Reviewer’s report

Title: Frequency and Patterns of Second-line Resistance Conferring Mutations among MDR-TB isolates resistant to a Second-line drug from eSwatini, Somalia and Uganda (2014-2016)

Version: 0 Date: 26 Mar 2019

Reviewer: Elisa Tagliani

Reviewer's report:

Authors describe the frequency and patterns of second-line resistance conferring mutations among MDR isolates phenotypically resistant to second-line drugs from three African countries: Uganda, Somalia and eSwatini.

Although the manuscript has greatly improved upon revision, additional changes should be made to consider the work suitable for publication.

One of the main concerns regards the interpretation of mutations as markers for resistance (line 259-260). In particular, authors miss to identify the gyrB mutations Asp500His and Asn538Asp that are in the QRDR as mutations conferring resistance to fluoroquinolones, and that MTB strains with these mutations have MIC for fluoroquinolones that are above the critical concentration (Malik, S., et al. PLoS One 7, e39754 (2012). Technical Report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis. Geneva: World Health Organization; 2018 (WHO/CDS/TB/2018.5). Licence: CC BY-NC-SA 3.0 IGO.

Authors should specify the gyrB numbering systems used (ref. to Maruri et al. J Antimicrob Chemother. 2012 Apr; 67(4): 819-831).

Line 261: The number of LFX/MXF resistant isolates lacking known drug resistance conferring mutations in the QRDR should be modified (N=5, not 6).

Results/discussion/conclusion and Table 1 should be modified accordingly.

Authors should define "high level resistance" (used in lines 61-62, 64, 262, 272) as opposed to resistance mutations. The gyrA mutations in position 88, 90, 91 and the gyrB mutation Asp500His and Asn538Asp are not associated with a high-level increase in MIC for fluoroquinolones (World Health Organization, Technical Report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis. Geneva: World Health Organization; 2018 (WHO/CDS/TB/2018.5). Licence: CC BY-NC-SA 3.0 IGO), so it is not clear why authors refer to them as high level resistance mutations.
According to Supplementary Table 1, sample TC54005 is susceptible to both OFX and MXF but resistant to LFX. This is a very unlikely situation suggesting that this is possibly a case of false resistance to LFX. Phenotypic DST for LFX should be repeated for this sample.

References should be revised.

Line 87: Ref 5. Malik S, et al. PloS one 2012, 7(6):e39754, is not the appropriate reference for the sentence "MDR-TB and XDR-TB are very difficult to treat as the drug regimens are lengthy, toxic, and expensive" [5].


Minor comment:

Line 63: Asp94Gly is repeated twice, replace one of them with Asp94Asn.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

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