Author’s response to reviews

Title: Molecular detection of rifampin and isoniazid resistance to guide chronic TB patients’ management in Burkina Faso.

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Author’s response to reviews: see over
Reviewer's report:
Title: Molecular detection of rifampin and isoniazid resistance to guide TB chronic patients' management: a feasibility study in Burkina Faso
Version: 1 Date: 9 April 2009
Reviewer: Hendrik Simon Schaaf

Reviewer's report:
Molecular detection of rifampin and isoniazid resistance to guide chronic tuberculosis patients’ management: a feasibility study in Burkino Faso
The authors present a study of laboratory evaluation of sputum samples of chronic TB patients, identified by sputum smear-positive results after failing a retreatment regimen, and how these results can help to improve the management of the chronic TB cases, would these tests be available in Burkino Faso. The reviewer's opinion is that the study was not very well designed, as it seems that specimens were obtained far into WHO regimen IV therapy in quite a large proportion of cases, which definitely could play a role in the study outcome. There were also other limitations as mentioned below. Despite this, there is merit in the study and the results are interesting – especially if it can be presented in a clear way.

The reviewer has the following comments:

Major compulsory revisions:

Abstract and title:
1. Title and elsewhere in manuscript: Should change “TB chronic patients” to “chronic TB patients”. I think that the title is somewhat misleading as the laboratory part of the study was done in Italy and not in Burkina Faso – it would only be feasible if the laboratory work can be done in Burkina Faso itself.

“Our chronic patients” has been modified to “chronic TB patients” as suggested and the entire title has been modified to “Molecular detection of rifampin and isoniazid resistance to guide chronic TB Patients’ management in Burkina Faso”.
At this purpose we would like to underline that the study started under the promise that the local laboratory would have been ready before the end of the study and therefore sample preparation (as a first step) and the full test (as second step) could have done in Burkina faso. Moreover, the head of the local laboratory was specifically trained in Italy. Unfortunately, the opening was delayed for technical reasons and the laboratory will be operational shortly. We decided not to postpone the study in response to a specific request of the National Tuberculosis Program (NTP) that needed the results for an appropriate management of patients under second-line drug treatment.

2. The aim in the background seems to be the wrong way round: As I understand the study, the researchers “…evaluated the molecular assay Genotype MTBDRplus for detecting DR-TB directly in clinical specimens as a means to a more accurate management of chronic TB patients in Burkino Faso.”

We agree and accept the suggestion. The text has been modified accordingly.

3. The method in the abstract does not clearly state on what grounds patients were selected as “chronic TB patients” – were they all supposed to be sputum smear-positive on microscopy? This is what I gather from the results because almost half the patients were not “reconfirmed as sputum smear-positive” which
The statement “One hundred and eight chronic TB patients (sputum smear positive after completing a re-treatment regimen for pulmonary TB under directly observed therapy) were enrolled in the study from December 2006 to October 2008” was added in the method section in the abstract.

Methods (Main body of manuscript):
4. Methods, page 5, study setting: The authors need to say what the HIV prevalence in the country/setting is where the study was done. Looking at the high rate of NTM infection in the chronic cases, the authors should give some information about NTM infection rates in the area. Lastly, the second part of the first sentence should be deleted because it is repeated in the third sentence.

HIV prevalence has been included in the section. To the best of our knowledge, data concerning NTM infection rates in the area are not currently available. The second part of the first sentence “a country where culture capacity is not yet in place” was deleted as recommended.

5. Methods, page 5, 2nd paragraph: What, in these patients, is category IV regimen? This is not a standard regimen, but could be different regimens as far as I understand. The readers not familiar with WHO TB guidelines will also not know what regimen IV is – please define accurately.

Treatment regimen has been defined as recommended.

6. Methods, page 5, 3rd paragraph: Why were all patients not enrolled at the beginning, before starting follow-on therapy? Was there any cut-off time for not further enrolling a patient, or were they all still smear-positive by the time of enrollment (not clear from current methods)? I think that two different early-morning specimens were sent to the two laboratories (same morning or different days?) can also be a limiting factor giving different results.

National Tuberculosis Program (NTP) and local clinicians asked for the enrolment of patients already under treatment in this study in order to verify the appropriateness of their classification. Sixty-one of them were enrolled in this study at the beginning of category IV regimen and 47 patients were enrolled at variable time during their therapy regardless of smear-microscopy (21 smear-positive, 26 smear-negative at enrolment time).

7. No mention is made of ethics or institutional review board approval for the study, especially in the light of taking specimens out of the country.

This study is part of a contract for technical assistance for the management of MDR-TB cases between the Ministry of Health, Burkina Faso, the San Raffaele Institute, Milan, Italy and the University of Brescia, Italy.

