Author's response to reviews

Title: Association of mutation patterns in gyrA/B genes and ofloxacin resistance levels in Mycobacterium tuberculosis isolates from East China in 2009.

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Author's response to reviews: see over
Dear Editor,

Thank you for your review of our manuscript (MS: 3380119547869476). We appreciate the concerns and suggestions provided by the reviewers, and have revised our manuscript accordingly. Our point-by-point responses are provided below, and text that has been added or modified from the original text is shown in the revised manuscript in red font. We know that your journal has high publication standards, so we have already had the language of this paper corrected by a professional language editing service that specializes in scientific manuscripts.

Upon review of our revised manuscript, we hope that you will find it acceptable for publication in *BMC Infectious Disease* and we look forward to your response.

Sincerely,

Zhenling Cui

**Responses to editor:**

*Comment 1: Please provide an email address for each author on the title page.*  
**Response:** The email addresses for each author are provided on the title page. [Page 1, Line 13-18]

*Comment 2: Experimental research that is reported in the manuscript must have been performed with the approval of an appropriate ethics committee. Research carried out on humans must be in compliance with the Helsinki Declaration (http://www.wma.net/e/policy/b3.htm). A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate. Informed consent must also be documented.*  
**Response:** The study was approved by the ethics committee of the Shanghai Pulmonary Hospital. Written informed consent was obtained from all the participants. This statement has been added in the manuscript [Page 5, Line 78-79].

**Responses to Reviewer #1 (Paolo Miotto):**

*Comment 1: Along the manuscript drug concentrations are reported sometimes as µg/mL whereas some other as mg/L. To help the reader I suggest using always the same concentration unit.*  
**Response:** Throughout the manuscript, drug concentrations were reported as mg/L.
The concentration unit µg/mL has been changed to mg/L.

Comment 2:
Materials and Methods
Paragraph “Drug susceptibility test”: I suggest “The drug susceptibility test (DST) of selected strains was…”.
Response: In accordance with your suggestion, “The drug susceptibility test (DST) of selected strains were…” was revised to “The drug susceptibility test (DST) of selected strains was…” [Page 5, Line 82].

Comment 3:
Acknowledgements
“This work was supported by the grant 10QA11405800 from the Shanghai Rising-Star Program to [PI]”.
Response: The acknowledgements were revised. This work was supported by the grant 10QA11405800 from the Shanghai Rising-Star Program to Zhenling Cui [Page 14, Line 252-253].

Comment 4:
Carefully check text format (apex, spaces…). Abbreviations should be described at the first time of use; once introduced, abbreviations should be used along the all manuscript.
Response: There was one abbreviation in the Abstract, “minimal inhibitory concentrations (MICs)”. There were six abbreviations in the text, fluoroquinolones (FQ), ofloxacin (OFX), tuberculosis (TB), M. tuberculosis (MTB), quinolone resistance-determining region (QRDR), nitrate reductase assay (NRA) and analysis of variance (ANOVA), which were used throughout the manuscript.

Comment 5:
Materials and Methods
Paragraph “MIC determination”: replace “according to the reference manual [20]” with “as described by Kumar and colleagues [20]”.
Response: “…according to the reference manual [20]” was replaced by “…as described by Kumar et al [24]”. The references themselves were not changed, but the numbering of the references was changed [Page 6, Line 90].

Comment 6:
Results
Paragraph 1: I suggest “S95T mutation in gyrA is a natural polymorphism”; please provide appropriate reference.
Response: Reference [25] was provided [Page 8, Line 127].

Comment 7:
Results
Paragraph 2: please report percentages using only one decimal, as along all the manuscript.

**Response:** The report percentages used only one decimal throughout the manuscript.

**Comment 8:**
**Discussion**
Carefully check for the use of abbreviations (e.g. *M. tuberculosis*, fluoroquinolone, ofloxacin... all these have been already introduced in the manuscript; please use abbreviations also in the discussion section).

**Response:** The use of abbreviations has been checked. The abbreviations for *M. tuberculosis*, fluoroquinolone and ofloxacin were used throughout the manuscript.

**Comment 9:**
**Discussion**
Paragraph 3: I suggest “amino acid modification at codons 90, ...”.

**Response:** “…amino acid modification at codons 90, …” replaced “..amino acid modification in codons 90, …” [Page 10, Line 183].

