Author’s response to reviews

Title: Genotyping and Drug Susceptibility Testing of Mycobacterial Isolates from Population based Tuberculosis Prevalence Survey in Ghana

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Author’s response to reviews:

THE EDITOR-IN-CHIEF

BMC INFECTIOUS DISEASES

Dear Sir/Madam,

RE: INFD-D-17-00134 - GENOTYPING AND DRUG SUSCEPTIBILITY TESTING OF MYCOBACTERIAL ISOLATES FROM POPULATION-BASED TUBERCULOSIS PREVALENCE SURVEY IN GHANA

Thank you very much for forwarding to us reviewers’ comments on the above-titled manuscript. The necessary modifications have been effected in the text and we trust the article will be considered for publication by the Editorial Committee.

Below are responses to the comments.
EDITOR COMMENTS

1. We think that additional file 2 contains enough information about individual participants to compromise anonymity. As a result we would like to request this is removed.

Response

Additional file 2 removed as requested.

2. Please include a figure legend for additional file 1 in your main manuscript.

Response

This information has now been added.

3. Please ensure that your manuscript contains a Declarations section before the references, with ALL of the following subsections:

* List of abbreviations

* Ethics approval and consent to participate

* Consent for publication – as you are not publishing identifying patient data, you can write "Not applicable" in this section

* Availability of data and materials

* Competing interests

* Funding

* Authors’ contributions

* Acknowledgements.
Response

All information under the various subsections have been provided.

REVIEWER REPORTS

Reviewer 1 (Mahmud Wasim Hanif)

Title: Genotyping and Drug Susceptibility Testing of Mycobacterial Isolates from Population based Tuberculosis Prevalence Survey in Ghana

Comment: The title should be reframed omitting the words Population based Tuberculosis Prevalence survey.

Response

This was a follow-up study to the Population based Tuberculosis Prevalence Survey in Ghana whereby isolates obtained were used. Hence authors believe that the words ‘Population based Tuberculosis Prevalence Survey’ must be included in the title to reflect the source of the isolates.

Method section

1. In line no. 80, it is mentioned that 361 culture isolates including MTBC & NTM were taken for the study.

Comment: Detailed procedure regarding how there identification has been done is not mentioned in the methodology.

Response

Detailed procedure on how the culture positive isolates were identified as MTBC and NTM has now been added to the Methodology.
2. In line no. 95, it was mentioned that all the assays were run according to the manufacturer's instructions.

Comment: (a) DNA extraction procedure was not adopted as per the kit manufacturer’s instructions.

Response

The kit contains reagents for PCR and hybridization process. However, the manufacturer has another kit (GenoLyse®) which is sold separately for DNA extraction. According to the manufacturer, other extraction methods producing amplifiable DNA from bacteria can be used (http://www.hainlifescience.de/en/products/microbiology/mycobacteria/tuberculosis/genotype-ntbc.html). The “An in house DNA extraction method was used” has been replaced with “The boiling (heat killing) method of DNA extraction was used” in the DNA extraction subsection in the methodology. Another step in the extraction procedure has been added.

(b) Detailed description of the procedure adopted is not given.

Response

The boiling (heat killing) method of DNA extraction was adopted. Detailed description has been provided in the DNA extraction subset section of Methodology.

3. In line no. 96, it was mentioned that there were four different kits used.

Comment: Description given for only one method: Genotype MTBDRplus. Brief description to be given about the use of other kits also.

Response

All four kits contain the same set of reagents (Denaturation, Hybridization, Stringent, Rinsing, Conjugate, Substrate) and follow the same procedure for running the assay. However, they differ in the type of probes (oligonucleotides) on the test strip. Hence the procedure described in the methodology represents all the kits used. That notwithstanding, procedure for each of the four kits has been referenced in the text (9-13).
Results section

1. In line no. 138, A total of 361 mycobacterial isolates consisting of 165 (45.7 %) MTBC and 196 (54.3 %) NTM was described.

Comment: Description to be given as how grouping of MTBC and NTM done.

Response

Detailed description has now been shown in the Methodology section.

2. In line no. 138, it is mentioned that percentage of NTM is 54.3%.

Comment: Percentage of NTM found was quite high; denominator used for calculation is not appropriate.

Response

The anomaly has been rectified. It is now 120/361 (33.2%).

