Reviewer's report

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

Version: 5 Date: 3 November 2008

Reviewer: Stefan Niemann

Reviewer's report:

Dear Sirs,

Tanveer et al. have revised the paper according to the comments made by the reviewers. Several parts of it read now much better. However, there are still significant revisions necessary that are detailed below.

Sincerely yours

Stefan Niemann

Major Compulsory Revisions:

1. Visual check of the genotype classification shown in Fig. 1 reveals questionable cases (see below). Therefore, I would suggest that additional methods will be used to improve this (discussed below and in previous review) or that the data will be checked once again maybe with help of an expert in the field.

2. Introduction line 128. The use of the TBD1 deletion alone makes not really sense. It is well known that CAS strains carry this deletion while EAI do not (e.g. see MIRUVNTRplus webpage). Additional deletion typing might be used to confirm lineage classification e.g. RD750 for Delhi/CAS strains or RD239 for EAI strains. This applies also for the abstract were I would suggest to delete lines 67-68.

3. Results line 227. Please add a number and percentage for CAS strains here. A valid definition of the classification of the CAS strains should be provided. I would not group the U and Manu strains with the CAS strains. Here additional deletion analysis of lineage specific deletions can confirm classification (see point 4).

4. Results: I would suggest to separate the presentation of genotype and cluster data. The results section is really confusing and not easy to read. Follow a strict read line starting with genotype classification (Table 1) and ending with association analysis. Table is very important and must be notified in the text of the paper. One comment here: Why didn’t you classify orphan types in genotypes?

5. Results line 239. The spoligotypes of PAK 16 and 35 might be similar to Haarlem strains by cluster analysis, but they are definitely no Haarlem genotype
strains (More likely EAI). Overall, genotype classification appears to be questionable for several isolates and a revision of this is necessary.


7. 

• Discretionary Revisions

1. Introduction line 113: “Certain genotypes. E.g. Beijing…..have been associated with a number of major outbreaks” Please clarify this sentence. A number of epidemiological studies have revealed transmission of TB strains in a number of different settings. Here, strains of nearly all described genotypes have been involved depending on the geographical setting.

2. Introduction line 113-120: The whole passage is not very clear and a mixture of not very well related and confirmed statements. The authors should avoid overstatements and try to present in their introduction valid facts that were represented with a “red line” in it. For example, the fact that a Haarlem outbreak has been reported does not necessarily mean that Haarlem strains are associated with outbreaks and extended transmission everywhere. Please present a careful overview on the global and local population structure and avoid over interpretation here. Indeed, there is a association between particular phylogenetic lineages and disease characteristics such as Beijing and MDR TB. However, this should be presented correctly and more carefully.

3. Introduction line 124: There are numerous studies that confirm the association between Beijing and resistance. Please use a correct citation here (e.g. Glynn et al2002).

4. Methods line 200. I would like to know how you performed you comparison with the SPOLDB4 database: strains by strain?

5. Results deletion analysis. How many strains were tested in total? Your finding is well known and not very helpful for you study. It would be better to use selected deletion typing for lineage classification. E.g. do Pak 15 and 35 strains have the TBD1 deletion or not?

6. Discussion line 309. Please add correct citation here.

7. Discussion line 327. This has been confirmed before.

• Minor Essential Revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the
statistics.

Declaration of competing interests:
I declare that I have no competing interests