Reviewer's report

Title: Genotyping and Drug resistance patterns of M. tuberculosis strains in Pakistan

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Reviewer: Richard Huard

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Overall Impression:

The authors provide a survey of the population structure of M. tuberculosis strains causing tuberculosis in Pakistan along with resistance patterns and other demographic comparisons. The work is novel and important, as well as complete and well-presented. Although the quality of written English is satisfactory, there are small errors throughout the manuscript that a second proof-reader could catch and correct. In the future, the authors should remember include line numbers in draft manuscripts in order to aid the Reviewer in making remarks. Below are specific comments that the authors must adequately address before the article is ready for publication (ie. Minor Essential Revisions). Major compulsory revisions are noted.

Specific Comments:

Abstract:

1. the use of terms "unique" in place of non-clustered with respect to spoligotypes is confusing. Are they unique because they've never been seen before or not in SpolDB4 (orphans) or are they simply single spoligotypes not part of a cluster?

2. Similarly, the use of the term "homologous" is nonconforming to accepted nomenclature and lacks specificity when "variants" or 'derivatives of" a major spoligotype signature is more clear.

3. The Conclusions statement beginning with "Lack of association of" does not make sense. Lack of MDR in CAS1 strains would suggest that appropriate therapy is delivered. More appropriate conclusions here are required.

Introduction:

1. Overly long with content that is not relevant to the study data and a somewhat illogical order of presentation. Efforts in addition to the following suggestions should be made to streamline it better.

2. Paragraph 1 is OK except that MDR should be defined "that is, resistance to rifampin and isoniazid"

3. Paragraph 2 should be cut, it is superfluous.

4. Paragraph 3 should be moved back in the introduction. EAI strains are
introduced before spoligotyping which defines EAI. The definition of TbD1 is confusing. TbD1 is present in modern Mtb and intact in ancestral Mtb strains. The final statement starting with It is further suggested is logically flawed. There are few SNPs known that define specific Mtb lineages and none of these are resistance related, perhaps excepting the pncA mutation in M. bovis conferring PZA resistance. No restricted geographical localization has been found for the major SNPs conferring resistance to Rif or INH. This sentence should be cut.

5. Paragraph 4: MIRU is not synonymous with IS6110 RFLP. SpolDB4 is not defined. The sentence starting with Studies reporting strain diversity is out of place and should be moved somewhere else. The BCG info is not necessary to understand the current study and should be cut.

6. Paragraphs 5 and 6 should be merged and reorganized significantly. The authors are in factual error with regard to the prevalence of the Beijing family. The prototypic Beijing spoligotype is the single predominant signature in SpolDB4 but Beijing-family strains account for a smaller proportion of TB cases worldwide than T, LAM, and Haarlem (see Brudey et al. BMC Microbiol. 6:6:23). Please correct.

7. Paragraph 7: The definition of XDR is incorrect. The correct definition is MDR plus resistance to any fluorquinolone and resistance to at least one second-line injectable drug (amikacin, capreomycin, or kanamycin). Without a solid definition, the authors conclusions with respect to XDR-TB are suspect (Major compulsory revision).

8. Paragraph 8: ancestral not ancient

Results section:

1. Paragraph 1: extrapulmonary (n=76) is stated twice. Why so few strains from Baluchistan? Five strains is hardly a representative sample and the authors should temper their conclusions with respect to this province a note in the discussion is called for (Major compulsory revision). Spelling for Baluchistan is different in Figure 4.

2. Paragraph 4: stating % homologies is noninformative and unnecessary cut.

3. Paragraph 6: All ST info should be present in Figure 2 and so should be cut from the text of the paper. As mentioned above the use of homology should be altered.

4. Paragraph 7. Same with Unique strains also confusing with U spoligotype (= unknown) designation that is used.

5. Final paragraph: is the definition of XDR-TB correctly applied? It is confusing based upon the lines above in this paragraph and the lack of a definition outside of that hidden in the Methods. The XDR data should be in a paragraph separate from the other first line drugs. The p value for MANU1 is different in the Abstract compared to here. The breakdown of the number of strains in Pak, U, unique, and MANU1 that were XDR is not given. The most MANU1 XDR strains possible is 5, if only one or two the relevance of your statistical association is suspect.
(Major compulsory revision).

Discussion:

1. Paragraph 2: the authors should temper their enthusiasm for the idea that unique strains causing extrapulmonary disease are more virulent and so more genetically variable. To the contrary, the body of literature to date supports that unique strains are less virulent. They are certainly less transmissible.

2. Paragraph 3: SST? The authors have also fallen prey to the disregard of certain other authors to previously established nomenclature. So-called TbD1-intact EAI strains are not related to TbD1-deleted EAI at all. In fact they are CAS strains (see Lancet 7:328 that is their attempt to sort out the mess they created). The authors should modify their discussion accordingly.

Figure 2: these data would be more comprehensible and associations easier to make if the spoligos were arranged according to Binary rather than Class (eg group Pak 1-12 and CAS). Classes are missing (such as LAM9, LAM6, and Pak 34-36) â## all should be included.

Figure 4: legend should overtly say that nonclustered strains are not included. (Very nice image).

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

'I declare that I have no competing interests'