Discussion section

1. In line no. 192, percentage of M. africanum (2.4%) was very lower than what was reported worldwide.

Comment: This data has no significance to the study.

Response

The data has been removed from the discussion in the main text.

2. In line no. 204& 205, the observed rate of MDR is 6.7% which is higher than rate reported earlier
Comment: This statement is not appropriate as the sample is too low (N=161) to calculate the rate of MDR.

Response

The observed MDR rate has been omitted from the text due to low sample size. Only the number of isolates resistant to both isoniazid and rifampicin has been reported. Discussion on this result has been revised.

3. In line no. 216 & 217, it was mentioned that, the remaining unidentified NTM species (39%) could be other acid fast gram positive bacteria whose correct identification was beyond the scope of the assays used.

Comment: (a) In methodology section, no such process description was given whether these were actually identified as gram positive bacteria.

Response

No further tests were done to actually identify these unidentified NTM species as gram positive bacteria beyond the interpretation chart provided by the manufacturer of the GenoType Mycobacterium CM and GenoType Mycobacterium AS kits. The chart provides that all samples which produced only two bands (Universal Control & Genus Control) are to be classified as high G+C Gram positive bacteria.

(b) This means large number of NTM species were not identified (39%) shows limitations of the study.

Response

The limitations are stated in the last part of the discussion section.

Conclusion section

1. In line no. 238, it was mentioned that, Diverse species of NTM were found in sputum specimens from presumptive TB cases in Ghana.
Comment: This is generalized statement and not derived from the data of the study. Moreover, it was mentioned in the study design that only positive culture isolates were used not direct specimens.

Response

Statement has been revised to reflect data from the study in the text.

Reviewer 2 (Didi Bang)

This paper describes the evaluation of the commercial line probe assays without including golden standards and includes both differentiation within the Mycobacterial complex, NTM differentiation from Mycobacterium tuberculosis and drug resistance for the first-line drugs from isolates obtained in a previous nationwide prevalence study in Ghana and at the same time describes risk factors from the prevalence study.

The reviewer’s main concern is the lack of appropriate golden standards and that the 361 Mycobacterium tuberculosis isolates obtained from 8,175 participants whom are not described. The aim of the study both in the abstract and text did not include risk factors, which are described in the results and discussion section.

Response

The World Health Organization (WHO) also recommends the use of only LPA without the need for conventional culture under certain conditions (http://www.who.int/tb/publications/factsheet_tb_fllpa.pdf). In this study, LPA was used as an appropriate molecular technique to achieve our aims of genotyping and drug susceptibility testing of mycobacterial isolates obtained from a previous TB prevalence survey in Ghana. Under extreme circumstances such as paucibacillary growth of majority of the archived isolates after subculture phenotypic testing was a challenge. Moreover, the aim of the study was not to evaluate the usefulness of commercial line probe assay which have been extensively studied and shown in many countries including Ghana to be useful for rapid differentiation of mycobacterial isolates and their drug resistance patterns.

The 8,175 participants have been described in the study design subsection.

Results and discussion on the risk factors have been omitted from the main text.
The authors conclude despite not having evaluated towards a golden standard that the line probe GenoType Mycobacteria assay series are appropriate tools for rapid differentiation of mycobacterial isolates and drug susceptibility testing in Ghana. The reviewer suggests that the authors include conventional Mycobacterial culture identification and drug susceptibility methods and avoid including risk factors from the prevalence study, without including them in the aim. To ease reading the findings from this study may be split into several smaller manuscripts.

Response

The concluding statements have been revised.

Risk factors from the prevalence study have been omitted since they were not included in the aims of the study.

The presentation of the findings have been structurally revised to ease reading. We therefore think they can be in one manuscript.

The performance findings of the HAIN line probe assays Genotype MTBC for species identification within the Mycobacterium tuberculosis complex, CM/AS for differentiating Mycobacterium tuberculosis complex and NTM and the GenotypeMTBDRplus compared to conventional phenotypic DST have been extensively studied and shown. The use of the line probe for determination of Drug resistance of NTM has less published. However, the use of these methods may be of importance in the African setting, such as Ghana, where studies of rapid differentiation of the Mycobacterium complex from NTM and rapid DST is of importance. Did the authors consider testing the DR LPA directly on smear positive specimens? The LPA is also available for testing second line drugs and may improve the manuscript if a golden standard is included.

Response

We did not test the DR LPA directly on smear positive specimens because the study used only culture positive isolates.

