**Author’s response to reviews**

**Title:** Underestimated pyrazinamide resistance may compromise outcomes of pyrazinamide containing regimens for treatment of drug susceptible and multidrug-resistant tuberculosis in Tanzania

**Authors:**

Saumu Juma (s.pazia@kcri.ac.tz)

Athanasia Maro (athanasia.maro@gmail.com)

Suporn Pholwat (sp4vs@eservices.virginia.edu)

Stellah Mpagama (s.mpagama@kcri.ac.tz)

Jean Gratz (jean.gratz@gmail.com)

Alphonse Liyoyo (a.liyoyo@kcri.ac.tz)

Eric Houpt (ERH6K@hscmail.mcc.virginia.edu)

Gibson Kibiki (g.kibiki@gmail.com)

Blandina Mmbaga (b.mmbaga@kcri.ac.tz)

Scott Heysell (SKH8R@hscmail.mcc.virginia.edu)

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**Author’s response to reviews:**

Kilimanjaro Clinical Research Institute,
Moshi, Tanzania,

08 May 2018.

The Editor,
BMC Infectious Diseases.

Dear Editor,

Regarding Manuscript entitled Underestimated pyrazinamide resistance may compromise outcomes of new pyrazinamide containing shorter course regimens for multidrug-resistant tuberculosis in Tanzania by Saumu Pazia Juma et al.
We are grateful for the comments and suggestions by the reviewers regarding our manuscript. In the following we have addressed the detailed comments made by the editor, reviewer #2 and #3 and indicate the changes we have made. Also with this report we have included a thank you note to reviewer #1 for their time to read and comment this manuscript.

No. comments Responses

Reviewer No. 1

Well designed study and comprehensively written paper. This study adds knowledge to the growing pool of data on PZA resistance associated with multidrug resistance and treatment outcome in the context of Tuberculosis.

Half of the MDR-TB patients presented with PZA resistance as was analysed with the conventional culture based method. This is comparable to what has been published from other locations. In contrast, a higher than previously observed percentage of PZA resistance was found in non-MDR-TB patients. Respective strains were less likely to be M.bovis (which is intrinsically resistant to PZA) as the mutations differed from M.bovis related mutation. The underlying, possibly epidemiological cause will be further studied. Treatment failure was high as 80% in PZA resistant MDR-TB however sample size is small (N=6) so if at all this observation shows a trend and needs to be confirmed in a larger data set. By combining the molecular pncA test with DST for PZA the two-by-two table (Table1) shows that in this setting the molecular test has a good NPV but a lower PPV. This highlights the culture based PZA test to still be the reference method that should be performed for the analysis of the PZA status.

Thank you very much for your time and positive comments, and we agree with your assessment.

EDITORS COMMENTS

Perhaps the authors could rephrase the background section to just reflect current treatment recommendations in which Pyrazinamide is part of the standard 6-month regimen for TB. So rather not refer to older treatments that were longer in duration but are no longer recommended.

Thank you. Rephrased

As in the abstract, the focus should be on the current 6-month regimen in which Pyrazinamide is included; there is no need to refer to 9-12 month treatments.

Thank you for the observation. Rephrased

The authors should include references to WHO recommendations for standard and MDR-TB treatment.
Thank you. Done

When were the sputum samples tested? Was this done directly after collection or were they batched and analysed later? Because if they were analysed immediately, should the results not have been reported back to patients and clinicians to modify treatment if necessary?

Thank you. The sputum samples were not tested immediately as they arrive to the lab, but rather tested in batch. However, results were communicated to the treatment doctor at KNTH for decision making in case this was needed.

Did patient samples get tested by both phenotypic assays and pncA testing? If not, what were the reasons for specimens not being evaluated with both tests?

Thank you. Both phenotypic assays (MGIT PZA) and pncA (HRM) testing was done for all 91 isolates but not all M. tuberculosis isolates underwent Sanger sequencing of the pncA gene to interrogate the MGIT and HRM discrepancies. Pg 7. This is also now mentioned specifically as a limitation in the Discussion.

During the study period, were all patients admitted for drug-susceptible or MDR-TB treatment included in the study, or was there some selection?

Thank you. We have now included this information in a more detailed description of the cohort on Pg 4.

Could the authors briefly describe sputum collection procedures? And transport of samples to the laboratory?

Yes, and we have now added information on sputum collection procedures and sample transportation to the Detailed description on Pg 4 - 5.

Regarding the informed consent procedure, was informed consent also obtained from the drug-susceptible patients who provided sputum samples?

Thank you. Written consent was provided by all MDR-TB and non-MDR-TB patients Pg 11.

Page 6, first sentence, is unambiguously described. Presumably all patients that were confirmed with isoniazid and rifampin resistance were initiated on treatment and demographic and clinical data extracted for all of them?

Thank you for the observation. We have removed the sentence, “Later confirmed to be resistant to isoniazid and rifampin (MDR-TB)”. All patients with MDR-TB were initiated on treatment per hospital protocol, and those with MDR-TB had treatment outcomes data collected for the purpose of this study Pg 6.
It would be helpful to introduce subheadings to the methods section, e.g. setting and study population, laboratory analysis, data analysis, etc.