**Comment 10:**
**Discussion**
Paragraph 4, statement 2: eliminate “But”.

**Response:** “But” was eliminated [Page 11, Line 206].

**Comment 11:**
**Discussion**
Paragraph 4: add “[according to] several published studies”.

**Response:** Accoding to the comment 11, “a 772bp fragment of *gyrB* including the reported mutation codons was sequenced according to [10,17,31,32]” was replaced “a 772 bp fragment of *gyrB* was sequenced that included the codons in which mutations have been reported [10,17,31,32]” [Page 11, Line 208-209].

**Comment 12:**
**Tables.**
I suggest to provide data as follow:

<table>
<thead>
<tr>
<th>Codon mutation</th>
<th>Nucleotidic change</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(Value 1)</td>
</tr>
<tr>
<td>…</td>
<td>…</td>
<td>…</td>
</tr>
</tbody>
</table>

and then report range and median values in the text (results section) or in the table on other columns.

**Response:** Table 1 and 2 were revised as follows. The range and median values of the MICs were showed in the text [Page 8, Line 139-141].

**Table 1:** The patterns of *gyrA* mutation and OFX MICs profile of OFX-resistant
Table 2: The patterns of gyrA/B mutation and OFX MICs profile of 11 OFX-resistant MTB strains.

<table>
<thead>
<tr>
<th>Codon mutation</th>
<th>Nucleotidic change</th>
<th>gyrB</th>
<th>gyrA</th>
<th>MICs (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. of strains</td>
<td>MIC (mg/L)</td>
<td>2</td>
</tr>
<tr>
<td>D94A</td>
<td>GAC-GCC</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>D94G</td>
<td>GAC-GGC</td>
<td>5</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>D94N</td>
<td>GAC-AAC</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>D94Y</td>
<td>GAC-TAC</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>D94F</td>
<td>GAC-TTC</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A90V</td>
<td>GCG-GTC/GTG</td>
<td>6</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>S91P</td>
<td>TCG-CCG</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>A90V&amp;D94Y</td>
<td>GCG-GTC &amp; GAC-TAC</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comment 13:

Materials and Methods

Paragraph “Selection of strains”: the Authors state that clinical isolates were collected from epidemiologically unlinked cases. Please report how did you performed epidemiological selection (e.g. based on genotyping techniques such as IS6110 or similar).

Response: The methods for the epidemiological selection (IS6110 restriction fragment length polymorphisms) were added and the relevant reference [20] was also added [Page 5, Line 71-72].
Comment 14:
Materials and Methods
Paragraph “PCR”: please report GenBank accession numbers for each gene.
Response: GenBank accession numbers for gyrA (GenBank accession no.: 887105) and gyrB (GenBank accession no.: 887081) were added [Page 7, Line 109-110].

Comment 15:
Materials and Methods
Paragraph 3: please explicitly state why D94F mutation has been excluded from the ANOVA analysis.
Response: The D94F mutation has been included in the ANOVA analysis in this manuscript, and the results of the ANOVA were calculated afresh [Page 9, Line 145 and 149].

Comment 16:
Discussion
The discussion section could be further deepen and modified in order to be more exhaustive.
The Authors refer to the paper by Yin; since the paper was about levofloxacin, I suggest to made some appropriate comments.
I suggest to refer and compare your results with those obtained by Sun Z. et al in Int J Antimicrob Agents 2008 (vol. 31) on OFX-resistant strains.
Response: The discussion section was modified and extended. We have compared our results with those obtained by Sun et al. [Page10-11, Line 185-193]. We also added an analysis and comparison with the results against those obtained by Yin et al. [Page 11, Line 193-199].

Comment 17:
Discussion
Paragraph 4: the Authors state that some mutations found are not related to OFX resistance. Even though, in principle, these statements may be acceptable, because the lack of an experimental proof-of-principle (e.g. cloning mutations in well characterized/reference strains) I suggest to better clarify that these statements are hypotheses/inferences.
Response: We have clarified that these statements are hypotheses/inferences. We are currently performing experiments (e.g., cloning mutations in reference strains) to verify the effect of gyrB mutation on OFX resistance in MTB strains [Page 12, Line 236-240].

