Author's response to reviews

Title: The impact of drug resistance on the risk of tuberculosis infection and disease in child household contacts: a cross sectional study

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Author’s response to reviews:

Dear Editorial Team,

We would like to thank the editorial team and the reviewers for going through our article so thoroughly and for providing such a detailed and helpful assessment. We set out below our responses to the comments and attach a revised manuscript as well as one with the changes marked as tracked.

REVIEWER 1

Comment

The introduction could be worded more concisely. For instance the first sentence could read "in 1953 Middlebrook and Cohn demonstrated that mycobacteria...."

Page 7 lines 1-30 could be reworded to make the points a little more clearly. As far as I can ascertain the major points are:

1. Mathematical models that assume fix fitness cost suggest that drug resistant TB will remain a localized problem.
2. Models that include heterogeneity in levels of fitness point to global spread of the drug resistant TB epidemic.

3. Understanding fitness of drug-sensitive and drug resistant organisms is therefore important.

Response

Thank you for this observation. We agree that the three points made above are the central points that we want to get across to readers in this final paragraph and have removed the second and third sentences to add clarity. We have revised the first sentence as suggested by the reviewer.

Comment

Methods: it's unclear to me whether the clinicians assessing the different cohorts were the same or different. Some indication of this could be given in the methods or the results. If they were significantly different that is a potential confounder that should be acknowledged in discussion.

Response

This is an important point and if it was unclear to the reviewer then there it is likely that it will be unclear to readers. Although different clinicians assessed children in the two studies, all children were assessed by clinicians working at the same academic research centre undergoing standard training. Standard diagnostic approaches and criteria were used. We have edited the methods to reflect this and have acknowledged this in the discussion section.

Comment

Children suspected disease had undergone microbiological testing. Although differences would be subject to a very large number of confounders and the numbers will be small it would be worth noting what proportions of DS and MDR contacts with suspected disease had cultures and how many had positive cultures. This is NOT essential if the data are difficult to retrieve but would be helpful. Certainly equal proportions of culture-positive disease in both cohorts or more culture positive disease in the DS-exposed children would help make the authors' case around fitness.

Response

As stated in the methods section, in both cohorts, all children with symptoms suggestive of TB or with suggestive chest radiograph findings all underwent standard gastric aspirate sampling, completed by research staff, which was sent for MGIT culture. Therefore all of the children who were suspected of having TB and all treated for TB (in both cohorts) had samples sent for mycobacterial culture. Although the optimal sampling strategy was for children to have at least two samples sent (and ideally three), a small number of children will only have had one sample sent. Data on the number of samples sent for each child are unfortunately not available. However, of the children treated for TB, in the DS-TB cohort, 7 out of 27 (26%) were culture
positive and in the MDR-TB cohort, 3 out of 15 (20%). These proportions are thus similar in both cohorts. This information is stated at the end of the second paragraph of the results.

Comment

The authors discuss limitations in detail. One limitation not discussed is that the clinicians were not blinded to the sensitivities of the index strain. Since treatment of DS-disease is shorter and involves less toxicity than treatment of MDR disease it is possible that clinicians would be unconsciously more inclined to label children with questionable radiographic abnormalities as having disease if the strain was DS than if it was MDR. This, again, is a limitation that does not argue against publication of these data but it should be acknowledged in some brief form.

Response

This is a really good point. Although all children were investigated using identical protocols, diagnostic tests and although identical, standardised definitions were used to define TB disease, this element of bias is impossible to avoid completely. We have added a sentence to this effect in the limitations section.

Comment

Table 1 notes a * for p<0.05, but there are no characteristics that are flagged as attaining this significance. Is this an omission? Perhaps it should be dropped if there are no variables where the differences attain this level of significance.

Response

Thank you for this observation. We have removed the reference to p-values in the 0.05-0.01 range from the table key.

REVIEWER 2

Comment

Page 9, Lines 42-43. TB disease among child contacts is defined as multiple categories, but the Results section reports only disease vs. no disease. Please explain whether how the final binary variable was defined.

Response

Thank you for this point. If a child was investigated for TB disease they were ultimately categorized into one of five categories: confirmed, probable, suspected, unlikely or not TB. However, we agree, that it is perhaps unclear to say that TB disease was categorized as “confirmed”, “probable”, “suspected”, “unlikely” or “not TB”. We have removed the last two categories from the investigations section in the methods since we did not analyse these
categories as TB. For analysis purposes, we combined all children with “confirmed”, “probable” and “suspected” TB into the category of “TB disease”, with all other children (those not investigated for TB and those investigated for TB and found to have “unlikely TB” or “not TB”) being defined as not having TB. We have clarified this in the analysis section of the methods.

Comment

The statistical analysis section does not mention accounting for household-level clustering (e.g. using GEE or mixed effects modeling). Assuming there are some households with multiple children exposed, one cannot assume independence between observations. Please explain why within-household clustering was not accounted for in your analysis.

