Author's response to reviews

Title: Epidemiology of Anti-tuberculosis Drug Resistance in a Chinese Population: Current Situation and Challenges Ahead

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Author's response to reviews: see over
Dear editors and reviewers,

Thanks a lot for the comments on our manuscript entitled with “Epidemiology of Anti-tuberculosis Drug Resistance in a Chinese Population: Current Situation and Challenges Ahead”. We carefully read and revised it again. Here are the responses.

Reviewer: DIMITRIOS PAPAVENTSIS

1. The aim of the study, to provide information for the Jiangsu region, not previously included in the Chinese surveillance system, is well defined.

2. The methods used for sampling and data, have been developed by the WHO/IUATLD, and are appropriate and well described. Authors should provide reference for the sampling method and the DST proportion method and critical drug concentrations used (e.g. the 2008 WHO/HTM/TB/2008.392).

   Answer: In the revised version, we added the reference [Guidelines for surveillance of drug resistance in tuberculosis(WHO/HTM/TB/2009.422)].

Did the authors perform any molecular techniques regarding M.tuberculosis identification and drug-susceptibility testing, and if yes, which?

   Answer: Currently, we are performing several molecular techniques such as Spoligotyping and MIRU, to explore the association between specific genotypes of MTB strains and drug resistance. The data have not been published.

3. The data obtained are clearly presented in the Results section. Some points that should be clarified include:

   a. Was non-tuberculosis mycobacterium cases (n=24) identified based solely on PNB growth? Were these isolates identified at the species level? If an additional method was used, it should be included in the Methods section.

   Answer: Identification of MTB was done using the p-nitrobenzoic acid (PBN) and thiophene carboxylic acid hydrazine (TCH) resistance test. Growth in LJ medium containing PNB indicates that the bacilli does not belong to the MTB complex.

   b. Apart from drug mono-resistance, it would be interesting to present "ANY" drug resistance, at least for INH and RIF (table 1).Wouldn't it be better to show this in a pie chart or another graph?

   Answer: We added a graph (Figure 2) to illustrate the proportion of drug resistance.
c. Please include p values described in the text (page 9), also in Table 2 (statistical significance between new cases and previously treated).

Answer: We added \( \chi^2 \) and P values in the revised version.

d. Based on the patient location, proportion of MDR among new cases in the south region, seems to be significantly less compared to the other two regions vs. what is being described for the previously treated cases (Table 3). Please add a comment on the possibility of primary transmission of MDR-TB strains.

Answer: In the revised version, we added a comment on the possibility of primary transmission of MDR-TB strains.

“Geographical variation of drug resistance was observed in the present study, where a higher proportion of MDR-TB among new cases was found in the central or north part as compared with the south area. Understanding the patterns of transmission is important in TB control because acquired resistance and primary resistance require different control strategies. To prevent primary drug resistance, new technologies for early distinguishing drug resistance and adopting effective measures to block the transmission are important. To reduce acquired drug resistance, development of high-quality drugs and strength of patient management are needed. Lower proportion of primary MDR in the south part indicates that TB control in this region is rather successful and lacks substantial transmission of MDR strains as compared with the central and north part of Jiangsu. However, similar higher proportion of acquired drug resistance among TB patients in these three regions also caused concerns for strengthening drug development and patient management.”

e. Please change “P for rend” to “P for trend” (Table 4)

Answer: We corrected this error in the revised version.

f. If any data are available for quinolones or injectable drugs (amikacin/capreomycin), it would be of special interest to be included.

Answer: In this study, we only tested drug susceptibility to the four first-line anti-tuberculosis drugs. We would like to explore the resistance to the second-line drugs in the future.
g. It would also be of great interest to report data regarding native population vs. immigrants, as Jiangsu is a province absorbing a large number of non-permanent residents. What is the OR for MDR-TB of the moving population vs. native population?

Answer: As the reviewer mentioned, Jiangsu is a province absorbing a large number of non-permanent residents. The role of migration in the transmission of MDR-TB is an interesting topic. In this study, we didn’t specifically design questions to distinguish the moving population from the native population. However, one question might be associated with this topic: “How long have you been living here”? We analyzed this variable, but found no significant association. One possible explanation might be a large part of moving population preferring to return to their home when they were suspected as TB. We think a revised epidemiological design is needed to further analyze the role of migration on MDR.

<table>
<thead>
<tr>
<th>How many years have you been living here?</th>
<th>MDR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No, n(%)</td>
<td>Yes, n(%)</td>
</tr>
<tr>
<td>&lt;2</td>
<td>59(3.88)</td>
<td>13(4.29)</td>
</tr>
<tr>
<td>2-5</td>
<td>108(7.10)</td>
<td>14(4.62)</td>
</tr>
<tr>
<td>5-</td>
<td>1,354(89.02)</td>
<td>276(91.09)</td>
</tr>
<tr>
<td>Total</td>
<td>1,521</td>
<td>303</td>
</tr>
</tbody>
</table>

χ² = 2.55, P=0.279

4. The discussion and conclusions are adequately supported by the data, however, some parts of the discussion could be shorter in extent.

a. Additional explanation should be given regarding the higher proportion of MDR-TB in the central and northern parts of the province compared to the southern. Why is there such a difference for younger patients in the south region (page 12)?