Results:

7. Results, page 8, first paragraph: The authors state that all chronic TB cases were confirmed sputum smear-positive when diagnosed – were these smears checked by the research team or were these the results from the local laboratories? It is quite concerning that there is such a big difference in smear results with 1/3 of patients not yet on treatment having a smear-result discrepancy?
We agree with the referee and we are now addressing this issue with the NTP. All the cases enrolled at month 0 were found smear-positive at the enrolment centres. Some of them were not reconfirmed as smear-positive in Italy. We specified that 26 patients included in the study during category IV therapy follow-up were already smear-negative at the time of the enrolment. The overall quality of the direct smear examination in some areas of the country is an issue of concern. QA is presently a priority for NTP authorities. One of the public health consequences of this study is that evidence was provided on the need to tackle this key issue. Smear-positivity was not the criterion used for the enrolment of patients during therapy (M1-M30).

8. Results, page 8, sputum smear positive sample analysis: This part is difficult to follow. Most of the numbers of the GenoType MTBDRplus test results again appear in Table 1, but then the percentages are different because different denominators are used, which is confusing. I think that it may be better to present numbers and percentages only in table 1, not to be repeated in text, and to add the culture results to the table (or if too difficult, only present the culture result comparison in text), showing how these compare. The text can then be used to mention the complexities of the results. Some important findings are presented, such as discrepancies between culture and GenotypeMDRTBplus methods, but it gets lost in a maze of results and grammatical problems. Table 2 is very helpful and clear.

The text has been simplified but some percentage has been left for matter of clarity. Culture and culture DST has been included in Table 1 as recommended. Enrolment time was also added.

9. Results, page 9: Frequency of mutations: last sentence of 1st paragraph: Were these rifampicin-resistant strains diagnosed by the absence of WT probes confirmed by culture and DST?

The following paragraph was added to the results section: “Two of them were confirmed as RIF-R by DST. Three cases bearing the following mutations in rpoB (M515I+H526N, L533P, and H526N respectively) resulted RIF-S by DST and were further investigated. Minimum inhibitory concentration (MIC) evaluation was performed on the strains. The two strains harbouring L533P and H526N, respectively, despite resulting RIF susceptible, showed a slight increase in the MIC value (0.5 µg/mL). The strain carrying the 515I+H526N substitutions showed a MIC of 2 µg/mL.”

Discussion:

10. Discussion, paragraph 3. The study method was not very inductive for good results from the GenoType MDRTBplus, as many of the specimens were obtained (long?) after category IV treatment was initiated – the results obtained could therefore be incorrect, with original organisms already been removed. I think these results should therefore be interpreted with some care.

We agree about the limitations of study design: as already specified, the study was performed with the aim of supporting and potentially improving the patients management in a setting lacking laboratory facilities. An additional aim of the study is, of course, to show how the test can be used in support to the clinical management of patients.

11. Discussion, page 11, 4th paragraph: the results mention 2 rifampicin monoresistant cases by MDRTBplus method being identified as MDR TB by culture (therefore two INH resistant cases missed)

The sentence “In addition, two MDR-TB cases has been reported as RIF-monoresistant strains by the molecular assay.” has been added as recommended.
12. Discussion page 12, 2nd paragraph: The authors need to explain better why there were such huge discrepancies between sputum smear microscopy results between Burkino Faso and Italy – sampling time is not clear, and in many cases both samples were taken before treatment was initiated (according to methods)

We specify that 26 patients included in the study during category IV therapy follow-up were already smear-negative at the time of the enrolment. Some patients were defined chronic TB cases based on smear microscopy results performed at peripheral centres. The overall quality of the direct smear examination in some areas of the country is an issue of concern. QA is presently a priority for NTP authorities. One of the public health consequences of this study is that evidence was provided on the need to tackle this key issue.

13. Discussion page 12, 3rd paragraph: Why were suboptimal methods of transportation used?

Unfortunately one shipment was not delivered promptly and they were stored under sub-optimal conditions.

14. Discussion page 12, 1st paragraph – the results presented in this paragraph need to be moved to the results section

The paragraph has been moved in the results section as suggested (see results comment n 9) and replaced with the sentence “Further evaluation on the three strains carrying substitutions in rpoB gene allowed to identify increased MIC values”.

15. The conclusions on page 14 should be part of the discussion – as in my second comment I think that the aim should be turned around. Further in the same paragraph, the authors now call it the Hain molecular test, while previously (and more correctly) referring to the Genotype MTBDRplus test. At the end there is another conclusion which is more correct.

Aim has been modified as previously suggested. “Hain” has been modified in “GenoType MTBDRplus®”. Conclusions have been re-arranged as suggested.

16. The manuscript needs major grammatical revision

English revision has been performed by two mother-tongue TB and infectious diseases experts colleague, who has been acknowledged for his effort.

Minor comments

1. Abstract, results, 5th line: no-tuberculous mycobacteria (not uppercase M)

The word has been corrected as recommended.

2. Abstract, results, page 3 – INH mutations – need to see the numbers

The number has been added as recommended.

3. Abstract, conclusions: should state “sputum specimens”. Also, it may contribute to limiting the emergence of drug resistance (although this is logical, it is not part of this study’s results and therefore can probably not be a conclusion)
The inappropriate conclusion has been deleted as recommended.

