Author's response to reviews

Title: Recent-Transmission of Mycobacterium tuberculosis (MTB) strains among Iranian and Afghan Treatment failure Cases: Use of IS6110-Fingerprinting

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Author's response to reviews: see over
Answer to question:

Reviewer 1 (Dr. M. Filipenko)

Specific Comments

Material and Methods:

- For identification of M. TB a new line has been added in Methods (page 6, bacterial isolation, last paragraph). "All isolates were identified as M. tuberculosis by using biochemical tests, including production of niacin, catalase activity, nitrate reduction, pigment production and growth rate." [15]

- Name of health centre and city has been included in setting section and data collection page no 6.

- "Setting: The National Research Institute of Tuberculosis and Lung Diseases, which acts as the reference unit for National Tuberculosis Program, is the only centre for diagnosis and treatment of relapse patients.

- And in Data collection, The study was conducted from June 2005 to June 2007. Generally, all health facilities in Tehran refer their TB suspect to National Reference TB laboratory (NRL) Tehran, Iran for susceptibility and identification test.

- About drugs profile and its results, drug concentration added in page 7 paragraph 3: Drug susceptibility testing against isoniazid (INH), rifampin (RIF), streptomycin (SM), ethambutol (ETB) and pyrazinamide (PZA) were performed by the proportional method on Lowenstein-Jensen media at a concentration of 0.2, 4.0, 4.0 and 2.0 µg/ml, respectively. [16]

- And in the results section new paragraph in page 8 has been included: "As shown in Table 1, one hundred and thirty one Iranian (131, 65%) and thirteen Afghani cases (13, 22%) were susceptible to all 4 drug tested. The results showed that seventy two patients (72, 28%) were MDR-TB cases. Notably, 38; 52.7% of MDR-TB cases were isolated from Afghan immigrants. Twenty patients (20, 47%) had mono drug resistant strains (9 were INH, 7 were SM, 3 were RF and 1 were ETB) and twenty two patients (22, 52%) had a combined resistance.

- Amount of chromosomal DNA and PUVII enzyme included in page number 7.

- About dendrogram, the new line in page number 7 added: "The dendograms were generated by the hierarchic unweighted pair group method analysis (UPGMA) clustering algorithm."

Results:

The information like nationality, susceptibility results and age and gender, and spoligotyping patterns in each cluster were also included in figure 1.

- AS suggested by second reviewer, the data of spoligotyping in material, results and Discussion were included.

Discussion: Since the spoligotyping results has been included, I changed the discussion parts and I mentioned about our previous work as kindly suggested by reviewer.

For English correction I had send it to my prof (S. Hoffner, Swedish Institute) and he critically revised the manuscript.
Thanking you

Answer to question:

Reviewer 2 (Dr. C. Sola)

about definition of new cases versus replaces , the paragraphs in page 6 has been included. Patients included in this study had at least two episodes of TB, with cure as the outcome of the first episode. According to WHO criteria, cure was defined as the completion of a course of six to eight months of directly observed combination therapy (with isoniazid, rifampin, and pyrazinamide in a single tablet), compliance (attendance for the course of therapy, with at least 80 percent of prescribed dose taken) and a sputum culture positive for MTB at diagnosis and at least one negative sputum culture at the end of treatment. Recurrence or relapse was defined as development of culture positive for MTB and symptoms consistent with tuberculosis after the patient had completed a course of treatment and had been confirmed cultured negative and clinically recovered [11].

For methods:

I had rewritten the whole introduction section and included the following sentence in page number 5: However, since RFLP analysis with IS6110 alone may be inconclusive for strains carrying few copies of IS6110 [13], we also used an alternative PCR-technique called spoligotyping. The technique detects various non-repetitive spacer sequences located between small repetitive units (direct repeat DR) in the chromosome on MTB complex [14].

Also I included the data of spoligotyping in material, results and Discussion.

As kindly suggested by reviewer I had changed the discussion totally and included our previous observation.

Thanking you.