Thanks for the observation. We have inserted subheadings as recommended. The subheading for Data analysis was also added.

Was the database anonymized?

Thank you. The database was anonymized as it has used only unique study ID for the record for confidentiality.

What was the rationale for comparing pre-treatment characteristics and treatment outcomes only for those with MDR-TB? It would have been interesting to know whether there were differences between these characteristics also in the group with non-MDR pyrazinamide resistance.

We agree that it would have also been interesting to compare pre-treatment characteristics between those non-MDR with and without pyrazinamide resistance, but we did not have support to do so for all patients and as this was an observational cohort, we focused on people with MDR which were more reliably traceable through their entire treatment course.

Can the authors comment on whether there were lab controls in place to test for cross-contamination?

Thank you. We added the information on the method part of the manuscript “and with each run M. tuberculosis H37RV, known to be pyrazinamide susceptible, was include as a control.” Pg 6.

Page 7, row 17: “yet of those 14”, not clear from the text to which 14 the authors are referring to.

Thank you. We have added this clarifying change, “Yet of the 14 that were susceptible by MGIT PZA but had mutation in pncA, 4 (28.5%) demonstrated HRM results with percent similarity of 50-59% with the wild type sequence” Pg 7.

Row 39-40: those who initiated antiretroviral treatment, was it before MDR-TB treatment initiation or at the time of sputum collection? Now it is referred to both, which is confusing.

Prior to hospitalization, pre-treatment sputum collection and MDR-TB treatment initiation. This is now clarified on Pg 7.

Reference to prior studies from Tanzania (p7, row 43) should be in the discussion.

Thank you. Changed accordingly. The reference has moved to the Discussion.
The sample size is extremely small and may not be sufficiently powered to identify differences between those with and without PZA resistance and those with and without treatment success.

Thank you. We acknowledge the small sample size in the Results and Discussion.

Page 8, first sentence: you cannot state that a prior history of TB treatment was even slightly more common in patients with PZA susceptible TB. In one group 13 out of 15 patients received prior treatment, and in the other 12 out of 15. This is almost the same, and not statistically different.

Thank you for the observation. The whole sentence is removed. Pg 1 and Pg 8.

Replace “trended towards association” with “was associated”, both in main text as in abstract.

Thank you. Changed accordingly.

The authors must be careful not to overstate their conclusions. Instead of saying (p8, r 46) “was associated with an important trend toward treatment failure” – the authors can mention that the proportion of patients failing treatment was higher in the group with PZA resistance, but they should highlight that this was not a significant finding. They should then explain that this is most likely not significant because of the small sample size.

Thank you. We acknowledge the small sample size in the Results and Discussion section.

Can the authors comments on the reason why not all MTB isolates underwent Sanger sequencing of the pncA gene?

Thank you. “Due to funding constraints we were not able to perform Sanger sequencing of the pncA gene for all isolates” Pg 11. The samples were randomly selected in order to meet the number for which funding was available.

The conclusion is a repeat of the last paragraph of the discussion

Changed accordingly.

REVIEWER #2 COMMENTS

Background and results: Although rare and eventually replaced with other drugs in the regimen, baseline Lfx and Km resistance among MDR-TB patients is important to include in Table 2 (as part of clinical characteristics describing patients with and without Z resistance) and Table 3 showing treatment outcomes. As they are crucial drugs, individualized treatment using weaker agents as replacement may have contributed to treatment failure.
A more careful review of the entire manuscript is advised and correct discrepancies between Table 1 figures and the text referring to the table, rounding off of figure (MDR among 91 patients), percent on ART among 6 HIV-positives.

Correct the inaccuracy using reference 7; only HIV status is mentioned as a predictor of mortality in this article, and not Z resistance. Cite reference for MDR-TB treatment outcome definitions (completion, interruption) used in this paper.

Careful review is needed to correct typographical and grammar errors in different parts of the manuscript.

Unfortunately for these analyses we only reported PZA resistance. In prior studies from this setting conventional Lfx and Km resistance is rare, and we discuss/cite this on pg 5.

Correction done. Thank you for the observation, these were the results for the Sanger sequencing that’s. Pg 8, result section.

WHO reference added.

Done.

Use the latest Global TB Report as reference (2); use "loss-to-follow-up" instead of "default" (patient-centered terminology).

We agree with the importance of these language changes, and have done so accordingly.

- Discussion: To the reviewer's knowledge, no consensus recommendation has been made to extend treatment of Z resistance among DS-TB patients. Otherwise, cite the reference. Provide a more focused discussion.

Mentioning rates of prevalence of Z-resistance in other settings would fit better in the Background rather than expressed lengthily in the Discussion.