Response

This study was designed to limit the potential impact of household-level clustering, by limiting the number of children recruited per household to four. Given that we only included children under five in this analysis it was rare for there to be more than 2 children under five in a household. In the two cohorts, less than 50% of the total sample of children enrolled were members of households from which multiple children were recruited. In past analyses of these data, clustering was accounted for using GEE models, however, using these methods did not have a material impact on our results, suggesting that the recruitment methods use were sufficient to address these effects.

Comment

Page 10, line 17 states that a DAG was used to identify variables a priori for the multivariable models. Typically, this implies that all of the variables that conceptually should be included in the final models were, in fact, included. However, not all of the variables reported are included in Table 2. Please clarify the process by which variables were selected for inclusion.

Response

Two DAGs were used, one for the infection model and one for the disease model. We have amended the wording of this section of the methods to specify which variables were included in each model.

Comment

Please justify the inclusion of smear positivity in the final model for infectivity. It seems that application of DAG would have identified smear positivity as a descendent of MDR TB and, therefore, in the causal pathway between MDR TB and infection. Conditioning on smear positivity could, therefore, attenuate the overall relationship.

Response
We thank the reviewer for this observation. Although there are some circumstances under which smear status could precede MDR-TB status on the causal pathway (for example in the case of resistance acquired during prolonged treatment for advanced disease), most often smear status will indeed be on the causal pathway between MDR-TB status of the adult and the infection status of the child. Therefore, we agree with the reviewer and we have removed this variable from the infection model. Its removal only slightly altered the results, increasing the OR for infection from 1.80 to 2.05.

Comment

IPT is mentioned in the statistical analysis section (page 10, line 30), but not described in the results or the table. While this should not affect the findings (presumably, children exposed to MDR TB were not given IPT), it would be of interest to include this data in Table 1. Please also mention clinical care, if any, provided to children exposed to MDR TB (i.e. preventive therapy, active follow-up, etc.)

Response

For this study we evaluated children soon after the index case had been diagnosed. Unless the child had recently been exposed to a different TB case then the child would not already be on preventive therapy. In some situations, children were seen as contacts of MDR-TB where TB had been diagnosed in the source case through smear and assumed to be DS-TB and the child started on isoniazid preventive therapy. Only when the drug susceptibility testing was found to be MDR-TB was the child seen in our clinic. However, for both the cohorts only a handful of children (<5 in each cohort) were already on preventive therapy when seen by our research teams. We had assumed that this would be an important factor in evaluating risk of infection but given the small numbers we removed it from descriptive outputs in the tables. However, we agree with the reviewer that as it is, it is a bit confusing to list it in the methods and then not refer to it again. We have therefore removed this from the methods section.

All children exposed to MDR-TB received preventive therapy based on a local standard of care regimen, consisting of ofloxacin, high-dose isoniazid and ethambutol at the time. All children with drug-susceptible TB were referred for standard 6 months of isoniazid once daily. We have now described this in the methods section.

Comment

Please explain why children with previous TB treatment were included in the analysis for infection. It seems that it would be difficult to interpret TST results among such children. Many of those would, presumably, be misclassified as new infection.

Response

This is an interesting point and one that was discussed extensively within the team. What we would really have liked to have determined is what characteristics of the source case and the child and the interaction between them have led to TB infection in the child during this episode.
of TB in the source case. What we have is much less perfect. First we have TST as a proxy for TB infection and it is clear that this is not a perfect measure of infection. Second, there are many reasons why children may have a positive TST, other than caused by the most recent interaction with a TB case: 1) Children may have been exposed previously to TB at some time in their life, 2) the impact of BCG or environmental mycobacteria (NTM) may have led to a false positive TST and 3) obviously previous TB disease in the child may lead to a positive TST. However, TST is not universally positive in those with TB disease – in children with confirmed disease only about 70% have a positive TST. We felt that rather than excluding children from the analysis for having other reasons for a positive TST, we would include them, quantify them and include these factors in the multivariable model so that other factors are adjusted to account for them. As can be seen from the results, only a few children in either cohort had been previously treated for TB disease and these children were not universally TST positive. We also find a very low proportion of NTM amongst mycobacterial isolates amongst children in our setting. We have expanded the section in the discussion on limitations to describe this.

Comment

The Discussion section provides too much detail about HIV infection and other factors, and not enough discussion about the main finding pertaining to the relationship between MDR TB and infection/disease. Please expand your discussion of the relevant literature listed in page 15, lines 27-45 to improve contextualizing your results. For example, how is the design of your study similar or different than the previous ones? How do your findings extend current knowledge? What are possible reasons for disparate findings in the different studies and settings?

