Reviewer’s report

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

**Version:** 0  **Date:** 05 Jun 2017

**Reviewer:** Didi Bang

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05-06-2017: Review Ms Ref. No. INFD-D-17-00134

Title: Genotyping and Drug Susceptibility Testing of 1 Mycobacterial Isolates from Population based Tuberculosis Prevalence Survey in Ghana

Reviewer comments:

Tuberculosis (TB) is a major public health problem in Ghana. Mycobacterium tuberculosis complex (MTBC) and Non-tuberculosis Mycobacterium (NTM) infections differ clinically, making rapid identification and drug susceptibility testing (DST) very critical for infection control and drug therapy.

The aim of the study aims was to use the World Health Organization approved line probe assays to differentiate mycobacterial isolates obtained from a tuberculosis prevalence survey performed in Ghana and to determine their drug susceptibility patterns.

The study design was retrospective, where a total of 361 mycobacterial isolates were differentiated and their drug susceptibility profiles determined using the commercial GenoType Mycobacterium Assays: MTBC and CM/AS for differentiating MTBC and NTM as well MTBDRplus and NTM-DR for DST patterns of MTBC and NTM, respectively.

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 reviewers main concern is the lack of appropriate golden standards and that the 361 Mycobacterium tuberculosis isolates obtained from 8,175 pariticipants 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.
The study revealed that of the 361 isolates, 165 (45.7 %) had Mycobacterium tuberculosis by the line probe MTBC assay while 196 (54.3 %) had line probe NTM. The line probe MTBC consisted of 161 (97.6 %) Mycobacterium tuberculosis and 4 (2.4 %) Mycobacterium africanum. Fourteen different NTM species were identified with majority (21.4 %) being M. fortuitum. Eighteen (10.9 %) and two (1.2 %) of the MTBC isolates were isoniazid and rifampicin monoresistant respectively while 11 (6.7 %) were multi-drug resistant (MDR).

Thirty six NTM isolates belonging to the M. avium complex and M. abscessus complex were all susceptible to macrolides (clarithromycin, azithromycin) and aminoglycosides (kanamycin, amikacin, and gentamicin).

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.

I have the following comments:

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.

Did the authors consider preforming 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?

Was there a golden standard in form of conventional phenotypic LJ based or liquid culture DST only performed on isolates with a LPA results?
Which method was used for MTBC identification routinely?

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.

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?

Methods section:

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

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

Line 107: Describe "AM-A"

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.

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

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?

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. 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?

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

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics
Quality of written English
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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