While no consensus recommendation exists, we have now cited the Curry Center guideline that recommend a minimum of 9 months for PZA mono resistance, which is a practiced adopted in many settings with access to PZA susceptibility testing. [Curry International Tuberculosis Center. Drug-Resistant Tuberculosis: A Survival Guide for Clinicians, Third Edition. CITC, Washington, DC 2016. http://www.currytbcenter.ucsf.edu/sites/default/files/tb_sg3_book.pdf]

We have shortened this comparative discussion.

Page 7, first paragraph of the results, and Table 1: the numbers in this section are unclear to me. The authors mention 23 discrepancies, when in Table 1 there are only 17. I don't understand what the authors refer to when they say "Those 14". In the footnote of Table 1, there is a reference to an asterisk which is not present in the Table; in any case, the results presented in the footnote should also be present, at least by a reference, in the text.
Thank you. Corrections done. 14 were susceptible by MGIT PZA but resistant by HRM.

I could not find in the text a reference to how many DS-TB patients have been previously treated for TB; as the authors correctly mention in the discussion, this is a crucial element to interpret the incidence of pyrazinamide resistance; if this information is not available, this should be acknowledged in the limitations.

Thank you for the observation. We acknowledge the missing information about the outcome of this population. See Pg 9.

Recommendations: MGIT remains to be the currently WHO-recommended DST to Z with all its drawbacks and reproducibility issues; global advice is needed for endorsement of a more reliable test. Consider to recommend to specifically review Z resistance in similar settings where retreatment is common.

Thank you. Information added. Pg 11.

REVIEWER # 3

Title: I find the title misleading, as the shortcourse MDR-TB treatment is only mentioned in a couple of passages of the manuscript and patients in the study received the conventional treatment. I would rephrase the title by underlining the high incidence of pyrazinamide resistance in both DS- and DR-TB cases, and the potential impact on any pyrazinamide-containing regimen.

Thank you for the suggestion, and we have now revised the title:

Underestimated pyrazinamide resistance may compromise outcomes of pyrazinamide containing regimens for treatment of drug susceptible and multidrug-resistant tuberculosis in Tanzania.

Page 8, line 40-46: I suggest that the authors state clearly that the "important trend" did not reach statistical significance, mostly due to the small sample size; I would also be less assertive in the conclusions.

Thank you. We acknowledge the small sample size in the Results and Discussion section.

In the Discussion, the authors should discuss the small sample size, in particular for DR-TB, as a major limitation.

Thank you. We acknowledge the small sample size in the Results and Discussion section.

Page 1, line 40, and page 3, line 23: replace "role-out" with "roll-out"

Thank you. This has been revised and rewritten accordingly.
Page 1, line 35: I would suggest using the full word pyrazinamide, instead of PZA, throughout the text

Thank you. Changed accordingly in the abstract. We defer to the copy editor and journal style to determine if this should be the case throughout the main text.

Page 1, line 47: I am not sure to see why pyrazinamide would be "increasingly important" in novel MDR-TB trials.

Of the 19 current MDR or XDR trials, 17 include pyrazinamide. For example, NC005, a novel regimen including bedaquiline, pretomanid, moxifloxacin and pyrazinamide for short course treatment of MDR was based on pre-clinical and later human culture conversion studies that pyrazinamide enhances the activity of novel agents. The same rationale for endTB, as mentioned by the Reviewer later.

Page 2, line 33: remove "even"

Thank you. Changed accordingly

Page 2, line 38: remove "only"

Thank you. Changed accordingly

Page 3, line 33: in the sentence as it is, it seems that WHO guidelines are followed mostly (or only) in TB endemic settings, which is not the case; please rephrase

Thank you. Changed accordingly

Page 3, lines 38-43: Reference 6 does not appear to be appropriate for this sentence

Thank you. Reference changed

Page 3, line 53: I would suggest replacing reference 8 with a reference to the WHO 2016 MDR-TB guidelines, which include guidance on the shortcourse treatment

Thank you. Reference changed.

Page 3, line 53: since you mention the synergy between pretomanid and pyrazinamide, I would suggest to briefly discuss also the one between pyrazinamide and bedaquiline, and the trials including this combination (i.e., endTB)

Thank you, we agree. Information added. Pg 3.

Page 4, lines 50 and 52: replace "is" with "was"

Thank you. Changed accordingly
Page 5, lines 48-53: the sentence starting with "previously" might be more suitable for the introduction than for the methods

Thank you. Changed accordingly.

Page 6, lines 21-26: the sentence starting with "fluoroquinolone" might be more suitable for the introduction than for the methods

Thank you, changed accordingly.

Page 5, lines 48-53: the sentence starting with "previously" might be more suitable for the introduction than for the methods Thank you, changed.

Page 8, line 19: add "with" between "patients" and "treatment" Page 6,

Thank you, changed.

Page 8, line 21: remove "only"

Thank you. Done

Page 10, line 14: I suggest adding an appropriate reference after "previously"

Thank you. Done

Page 10, lines 21-30: I suggest dividing this sentence in two (stop the first after "common") to increase its clarity

Thank you. Done

Conclusions: I would stress that DST seems to be critical for both DS- and DR-TB care

Thank you. Done

Sincerely yours,

Saumu Pazia Juma