4. Background, page 4, ref 4-6: There also exists evidence in some recent articles that cure rates are not necessarily very low – this should also be presented

We have modified the sentence as requested.

5. Background, page 4, 3rd paragraph, last line: change “MTB drug resistant strains to “drug-resistant MTB strains”

The sentence has been modified as recommended.

6. Page 6, 2nd paragraph, 3rd line: abbreviations should first be written in full before used, e.g. DOT. In the following paragraph, what does (M0) stand for?

Acronyms and abbreviations have been written in full before use in the text.

7. Page 8 and elsewhere – the authors should decide to use drug susceptible or drug sensitive – the first is preferred (WHO guidelines). Using both terms interchangeably causes confusion.

The word “sensitive” has been replaced with “susceptible” as recommended.
Reviewer's report:
This paper by Miotto et al concerns a very important issue: the rapid detection of MDR Mycobacterium tuberculosis in a resource poor nation. In this study they tested a new commercial method for detecting the mutations responsible for INH and rifampin resistance using sputums that were collected in Burkina Faso and shipped to Italy for testing. In Burkina Faso there are no facilities for doing Mycobacterial culture, let alone sensitivity, so patients are assumed to have resistant organisms if they do not respond to anti-tuberculocous treatment. It was this group of patients that were the source of the sputa that were assayed, but unfortunately more than 40% of the samples were obtained after the patients had been started on a new treatment regimen. In one laboratory (in Milano) they did the PCR and did the hybridizations using GenoType MTBDRplus (Hain Life Science), and in another lab (in Brescia) they cultured the sputa in the MGIT system and did INH and rifampin sensitivities. The group in Milano also sequenced the rpoB, inhA, and katG genes and identified the resistance mutations. In general, the results with were quite positive and revealing, but the samples were less than ideal. For instance 1/3 of the samples taken before a new regimen was started and about half of the sample taken after therapy were smear-negative when re-examined in Italy. Thus, either the original assignments were in error, or there were relatively few organisms in the sputa because different samples were examined in Africa and Italy. However, even the smear negative sputa were useful in that 19/51 yielded a PCR result and of these 15/19 were not resistant mutants, showing the cases were clinically misclassified and those patients potentially were going to receive less than optimal therapy for their TB. Only 4/19 grew bacteria and 2 of those were MAC, not TB. One that was shown to be INH resistant by GenoType MTBDRplus assay did not grow, and another grew Nocardia, presumably a dually infected patient. The overall performance of the GenoType MTBDRplus was excellent with nearly all specimens yielding a result and there was concordance between the genotype results and sensitivity tests in nearly all cases; only 1 INH resistant strain was missed by genotyping.. Where there were discrepancies that authors were able to define the mutations and explain them.

I have several suggestions for minor revisions that I think will strengthen the paper.

1. Make a figure with a flow diagram showing how many specimens were collected before treatment, how many after treatment and divide them into smear + and smear – specimens and then show the results of analysis in the two labs.

   A flow diagram has been included (Figure 1).

2. In table 1 include the culture and sensitivity results.

   As suggested, culture and susceptibility results have been included in table 1.

3. In the discussion they should include much of what is in the conclusions about the usefulness of the test. This study strongly suggests that clinical criteria, promoted by the WHO and in widespread use, for diagnosing MDR Tb are inadequate because of too many false positives.
The sentence “Moreover, this study strongly suggests that sputum smear-microscopy as only
criteria to identify chronic TB patients is inadequate because of the risk of false positives.
” have been included as suggested.

4. They say the study was designed to test the feasibility of using GenoType MTBDRplus as a more
accurate test for managing patients in Burkina Faso. I think this study clearly shows the need for a
more accurate and rapid test, but not necessarily the feasibility, as this study was really done in Italy
and it is not feasible to ship specimens from Africa to Italy on a routine basis.

The title and text have been modified.

5. Unless they are sure that the strains they tested are not genetically related because of nosocomial
spread, I do not think it is justified to make the statement that the genetic basis of RIF resistance is
different in Burkina Faso than elsewhere. It may only be different because a resistant strain is
spreading in the institutions they are studying.

MIRU-VNTR genotyping technique was applied to the available isolates (n 11) harbouring the most
frequent mutation in \textit{rpoB} (D516V substitution). Despite 72.7% were belonging the Ghana lineage,
recent transmission could not be proven.
The paragraph was modified as follow: “Results obtained in our sample suggest that in Burkina
Faso the frequency of mutations involved in RIF resistance differs from that of other settings. The
commonest mutation is the D516V substitution in the hotspot region of \textit{rpoB} gene, detected totally
in 43.8\% (14/32) of RIF resistant cases. The S531L mutation is responsible of resistant phenotype
in only 9.4\% (3/32) in our study, whereas this substitution affects the majority of RIF resistant
strains in most of other countries worldwide“.