Comment 18:
Conclusions
Last statement: please better clarify the statement “Furthermore, our findings
indicate that the association of the extent of drug resistance and corresponding gene mutations varied due to different anti-TB drugs”.

**Response:** We have further clarified the statement, “Furthermore, our findings indicate that not all the patterns of gene mutations related to drugs reflect the resistance level of the corresponding drug for MTB drug-resistant isolates. The patterns of gene mutations related to rifampicin and isoniazid resistance reflect the resistance level of corresponding drug, whereas the patterns of gyrA/B mutation could not reflect the resistance level of OFX.” [Page 13, Line 245-249].

**Response to reviewer #2 (Claudio Köser):**

**Line 21-22:** R485L mutation missing.

**Response:** The R485L mutation was added into the Abstract [Page 2, Line 29].

**Comment 2:**

*Introduction:* Various mutations from clinical strains have been analysed before. Most notably amongst these studies is the fact that hyper-susceptibility to fluoroquinolones (FQ) has been described [1-4]. Moreover the possibility has been raised that low-level FQ resistance can be overcome using a high dose of moxifloxacin (at least in mice) [5]. The introduction should be adjusted to reflect these findings.

**Response:** The mechanism by which FQs bind to DNA gyrase and inhibit DNA replication was added to the Background and supported by the structural analysis and functional analysis of the enzymes of *M. tuberculosis* (MTB) DNA gyrase according to the references given by the reviewer. The possibility that low-level FQ resistance can be overcome using a high dose of moxifloxacin in a murine model was introduced in the Background section. [Page 4, Line 46-50]

**Comment 3:**

*Line 97:* Well done for including the version number for the Genbank accession no. Most researchers do not do this but it is essential.

**Response:** The version number for the Genbank accession no. of gyrA and gyrB was added in the manuscript [Page 7, Line 109-110].

**Comment 4:**

*Line 98 + 176:* Primers are fine but I get a different size for amplified fragments.

**Response:** The sizes of the amplified fragments were recalculated and the accurate sizes are now shown in the manuscript [Page 7, Line 111].

**Comment 5:**

*Line 112:* Add reference that S95T is a polymorphism.

**Response:** Reference [25] has been added [Page 8, Line 127].
Comment 6:
Line 121: Space missing after initial "and"
Response: A space has been added [Page 8, Line 134].

Comment 7:
Line 161: Space missing after "studies"
Response: A space has been added [Page 10, Line 174].

Comment 8:
Line 173-174: The list of mutated codons should be reviewed. Different studies do not always use the same numbering system. For example, some studies rely on the *gyrB* sequence deposited by Takiff et al. (Genbank L27512.1) whereas others use the numbering in Figure 2 of Takiff et al. [6]. To complicate things further, the start of *gyrB* has been recently revised by TuberculList which curates the sequence of the H37Rv laboratory strain:
Response: The amino acid numbering system of *gyrB* (GenBank: CAB02426.1) was added to the manuscript [Page 9, Line 152]. The list of mutated codons in paragraph 4 of the Discussion section was listed by a different numbering system [Page 11, Line 203-206].

Comment 9:
Line 179: was not found by Cui et al.
Response: The “T539P” mutation was deleted [Page 12, Line 211].

Comment 10:
Line 183: Mokrousov et al. [7] use a different numbering system. This is not the same codon.
Response: The manuscript has been revised to explain that Mokrousov et al. used a different numbering system from the one used in this study [Page 12, Line 225-226].

Comment 11:
Discussion: The authors should compare their results more closely with studies in which their mutations were described previously (e.g. D500N [5, 8, 9], [10], and R485C [11]– again, note the different numbering systems). Furthermore, they should mention that the *gyrA* S95T is a phylogenetically informative polymorphism whereas the polymorphisms detected by Cui et al. in *gyrB* are probably not [12].
Response: The results such as D500N, T539N and R485C of this study were analyzed by comparing to the corresponding reference in discussion section. [Page 12, Line 212-216 and Page 13, Line 229-233]. The *gyrA* S95T polymorphism was mentioned as phylogenetically informative [Page 11, Line 199-201].

Comment 11:
Table 1: Why are A90V mutants (9 and 15 stains) represented in two lines rather than
just one despite that they have the same base changes?

**Response:** The two lines representing A90V mutants have been merged into Table 1 [Page 21].