Response

Thank you for this comment. We have cut out quite a lot of the discussion of the epidemiology of HIV and age and focused on the literature around the impact of drug resistance on the risk of infection and disease in contact. We have described in some detail three studies that were only previously referenced to place our results in context. We discuss why results are variable.

Comment

Page 15, lines 7-10. Why did you choose not to include incident TB?

Response

This was a cross-sectional study to evaluate the risk of infection and disease at one time point soon after the diagnosis of TB in the source case. This allows an evaluation of risk factors for infection and disease in child contacts. After children were seen the two cohorts were managed in quite different ways and the studies were not designed to yield comparable data regarding incident TB. For the DS-TB cohort, which were referred for IPT to routine care TB clinics, the uptake of IPT was highly variable. In contrast, for the MDR-TB exposed cohort, children were treated by the study team. Thus, the important confounder of TB preventive chemotherapy could not be controlled for.
Comment

Page 16, lines 17-22. Please discuss the possible impact of the different inclusion criteria on the infection analysis. It seems that it could have led to differences in length of exposure, which could have biased the infection analysis towards increased effect.

Response

This is a really important point and we thought very carefully about this before comparing the two datasets. We acknowledge this potential methodological limitation and it is entirely possible that the length of exposure could have been different between the two groups, potentially leading to an increased infection effect seen. We have already discussed this in some detail in the limitations section of the discussion but have added to this to make the point further. It should be noted, however, that most of the DS-TB adult index cases had reported symptoms of >3 months, and in many adult TB cohorts, TB patients (both DS and MDR) have been symptomatic for many months before being diagnosed, although we were not able to formally document this.

Comment

Was time since symptom onset of the index patient recorded? This information would help better understand the causal pathway by which MDR TB affects infectivity.

Response

This is also a really important point. Unfortunately, the duration of symptoms in the index case was not recorded. Much of the data we collected on the index case was taken from the routine TB register so we were limited by the data fields that were used in the TB registers, which do not routinely document duration of symptoms.

Comment

It seems that MDR TB and DS-TB patients were drawn from different neighborhoods / populations that could have had different levels of TB transmission (as suggested by higher prevalence of previous TB among child contacts). Please describe how this could have affected the results.

Response

The two cohorts were drawn from overlapping and similar but not identical communities. The DS-TB cohort were recruited from three high burden communities in Cape Town whereas the MDR-TB cohort were drawn from all over the Cape Town Metropolitan area (including the three communities that the DS-TB cohort were recruited from). Although there is some variability in the burden of TB in different communities in Cape Town, the rates of TB in each of the eight sub-districts are fairly similar and very high – the pressure of TB infection is universally amongst the highest in the world. However, we fully acknowledge that this could have led to differences in results and have described this in detail in the discussion.
Comment

Related to above, was there information about previous TB history among other household members?

Response

This would have been very useful but unfortunately we did not record any details about previous TB treatment in other household members.

Comment

While controlling for BCG is appropriate, given that nearly 100% of DS-TB group and >80% of MDR TB group had BCG, I don't think controlling for this in the analysis eliminates the possibility of spurious findings introduced by misclassification.

Response

We would completely agree with this comment. As we responded to a previous comment above, there are a number of reasons why children may have a positive TST result other than due to M. tuberculosis infection from the most recent exposure. These include previous TB disease, previous TB exposure, BCG vaccination (which effect would typically wane after 2 years following BCG vaccination at birth) and NTM infection. While the effects of neonatal BCG vaccination do fade with age and while larger TST indurations (>15mm) are more commonly due to TB infection rather than BCG, it is possible that some of the TST responses between 10 and 15 mm, in younger children, were due to BCG and not M. tuberculosis infection. However, as discussed in our response to the previous comment, we felt it was more appropriate to include those children with risk factors for TST positivity in the final model and list this as a limitation in the discussion. Although we previously mentioned the limitations of TST in the discussion we have expanded this section to specifically detail this point.

Comment

Given the limitations described above, please remove the statement about implications in the Conclusions (Page 17). Instead, please describe how your findings inform the development of future studies that can more definitively answer this question.

Response

We have edited this section and edited to be more in line with the comments at the end of the abstract. As this is a conclusion we would like to write a brief conclusion at this point, bringing together the main messages of the study. We feel that this is not perhaps the place to discuss future work and further directions.

Comment
Figure 2 seems redundant and not helpful. I suggest removing it from the manuscript.

Response

We had wanted to present our baseline descriptive data stratified by clinical state (exposed uninfected, infection and disease) and had originally developed such a table. However, as authors, we felt it was more illustrative to present this information graphically. These descriptive data (as opposed to the model outputs) are not provided elsewhere in the article stratified by clinical state so we would dispute that these data are redundant – the figure is not a replication of data available elsewhere in tables/text. We would prefer to keep this figure but would leave it to the editorial team to decide if they feel it is better to keep the figure or remove it.