LPA for testing second line drug was not done as stated in the limitation.
Did the authors consider performing the extended version of the LPA GenotypeMTBDRsl for second-line DST testing on the isolates and this may improve the manuscript if a golden standard is included?

Response

LPA GenotypeMTBDRsl for second-line DST testing on the isolates was not done as stated in the limitation.

Was there a golden standard in form of conventional phenotypic LJ based or liquid culture DST only performed on isolates with a LPA results?

Response

There was no conventional phenotypic testing on isolates with LPA results as stated in the limitation.

Which method was used for MTBC identification routinely?

Response

BD MGITTM TBc Identification Test Kit

The English language needs improvement throughout the ms. I recommend that the authors make use of language editing services. Some sentences need completion or correction.

Response

The whole manuscript has been edited to improve spelling and grammar.
The title correctly reflects the study.

The introduction describes in detail the background of the study.

The objective of the study is formulated in a relevant way but does not reflect the risk factors from the prevalence study later shown in the results section and discussion.

Selection criteria of the isolates were not described well. There are some problems in the inclusion criteria of the clinical isolates the 8,175 participants are not well described. Did they all have Mycobacterium tuberculosis or how were the 361 culture positive isolates selected?

Response

The risk factors shown in the results and discussion sections have been omitted.

The selection criteria of the isolates have been described well in the study design subsection of the methodology.

They were all MGIT culture positive.

Methods section:

Line 84: "culled" means deleted from the database?

Response

The whole sentence containing the word “culled” has been deleted from the text.

Line 100: Why was an in house DNA extraction method used and not the one recommended by the manufacturer?
Response

The kit contains reagents for PCR and hybridization process. However, the manufacturer has another kit (GenoLyse®) which is sold separately for DNA extraction. According to the manufacturer, other extraction methods producing amplifiable DNA from bacteria can be used (http://www.hainlifescience.de/en/products/microbiology/mycobacteria/tuberculosis/genotype-mtbc.html). The “An in house DNA extraction method was used” has been replace with “The boiling (heat killing) method of DNA extraction was used” in the DNA extraction subsection in the methodology. Another step in the extraction procedure has been added.

Line 107: Describe "AM-A"

Response

AM-A (amplification mix A) has been described alongside AM-B (amplification mix B) in the Multiplex amplification with biotinylated primers subsection in the main text.

Results section:

Line 139-144: The age and gender, prior TB calculations perhaps warrant another aim and design to the study. Was gender and age not previously culled "deleted" in the methods section, see above line 84.

Response

These results have been revised accordingly.

Line 153-154 use international abbreviations for rifampin (RIF) and isoniazid (INH).
Response

The international abbreviations have now been used in the text.

Line 153-154 and Line 171-173: Of the 31…was it not 165.. the percentage of 10.9% is not 18 of 31 but 18 of 165. Likewise for 11 (6.7%). Again check numbers 12.2% is not 24 of 176 but of 196.

Response

The denominator used to calculate the percentage resistances has been changed in the main text. The new percentages are: 18/165 (10.9%); 2/165 (1.2%); 11/165 (6.7%).

Line 169: Mycobacterium gordonae is not considered a human pathogen but a contaminant from water. Do the authors have information about the clinical status of the participants?

Response

Information about the clinical status of the participants are not available.

Tables are clear.

The discussion includes information from the prevalence study. However, the discussion needs to be developed to more appropriately reflect the aim of the study, which needs to be clarified.
Response

The discussion has been revised omitting information from the prevalence survey to reflect the aim of the current study.

The authors conclude the study showed usefulness of the Genotype Mycobacteria assay series as appropriate tools for simple and rapid speciation and DST of mycobacterial isolates to enhance adequate and prompt treatment of mycobacterial infections in Ghana. However, it is of concern whether this conclusion without a evaluating the assays towards a golden standard?

Response

The concluding statement has been revised.

Line 216 39% unidentified NTM sounds to be a large proportion. Could the authors explore into why so large a proportion were not identified?

Response

This is a limitation in the use of GenoType Mycobacterium CM & GenoType Mycobacterium AS in differentiating NTM species in our study. We will explore the option of sequencing these unidentified species in future work.

The authors are standing by for any more clarification.
Thank you.

Yours faithfully,

PROF. KENNEDY KWASI ADDO
CORRESPONDING AUTHOR