PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Evolving rifampicin and isoniazid mono-resistance in a high multidrug-resistant and extensively drug-resistant tuberculosis region: a retrospective data analysis
AUTHORS	Mvelase, Nomonde Ritta; Balakrishna, Yusentha; Lutchminarain, Keeren; Mlisana, Koleka

VERSION 1 – REVIEW

REVIEWER	Emma Roycroft Irish Mycobacteria Reference Laboratory, Dublin, Ireland.
REVIEW RETURNED	23-Jul-2019

GENERAL COMMENTS	This is a very well-written paper on an extremely important and topical subject - i.e. the need for an accessible genotypic test for resistance to both rifampicin and isoniazid! It may not be possible, but the study would have even more statistical strength if the years 2015-2017 were also included. Also, the lack of Xpert data is a little disappointing, since that forms the basis of the 'diagnostic algorithm' change in 2011. The references range from 1970 to 2017. It might be necessary to perform a more up-to-date literature review prior to publishing the paper. Line 118 - 'cultures were performed' could be changed to 'specimens were cultured' 95% confidence intervals would be useful along with standard deviations Line 123 should read 'in total positive TB cases'

REVIEWER	Margaretha de Vos Foundation for Innovative New Diagnostics (FIND) Switzerland
REVIEW RETURNED	29-Jul-2019

GENERAL COMMENTS	Major comments: 1. The authors state that data was collected from isolates routinely done. However the authors failed to explain whether this collection included newly diagnosed patients (using Xpert MTB/RIF and following the routine diagnostic algorithm) or whether it includes re-treatment cases and isolates collected for patient treatment monitoring. If retreatment cases were included it may affect the

	results of the study. A flow diagram may allow the reader to better understanding the inclusion criteria used in this study. 2. I am not convinced that the methodology that the authors used to identify duplicate samples per patient is feasible. What was the unique identifier and for how many isolates were there not an unique identifier. I.e. for how many isolates did the authors need to remove duplicates by using demographic data, what type of demographic data was used. Please refer to McIntosh et al 2018 in Plos Med where the authors created a tool for this purpose. My understanding is that this is a complicated procedure and that even date of birth is not a reliable variable to identify duplicates. The removal of duplicates forms the basis of the final database used in the study and will affect the outcome of the results if not properly done. Can the authors please describe their methodology in detail and provide a flow diagram of the outcome. Which duplicate was removed (the first of the last isolate)? 3. If only data from Xpert MTB/RIF rifampicin resistant isolates were used the authors does not explain why there are INH-mono resistant isolates in the collection. If these are Xpert TB negative isolates from HIV positive individuals, this needs to be explain. 4. If data from all specimens received for culturing were received (including non diagnostic samples), it does not explain the decrease in cultures over the four years. 5. Is the high percentage of isolates receiving LPA realistic? Can the authors give a breakdown for the 15% that did not receive LPA (i.e loss of viability, contamination, no result). 6. How many isolates received pDST for the second-line antibiotics. The percentage decline of XDR is not a good reflection if there was a decline in isolates receiving pDST. 7. The decrease in number of isolates between 2012 and 2014 is not well explained. This was after Xpert was implemented and in theory the number should have increased as more patients would be referred for cultured (where the authors stated that before the implementation of Xpert only patients not responding to treatment were referred for culture). Minor comments: 1. All "MDR TB" and "DR TB" and "XDR TB" and "rifampicin resistant" needs to be hyphened. 2. WHO stats needs to be updated with the 2018 report 4. All "et al." needs to be italicized

VERSION 1 – AUTHOR RESPONSE

Reviewer 1: Emma Roycroft

1. It may not be possible, but the study would have even more statistical strength if the years 2015-2017 were also included. Also, the lack of Xpert data is a little disappointing, since that forms the basis of the 'diagnostic algorithm' change in 2011.
   Response: Thank you for your comment. We agree that the use of Xpert data would have added value into the study as we could make a direct comparison with the culture results. However, it was not possible to include it in the study. We used the 2011-2014 data because it was during this time that major changes were happening in the TB program that were

expected to influence culture confirmed TB. This data was also readily available as it was used to monitor the program during this time. In addition, there has been no subsequent changes in the TB diagnostic algorithm after the period of this study. However, it would be important to monitor any changes in the subsequent years.

