Author's response to reviews

Title: Prevalence and drug resistance profile of Mycobacterium tuberculosis isolated from pulmonary tuberculosis patients attending two public hospitals in East Gojjam zone, northwest Ethiopia

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Date: 1 May 2015

Author's response to reviews: see over
Cover Letter (Response to reviewers)

Dear Editor,

We hereby submit the revised version of a manuscript entitled "Prevalence and drug resistance profile of *Mycobacterium tuberculosis* isolated from pulmonary tuberculosis patients attending two public hospitals in East Gojjam zone, northwest Ethiopia" to be considered for publication in BMC Public Health.

In light of reviewers' comments, we have tried to provide the following justifications and revisions in the current version of the manuscript.

**Reviewer 1: (Solomon A Yimer)**

**Major Compulsory Revisions**

**Methods**

- Since our aim was to investigate the drug resistance profile of isolates from both smear-negative and smear-positive patients, we enrolled both groups (a total of 385 suspects which were calculated using a single proportion formula). Then we just tested isolates from culture positive patients. However, we didn't calculate the exact number of isolates (also considering the convenient sampling technique we used).

- We have incorporated more information about health centers and private health facilities in East Gojjam zone (lines 85-91).

**Results**
- The generalization is limited to study participants (title also modified a bit).

**Discussion**

- Now we have included points in the discussion part about the age group of 25-34 years (lines 272-277).

- We have expanded limitations part as suggested (lines 298-304).

**Minor comments**

- We have tried to fix grammatical issues as much as possible.

  **Thank you!**

**Reviewer 2: (David W Dowdy)**

  **Major compulsory revisions**

1. We have included 95% CIs for proportions in table 3 (for the combined resistance proportions) and in the body for the total proportion of any drug resistance for newly diagnosed and previously treated cases (lines 36-37).

    - We have also provided resistance proportions to a 0.01 level rather than to the 0.1 level (table 2, in results and discussion part).

2. Of course, we had subcultured each isolate once for the phenotypic sensitivity testing. Subsequent subculturinng of those failed or those with very few colonies was practically difficult due to the short period of time allocated to finalize the study and financial constraints (it was
MSc thesis). Some of the isolates (7 out the 31 lost isolates) had, of course, enough growth, but the DST result was indeterminate. The fact that we included smear-negative samples was also contributing for the loss (although it was not statistically significant, most smear-negative samples, about 34.5%, were with very few colonies and hence were not tested).

However, overall, the characteristics of patients whose isolates were not available for DST were not significantly different from those included for testing (also described in the body, lines 199-200).

**Minor essential revisions**

3. The word rate is now replaced by prevalence (line 40).

4. We have mentioned the possible change of proportion since the data was collected (line 286-288).

5. Now it is replaced with appropriate sentence and P value also provided (line 189).

6. HIV status was available for all enrolled suspects (it was an inclusion criteria) as described in line 180. In line 207(previous version and now line 212), the intention was to specifically describe the HIV prevalence among those whose isolates were tested. But the HIV status was still known for all enrolled patients.

7. The actual p value was 0.093 as indicated in table 3. It was typing error. But still we acknowledged your comments regarding the sample size and tried to couch our findings in a language of small sample size (described in the limitation, and also other parts, lines 265, 300-302).

8. We checked it and it was 0.022 (line 218).
9. Now the comparison is restricted to none overlapping or little overlapping confidence intervals (line 226-227).

10. Now the limitation part is expanded (lines 298-304).

11. Both crude OR and adjusted ORs are included (table 3).

**Discretionary revisions**

12. We have removed the genotype MDR part.

13. We have tried to reduce the text in results part.

14. We have also tried to reduce the comparisons and rather added some implications as suggested (lines 268, 276-278, 288-289).

**Thank you!**
Reviewer 3 :( Eyal Oren)

Major Compulsory Revisions

1- Methods, the comparison methods are described (lines162-164).

2- Results, the variable inclusion criterion into the multivariate model is now specified and variables clarified (lines 157-160).

3- Yes it was 0.022 (typing error) but now the adjusted OR is changed to 0.046, because we have included an additional variable (HIV status) into the multivariate model and age group is also reduced to 4 levels (as participants in the fifth age group don't have resistant strains, now it is merged with the fourth age group) table 3.

4- Now we have clearly shown both crude and adjusted ORs (table 3) and the potential confounder" HIV status" was also assessed ( Table 3, lines 158-159).

5- Discussion section, we could have been made more elaboration about Genotype MDR assay but we rather removed the genotyping result as suggested by other reviewer (David W Dowdy). The point was that it is difficult to compare the two methods (phenotypic vs. genotypic method) as the samples were very few (only three).

Major Essential Revisions

1. It was because the percentages were equal for both streptomycin and ethambutol in new cases, but still that was not clear and hence we have incorporated your comments (line 38-39).

2. We have cited a reference and tried to add supporting data for variations across regions (lines 69-70)

3. As we used the rapid HIV test algorithm, Stat-Pak kit was used to confirm those positive by KHB. But if positive by KHB and negative by Stat-pack (discordant results), Ungodly was used as a confirmatory test. We didn't use western blot due to financial constraints (Unigold indicated as confirmatory test, lines 109-110).

4- Now removed (as stated in major revisions, 5 above)
Discretionary Revisions

- It was because denominators were too small but now I have considered both percentages and frequency (lines 35-37).

- First we tried to identify the contamination by visual inspection of the culture (growth). In those contaminated tubes, there was a growth of microorganisms (which could be fungi and/or other bacteria) in less than 7 days indicating contamination as *M. tuberculosis* will not grow in this short period. Of course, we confirmed by AFB microscopy.

Thank you!