**Author's response to reviews**

**Title:** Intensified specimen collection to improve tuberculosis diagnosis in children from rural South Africa, an observational study.

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**Author's response to reviews:** see over
Dr. Jason Stout  
Deputy Section Editor  
BMC Infectious Diseases  

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Re: MS# 2028192016103557. Intensified specimen collection to improve tuberculosis diagnosis in children from rural South Africa, an observational study.

Dear Dr. Stout,

We thank you and both reviewers for your careful consideration of our manuscript and appreciate the thoughtful comments and questions provided. We have addressed each reviewer's suggestions, as detailed below and feel that the manuscript has been strengthened by these additions.

We hope that you find our improved manuscript suitable for publication in BMC Infectious Diseases.

Sincerely,

Tania Thomas, MD, MPH  
on behalf of all the co-authors.

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Reviewer 1:

Major Compulsory Revisions:

1. The authors state both in the abstract and the methods section that “All participants had blood, urine, and sputum collected.” However, in Figure 1, it shows that only 34/51 outpatients and 61/67 inpatients had sputum collected; in addition, urine was not collected from all subjects and 3/67 inpatients did not have blood cultures. The authors need to clarify this point and explain why some specimens (specifically sputum) were not obtained in all subjects.

   The methods has been clarified (see Abstract, page 3, lines 9-11; Methods, page 7, line 10; Additional information to explain the missing specimens has been added to the limitations section (see page 14, lines 12-16).
Some children did not have all specimens collected for various reasons including: inadequate fasting for sputum induction, inadequate time available for sputum induction, dry cough/insufficient specimen volume, inability to produce urine during the outpatient encounter, unsuccessful phlebotomy/insufficient specimen volume.

2. Regarding patient recruitment in the Methods section, the paragraph describing enrollment is somewhat confusing. From reading this statement it sounds like all patients were recruited from the district hospital (ie, inpatients), but this clearly was not the case. The authors should explain how outpatients were recruited and clarify this paragraph.

This hospital includes inpatient and outpatient settings; further information was added to make this clearer (see Methods, page 6, lines 8-9).

3. In the results section, the authors state that the sensitivity of CXR was 100% to diagnosis probable/confirmed TB; however, they admit this is likely due to the WHO case definition for probable TB (that includes an abnormal CXR). Given that CXR are used to categorize children as probable TB, I believe it would be best to not attempt to apply sensitivity/specificity analysis as it is misleading.

The information on sensitivity and specificity has been removed from the results section.

4. It was remarkable to see in Table 1 that 31% of all children reported “Prior TB.” It would be appropriate for the authors to comment on how a history of prior TB was obtained (and if it was verified), and why such a large proportion of their children report a history of prior TB.

Information on prior episodes of TB were reported by the caregivers and verified using the hospital’s TB DOTS registers, TB “Green Cards” and/or medical charts whenever possible. This information has been added to the Methods section (See page 7, lines 3-4).

This study was conducted in a TB endemic setting where HIV was very common (~30% HIV seroprevalence in local Ante-natal clinics, 65% HIV seroprevalence in this study). It is possible that re-infection in this community contributed to this high proportion.

5. Given only 50% of all children with suspected TB were actually treated for TB, it would be appropriate for the authors to include a summary statement or additional table detailing the non-TB diagnosis for children not treated for TB.

This study did not include additional testing to identify non-TB diagnoses. We have included this information on page 11, lines 10-11.

6. Was TST testing included in this study? If yes, TST results should be included in Table 1. If TST testing was not included, the authors should state this and provide an explanation as to why TST was not included.

Local TST testing was via the Tine test. Due to the lack of reproducibility of the Tine test as well as limitations in obtaining a sustained supply of PPD and quality control for Mantoux method of placement, TST data were not included. We have added this statement on page 7, lines 4-5.
7. The authors mention the limitations of the WHO classification for childhood TB, as well as other scoring systems in their discussion. They should emphasize in this section that 4/8 children with confirmed TB were only considered to have “possible TB”—further emphasis that clinical scoring systems are very limited.

*Information about this has been added, see page 12, lines 19-20.*

8. Although this study was performed prior to roll-out of Xpert TB/RIF testing, it would be appropriate for the authors to include some discussion of how this technology may assist in pediatric TB diagnostics, including its use on non-conventional clinical specimens (such as urine or stool).

*The authors agree that Xpert may aid the field of pediatric TB diagnosis. Newer diagnostics have been mentioned in the discussion section, with reference to Xpert.*

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**Reviewer 2**

Comments:

Title: explicitly state that the study took place in a hospital setting

*Due to concerns that the term “hospital setting” may add to confusion about the inpatient vs outpatient status of subjects included in this study, no changes were made to the title. Please see Reviewer 1, comment 2.*

Abstract: The fact that “MDR-TB was detected by urine culture alone in one child” and “urine ... provided one additional TB diagnosis” is misleading, as gastric aspirates were not collected in this child. I believe that the statement in the discussion “accurate diagnosis of MDR-TB and XDR-TB in children has great potential to save lives and reduce transmission but the low yield from current diagnostic tests remains a challenge” provides a better summary of the data.

*This child was not eligible for gastric aspirate based on age > 5 years. Information regarding age has been added (see Abstract, line 19 and page 21, Table 2).*

Introduction: It is not the prevalence of TB but the prevalence of MDR-TB that may make one consider whether TB treatment can be initiated without the need for culture confirmation in a child.

*The authors agree. This information has been clarified on Page 4, lines 17-18.*

Materials and methods:

- This is an observational study, not an efficacy trial

*This has been modified.*

- Children were classified as having possible, probable or confirmed TB based on WHO guidelines (reference?). There is a strong consensus in the field that the consensus clinical case definitions should be used in any evaluation of TB diagnostics in children. It is thus strongly recommended that for this manuscript, children are classified according to these definitions (ref 31).
The authors agree with the need for consensus clinical case definitions and have promoted their use. However, these guidelines were not available during this study, therefore a post-hoc classification will not be pursued. The guidelines used at the time this study was developed were the Provisional Guidelines issued by the WHO; the relevant citation has been added.

Results:

- According to figure 1, sputum was only collected in 66.7% of outpatient and GA in 70% of inpatient children. The reasons for not collecting specimens should be stated in the manuscript. If it is because parents refused or procedures failed, then this needs to be discussed as this would change the findings of feasibility of these procedures.

  Please see response to Reviewer 1, comment 1.

- Table 1: Given the small sample size, it is unlikely that any differences between groups will be statistically significant. It may be more informative to have the data presented by three groups (probable, possible and confirmed TB) instead of presenting the data in probable and confirmed together.

  Due to the small sample size of culture-confirmed cases, the groups in Table 1 have been combined and Table 2 has been used to highlight the key clinical and culture data.

- Table 2: please add information on age

  This has been added. (see page 22, Table 2)

Discussion:

- In the discussion on CXR, the low (21%) specificity should be highlighted as a limitation of using CXR in the diagnosis of childhood TB

  This has been added (see page 13, lines 6-7).