2. The references range from 1970 to 2017. It might be necessary to perform a more up-to-date literature review prior to publishing the paper.
   Response: The references have been updated

3. Line 118 - 'cultures were performed' could be changed to 'specimens were cultured'
   Response: Thank you, 'cultures were performed' has been changed to 'specimens were cultured' (Line 121)

4. 95% confidence intervals would be useful along with standard deviations
   Response: 95% confidence intervals have been added on the results section of the manuscript

5. Line 123 should read 'in total positive TB cases'
   Response: Sentence updated (Line 127)

Reviewer 2: Margaretha de Vos

Major comments:

1. The authors state that data was collected from isolates routinely done. However, the authors failed to explain whether this collection included newly diagnosed patients (using Xpert MTB/RIF and following the routine diagnostic algorithm) or whether it includes re-treatment cases and isolates collected for patient treatment monitoring. If retreatment cases were included it may affect the results of the study. A flow diagram may allow the reader to better understanding the inclusion criteria used in this study.
   Response: Thank you for your suggestion of a flow diagram, we have now included it as Figure 1 of the manuscript. This study looks at the specimens received in the provincial TB culture laboratory, so only TB culture results were analyzed. As this is a laboratory based study, there was no clinical information regarding previous history of TB. So, the findings include any patient with TB culture positive specimen irrespective of whether they were new cases or retreatment cases. We mentioned this fact as one of the limitations of the study (Line 268-269). We also gave indications for TB culture in line 61-63, which include:
   - Patients with Rifampicin resistant TB on the Xpert MTB/RIF
     Unlike in the Western Cape province where patients send two specimens in the initial diagnosis of TB (one for Xpert MTB/RIF and another one for smear/culture), in KwaZulu-Natal province only one specimen is sent for the initial diagnosis of TB using Xpert

MTB/RIF (Line 220-223). Thereafter, depending on the Xpert MTB/RIF results, a second specimen is taken for TB culture if the Xpert MTB/RIF results demonstrated rifampicin resistant TB.

- Patients suspected of having paucibacillary TB that could have been missed by the Xpert MTB/RIF (HIV infected, children and extra-pulmonary TB)
- Patients who fail TB treatment

2. I am not convinced that the methodology that the authors used to identify duplicate samples per patient is feasible. What was the unique identifier and for how many isolates were there not a unique identifier? i.e. for how many isolates did the authors need to remove duplicates by using demographic data, what type of demographic data was used. Please refer to McIntosh et al 2018 in Plos Med where the authors created a tool for this purpose. My understanding is that this is a complicated procedure and that even date of birth is not a reliable variable to identify duplicates. The removal of duplicates forms the basis of the final database used in the study and will affect the outcome of the results if not properly done. Can the authors please describe their methodology in detail and provide a flow diagram of the outcome. Which duplicate was removed (the first of the last isolate)?
   Response: The lack of a unique identifier in an ongoing issue in the South African context. Without a unique identifier, any deduplication method cannot be perfect. In order to remove duplicates, we used the MRM number (which is a number given by the laboratory to specimens from the same person), plus demographic details (Name, surname and date of birth). Only the first episode was included.
   We note the tool created by McIntosh et al, however, the KwaZulu-Natal (KZN) province is quite different from the Western Cape province in that almost all patients in the public sector are from the same tribe/language (the Zulus). So it is quite common for totally different patients to have the same name, surname and age. That is why we had to use a more stringent deduplication process.

