Reviewer's report

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

**Version:** 1  **Date:** 7 February 2008

**Reviewer:** Subhash Parija

**Reviewer's report:**

**Major Compulsory Revisions**

**Background**

The authors have mentioned that “In addition, Pakistan is surrounded by high TB disease burden countries such as, India (rank 1), China (rank 2), Afghanistan (rank 22) and Iran (rank 55) which may further facilitate TB transmission through the region[4]”. This doesn’t make any logic because the global ranking of Pakistan in terms of TB burden is 7th which is very high and it itself has the potential to transmit TB to other regions. This needs to be modified.

Authors should mention the recent rate of MDR-TB from Pakistan reported by health surveillances or WHO survey.

What about the nucleotide sequencing? Was the sequencing outsourced? If yes, please provide the details and a brief description of the methodology followed. Also, it would be better if the authors can provide the chromatogram of the sequencing analysis.

For MDR studies from where the reference sequence data was obtained. If obtained from Gene Bank, EMBL or DDBJ then authors are requested to mention the source. If authors have used their own data then they should mention the appropriate Accession numbers of the submission.

Regarding the drug resistance pattern the authors should give a concise description of the sensitivity and resistant pattern on scale; this would be useful information for the researcher working in the field.

Regarding the 205 Unique Cluster or Clade. Whether these unique Cluster or Clade of M. tuberculosis sequences were submitted to gene bank. If yes mention their accession number. If not, the authors are advised to submit the sequence to gene bank.

Also whether these unique clusters were subjected to BLAST in the genome database?

**Discussion**
The discussion could be more enriched by inclusion of more data from Pakistan regarding the M. tuberculosis prevalence and MDR pattern in last 10-15 years.

Methods
The authors should mention clearly regarding the sample collection and storage conditions under separate heading like “sample details”.

Whether the primer validation was done? If yes, then the authors should mention what has been done to validate the primers used in the study especially for Spoligotyping and TbD1 Analysis.

A brief description of “Spoligotyping” methodology must be given in the text.

There is no clear mention of the statistical analysis performed in the work. It would be better if the authors can provide a brief description of statistical analysis under separate heading.

Minor Essential Revisions
Abstract
In the abstract section the M. tuberculosis should be written as Mycobacterium tuberculosis in the first mention.

Abbreviations such as CAS, T1, EAI, CAS_DEHLI, U, MANU, TbD1, NWFP etc. should be mentioned in full form in the first mention.

Background
In the background section instead of using symbol (> ) the appropriate word like more than should be mentioned at least in the text.

References like WHO Report 2007 and WHO 2007 should be appropriately mentioned under reference section and should be indicated as reference number in text.

Result
There is repetition of the data in text and illustration (Fig 1 and 2). Author is advised to avoid the repetition on several occasions. Also the Figure 1 is not clear and resolving therefore the UPGMA data (dendrogram) cannot be appreciated well.

There is repetition of data in tables and text.

Discussion
In page number 12 the sentence {Prevalence of Beijing strains in our study at 3% (n=25) compares well with data from Delhi, where 8 % of 105 isolates are
thought to be of Beijing family[40].} should be rewritten as {Prevalence of Beijing strains in our study at 3% (n=25) compares well with data from Delhi, where 8% of 105 isolates are reported to be of Beijing family[40].}

In page number 12 the sentence the reference (http://www.pnas.org/cgi/doi/10.1073/pnas.052548299) should be appropriately mentioned in the reference section and should be numbered in the text accordingly. The same should be followed in other cases too as this has been repeated on many occasions.

Methods

This section is not structured properly. It should be structured with suitable subheadings to make the presentation clear.

**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 limited interest

**Quality of written English:** Acceptable

**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'