL and JCRC) the same?

Culturing was performed both on liquid (MIGIT 960) and solid media. The solid media used at JCRC was (Middlebrook 7H10 supplemented with Glycerol 10 % and 10% OADC) while at the National TB reference laboratory they used Lowenstein-Jensen slants. However, the protocol for sample (sputum) processing (NALC/Nacitrate-NaOH) in both laboratories were similar.

4: Please reference the chest X-Ray reading scheme used.


5: When stating that “Basal Metabolic Index” was calculated, do you not mean ”Body Mass Index”? True, we edited to mean “Body Mass index” throughout the manuscript: See line 123.

6: How was income stratified?

We dichotomized the income as 0-20 and > 20 USD per month as low and high respectively based on the Uganda national bureau of standards definition. www.ubos.org

7: 1746 cultures were stored - what proportion of the total number of patients enrolled does this represent? Were there a significant loss due to culture contamination / loss of viability etc?

The isolates used in this study represent 42 % of the total number of patients enrolled in the study. True, there were contaminations however loss of isolates was minimized by storing multiple cultures (2 to 3) at a given time point. For the isolates stored we didn’t have to re-culture (to face contamination or loss of viability challenges) we made a scraping off the frozen stored isolate for the DNA extraction. Live bacilli were not required for the genotyping to work.

8: 533 isolates were excluded from the analysis of clinical associations. Was the distribution of strain lineages in these 533 similar to the overall distribution?

The missing values were not from the same patient, it was heterogeneous. The proportion of strain lineages among the excluded patients was 58% (309/533), 28 % (149/533) and 14 % (75/533) for MTB Uganda genotype, Lineage 4 strains other than Uganda genotype and Lineage 3 respectively. These proportions are comparable (P>0.05) to the overall proportion noted. Thus, the by exclusion patients who lacked clinical information from our analysis didn’t negate our findings.

9: Was any association examined between incidence of TB in household contacts and strain lineage?

From the data set, 2 % (N=32) of the patients were categorized as incidence cases of TB. Of these 56 % (18/32), 25% (8/32) and 19 % (6/32) were infected with MTB Uganda genotype, Lineage 4 strains other than Uganda genotype and Lineage 3 respectively. This small number of incident cases did not allow for a robust analysis with our statistical model, and therefore we did not examine this variable for an association with MTB lineage.

B. Minor revisions.

1: I do not feel that Fig 1 adds anything to the manuscript - the data is presented in the text. It may be worth deleting this, and adding supplementary Table 3 (and possibly even supp Table 2 if space permits). Yes, we agree, we have deleted Fig 1 and left the text describing the data. We have included it as supplementary Fig 1.
Response to reviewer 2 comments

Dear Mark

Thank you for accepting to review our manuscript that we submitted to BMC infectious diseases and for the positive comments. We have looked at the comments and enclosed our responses point by point as shown below.

1. Since the propensity to cause cavitary disease is only one, very particular, measure of virulence, it might be better to avoid making generic statements about virulence (e.g., perhaps the title should refer to cavitary disease rather than virulence).

True we agree, we have changed the title to read “Long-term dominance of Mycobacterium tuberculosis Uganda family in peri-urban Kampala-Uganda is not associated with cavitary disease” See line 1-2.

2. More detail is required regarding the sampling framework. Did these represent all isolates in the study area over the period? How were participants selected? Did this differ between the two studies?

   • The MTB isolates (1746 isolates) analyzed in this study included all the isolates collected from peri-urban Kampala from 1995-2009. Additionally, Mulago hospital being a referral hospital, a TB patient coming from outside Kampala was recruited for as long as s/he agreed to stay around Kampala in a radius of 20 km for the next six month. The later scenario accounted for 8 % of the patients. The selection criteria for all the enrolled participants in both studies were the same. See line 104 and 118-120.

3. Were all isolates from a household or just one per household included?

   No, we considered an isolate per patient (whether same household or not) so as to understand the relationship between the strain and the outcome; hence we think the source of isolate will not bias the outcome in this case. However, when it comes to prevalence of MTB strain lineages, if more than one isolates was from the same household (especially if they are of the same lineage) this will overestimate the prevalence. In this study 84 % of the isolates were from index cases and thus they represent a single patient per household (isolate per household).

4. For how many isolates was the SNP assay confirmed by LSP-PCR?

   A total of 90 MTB isolates that were randomly selected were analyzed using LSP-PCR, the same samples were run in a SNP typing assay and the results were 100 % concordant.

5. Was there potential bias introduced when excluding patients for whom clinical information was lacking? Specifically, was the strain distribution similar amongst included and excluded patients?

   See response to comment 8 of reviewer 1.

6. What measure of virulence was used in the previous small study referred to in reference 20?

   Nahid-Payman et al 2010, defined virulence as cavitation, extent of lung involvement and high smear bacillary load at baseline. We also measured virulence using cavitation and extent of lung infection. The two variables highly correlated and we decided to use cavitation in the analysis. We have now changed the title to reflect this.