3. If only data from Xpert MTB/RIF rifampicin resistant isolates were used the authors does not explain why there are INH-mono resistant isolates in the collection. If these are Xpert TB negative isolates from HIV positive individuals, this needs to be explain.
   Response: Please refer to our response to number 1 above on the indications for TB cultures. So assuming that the TB diagnosis guidelines were followed, the INH monoresitant cases would be coming from either paucibacillary TB cases (missed by the Xpert) or treatment failures (probably patients failing first line TB treatment due to missed INH monoresistance). Line 201-202.

4. If data from all specimens received for culturing were received (including non-diagnostic samples), it does not explain the decrease in cultures over the four years.

Response: The cultures decreased because the majority of TB patients have susceptible TB, which according to the South African guidelines do not get a TB culture.

5. Is the high percentage of isolates receiving LPA realistic? Can the authors give a breakdown for the 15% that did not receive LPA (i.e. loss of viability, contamination, no result)?
   Response: By eliminating patients with rifampicin susceptible TB (which is the majority of TB patients), TB culture can then be performed in the remaining patients as described above. LPA was only performed on cultured isolates and not on clinical samples. All TB positive specimens got an LPA for TB drug susceptibility testing. The 15% that did not get an LPA consists of patients where clinicians only requested a phenotypic DST on the laboratory request form.

6. How many isolates received pDST for the second-line antibiotics. The percentage decline of XDR is not a good reflection if there was a decline in isolates receiving pDST.
   Response: All TB culture positive specimens received a pDST for both first line (rifampicin and isoniazid) and second line (ofloxacin and kanamycin) antibiotics. The number of XDR decreased but the proportion of MDR-TB that had XDR-TB remained relatively unchanged at 11%.

7. The decrease in number of isolates between 2012 and 2014 is not well explained. This was after Xpert was implemented and in theory the number should have increased as more patients would be referred for cultured (where the authors stated that before the implementation of Xpert only patients not responding to treatment were referred for culture).
   Response: This was one of the unexpected findings after the implementation of the Xpert MTB/RIF. We expected an increased in the volume/number of specimens from drug resistant TB cases. Instead, we found a substantial decrease although the proportion of RR/MDR-TB was steadily increasing. We believe that this is due to the change is the testing algorithm. In the manuscript, we give discuss several possible reasons for this (Line 211-229):
   - Patients with Xpert rifampicin resistant TB not getting a subsequent TB culture.
   - Patient loss to follow up. The second sample is only taken during the second visit when the MDR-TB treatment is initiated. During the time of the study, approximately 40-60% of RR/MDR-TB patients were initiated on treatment. So the second sample would not have been taken in cases where patients did not come back for treatment.

Minor comments:

1. All "MDR TB" and "DR TB" and "XDR TB" and "rifampicin resistant" needs to be hyphened.
   Response: This has been revised

2. WHO stats need to be updated with the 2018 report
   Response: The stats have been updated

3. All "et al." needs to be italicized

   Response: All et al has been italicized

VERSION 2 – REVIEW

REVIEWER	Emma Roycroft Irish Mycobacteria Reference Laboratory, Ireland
REVIEW RETURNED	30-Sep-2019

GENERAL COMMENTS	Thank you for addressing my comments. Best of luck with your future work.

REVIEWER	Margaretha De Vos Foundation for Innovative New Diagnostics, Switzerland
REVIEW RETURNED	27-Sep-2019

GENERAL COMMENTS	The authors have addressed my comments adequately. However I would like to ask the authors to add in the limitation section that the data presented is not prevalence data and that the results may be an underrepresentation as data from MTB positive cultures were used. Thereby loss to follow up patients and patients with contaminated/loss of viability cultures were excluded.

VERSION 2 – AUTHOR RESPONSE

Response to Reviewer 1: Emma Roycroft

-No response required

Response to Reviewer 2 : Margaretha De Vos

-I have included the suggested statement on the limitations section: Line 278-280