Author's response to reviews

Title: Molecular detection and characterization of resistant genes in Mycobacterium tuberculosis complex from DNA isolated from tuberculosis patients in the Eastern Cape Province, South Africa.

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Author's response to reviews: see over
RESPONSE TO REVIEWERS.

The authors are very grateful for the suggestion given by the reviewers to make the manuscript better. Below are the points raised by reviewers and responses to the reviewers highlighted in blue.

REVIEWER: Christian U Uhuru.

1) The use of English needs to be revised as the lexicon articulation is somewhat poor.

   The use of English has been revised as would be seen in the annotated manuscript.

2) Line 22; HIV in South Africa would not be referred to as “epidemic” but “endermic” hence correct accordingly.
   a. Line 22, the word “epidemic” was changed to “endermic” as suggested.

3) Page 3, line 54-55, the authors sounded too “alarmist” by using the phrase “and because of its poverty, illiteracy, TB and HIV; it is heading for disaster”.

   The sentence has been revised to reflect the situation in the study area hence, it now reads as follows; “The Eastern Cape is one of the poorest Provinces in South Africa and because of its poverty, the spread of TB is enhanced”.

4) Page 3, lines 60-61: the statement is incomplete “drug resistance arises due to improper use of antibiotics in chemotherapy of drug susceptible” what message are you conveying?

   The word “organisms” has been added. It now reads “Drug resistant organisms arise due to improper use of antibiotics in chemotherapy of drug susceptible organisms [6]”.

5) Line 61; “resistance” should be changed to “resistant”

   The word “resistance” has been changed to “resistant” on line 63.

6) Page 8, lines 200; what does the authors mean by the use of the term coloured race?

   Contextually, blacks are referred to as coloured and they have reported on black hence, the appropriate language should be used.

   Correction has been effected and we have replaced the word “Coloured” with “Mixed race”.

Reviewer: Zhiqiang Z. Zhang

Abstract

1) Page 2, lines 34-35; “Mutation results in 100% MDR, 40% pre-XDR and 98.9% of XDR ...”

This statement is misleading and needs to be rephrased. Does the result indicate progression to mutation or percentages of organisms showing varied levels of drug resistance consequent to mutation?

The statement has been changed to “Other percentages of drug resistance observed including 40% pre-XDR and 60% of XDR”.

2) Background
Page 2, lines 47-48; “Tuberculosis is a major public health concern a third’s of the world’s population is infected with MTBC” This statement is ambiguous so, recast. Having contact with MTBC is not same as infection from pathology/public health perspective. A typical example is the vaccination/immunization with BCG. This should not be included in the infection ratio while looking at the disease burden.

The sentence has been recasted to “Tuberculosis is a major public health concern and a third of the world’s population is infected with some members of MTBC [1]”.

3) Page 3, line 54; province should be written in lower case as it is not there as a noun.

Page 3, Line 54; province has been written in a lower case as suggested.

4) Line 59; “Anti-TB drug resistance” this phrase is misleading please recast

The sentence has been recasted to “Resistance of the organisms to TB drugs is a major public health problem that threatens the progress made in TB control worldwide”.

5) Lines 60-61; “Drug resistance arises due ... drug susceptible”. Please revise the statement as its present state conveys no meaning.
“The word “organisms” has been added. It now reads “Drug resistant organisms arise due to improper use of antibiotics in chemotherapy of drug susceptible organisms [6]”.

6) Lines 71-72; rephrase the sentence.
The sentence has been rephrased to “Inappropriate use of second line drugs used in the treatment of TB leads to amplification of resistance and development of XDR-TB [10]”.

7) Line 77; what gene was detected? In other words, what was mpb64 and IS6110 coding for?

“(mpb64 coding for immunogenic secretory protein specific for Mycobacterium tuberculosis complex and IS6110 insertion element found exclusively within the members of the MTBC)” has been added.

8) Methods
Line 82; Recast the subheading title as nothing in your write up showed documentation of the study area. I would suggest using “Sample Collection” alone.

As suggested, Sample area has been deleted. The subheading now reads “Sample Collection”.

9) Line 86; “culture over from January” what is this? “Demographic profiles including...” there was no documentation on demographic profile rather there are documentations on biodata. Please use the appropriate terms.

This sentence form part of the sentences that needed to be revised for language, it now reads …… “culture over a period of 24 months from January 2012 to 2013 December”. The phrase “demographic profile” has been deleted and word “biodata” used as suggested.

10) Line 127; the subheading is inappropriate, it does not reflect the work done hence, revise accordingly.

The subheading has been changed to “Polymerase chain Reaction for INH, RIF, Rrs and eis genes”. Then in line 163, the subheading “Sequncing of INH, RIF, Rrs and eis genes” was added to reflect the work that was done.

11) Line 128; poor lexicon usage

Line 128 has been changed to read “Resistant genes to first-line drugs were amplified using primers….”

12) Results and discussion
Line 178; the authors reported 5% of the samples to be positive to MTBC hence, why the contradiction of 100% here?

Indeed there is a contradiction in line 178 to what was said before/above. Therefore, the sentence was deleted as its explanation will only be a repetition of the above reported statement, “5% of the samples to be positive to MTBC”.

13) Line 190; this contradicts the methods section where the authors documented an assay procedure carried out. Did the authors run this section or simply go the results from another lab (NHLS)? Please clarify this.

The documented procedure was done, when we initially wrote the manuscript, we wanted to use only samples from NHLS, however, we decided to use the samples from the province because they were many, while those from NHLS were only 40. We should have deleted the sentence as it does not apply on this manuscript. Hence thank you very much for picking that up and informing us of the contradiction. The sentence has been deleted.

14) Line 195; “the drawback of the Seeplex ... members of the MTBC” expunge this statement as it is a repletion of what you have in lines 182-183.

Line 195; “the drawback of Seeplex…..members of the MTBC” has been expunged.

15) Line 199; replace “demographic data” with “biodata”

“Demographic data” has been replaced with “biodata” as suggested in line 199.

16) Line 200; replace “coloured race” with “mixed race” as black people are known as coloured.

The word “coloured race” has been replaced with “mixed race” in line 200.

17) Line 204; what was the bases for the classification of age group 0-14 as young people? 15 to 35 as youth? Etc. Please you the known standard of WHO/UNICEF for the classification of adolescents, youths and adults.

Our age group categorization was obtained from Stats SA for Nelson Mandela Bay Municipality (2011) which is the study site. Hence, changing to suit the WHO age classification will not correspond with the reality of our findings.

18) Lines 215-216; rephrase the sentence as it is contradictory.

The sentence has been rephrased to “However it could also be because of sampling bias where only samples from eastern part of the Eastern Cape were received irrespective of gender or race”. 
General comments

19) The manuscript would read better if the authors revise language use; improve upon lexicon use and eliminate personal idiosyncrasies.

Language use has been revised accordingly as would be seen in the annotated version of the manuscript.

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