Answer: In the revised version, we added a comment on the possibility of primary transmission of MDR-TB strains.

“Geographical variation of drug resistance was observed in the present study, where a higher proportion of MDR-TB among new cases was found in the central or north part as compared with the south area. Understanding the patterns of transmission is important in TB control because acquired resistance and primary resistance require
different control strategies. To prevent primary drug resistance, new technologies for early distinguishing drug resistance and adopting effective measures to block the transmission are important. To reduce acquired drug resistance, development of high-quality drugs and strength of patient management are needed. Lower proportion of primary MDR in the south part indicates that TB control in this region is rather successful and lacks substantial transmission of MDR strains as compared with the central and north part of Jiangsu. However, similar higher proportion of acquired drug resistance among TB patients in these three regions also caused concerns for strengthening drug development and patient management.”

b. What do the authors mean with “equivalent mono-therapy or bi-therapy” (page 12)?
Answer: Routine DST of MTB is not available at the country level of China. In many health facilities, TB patients are usually treated with the first line anti-tuberculosis drugs without knowing drug susceptibility test results. For example, if the MTB is resistant to INH and RIF, the combination of INH, RIF, SM and EMB equals to the bi-therapy of SM and EMB. Under this condition, the combined therapy might be equivalent to mono-therapy or bi-therapy.
To clarify this issue, we revised this sentence as “Even in some health facilities with necessary equipments, the results of susceptibility test can take as long as several weeks, when the patients with MDR-TB have already been treated with INH and RIF for a long time”.

c. Please give examples of specific rapid techniques needed (page 12)
Answer: We added examples of techniques for rapid identification of MDR-TB.
“Therefore, new strategies, rapid diagnostic tools[24,25] ( such as molecular line-probe assay), new anti-tuberculosis therapies, and effective vaccines—are urgently needed[26].”
Reference:

d. Again, why is there such a difference between the south and central/northern regions in the proportion of patients with previous treatment among different age groups (pages 13-14)?
Answer: In the revised version, we analyzed the role of geographical area in the risk of MDR-TB and we also gave a comment on the possibility of primary transmission of MDR-TB strains in different areas.

“Geographical variation of drug resistance was observed in the present study, where a higher proportion of MDR-TB among new cases was found in the central or north part as compared with the south area. Understanding the patterns of transmission is important in TB control because acquired resistance and primary resistance require different control strategies. To prevent primary drug resistance, new technologies for early distinguishing drug resistance and adopting effective measures to block the transmission are important. To reduce acquired drug resistance, development of high-quality drugs and strength of patient management are needed. Lower proportion of primary MDR in the south part indicates that TB control in this region is rather successful and lacks substantial transmission of MDR strains as compared with the central and north part of Jiangsu. However, similar higher proportion of acquired drug resistance among TB patients in these three regions also caused concerns for strengthening drug development and patient management.”

6. Limitations of the work are clearly stated in the last part of the discussion. Lack of evidence for XDR-TB prevalence, is an important limitation of the study.
Answer: We would continue to explore drug resistance to the second-line anti-tuberculosis drugs in the future by using current data and specimen.

7. The title and abstract are accurately describing what has been found in the study.
8. In general, the manuscript language is acceptable. However, the language has specific problems and grammatical errors which should be corrected. For
example:
- Abstract, background (page 2, line 3): “…a cause of concerns on tuberculosis…” could be replaced by “a cause of concern for tuberculosis…”
- page 2, second line from the end, and throughout the text: “middle part” to “central part”
- page 4, line 5: “the first two countries were India” to “the first two countries being India”
- page 6, line 1: “in this study, we aims to…” to “The aim of the present study was to…”
- page 7, last line, page 8, first line: “the bacilli are not belonged to the MTB complex” to “the bacilli did not belong to the MTB complex”
- etc.

Answer:
We corrected these language errors in the revised version.

Reviewer: Isabelle Devaux

General comments:
The topic (ant-tuberculosis drug resistance) is of public health relevance, in particular in China where TB incidence and MDR-prevalence are particularly elevated (as stated by the authors in the background section). The aim of the study is clear “to assess the drug susceptibility pattern of MDR-TB” focusing in one particular province of China which is Jiangsu. However this study presents some limitations that could be explored by further analyses and/or clarifications: source of data and recruitment process, selection of age categories, more detailed geographic representation of cases.

Abstract: multivariate analysis

Answer: In the revised version, we modified the abstract and replaced ‘Multivariate analysis’ with ‘Multiple logistic regression analysis’.

Multiple logistic regression analysis was performed to identify the risk factors for multidrug-resistant (MDR) bacterial infection. The strength of association was estimated by odds ratio (OR) and 95% confidence interval (95% CI).”

Age reference >62years what is the rational?

Answer: In the former version of the manuscript, we used tertile as the cutoff point for
the age where age was categorized as >=62, 41-62 and <41 years. As mentioned by
the reviewer, though it is a rational way in statistics, the results were difficult to be
used for comparing with other areas or countries. Thus, in the revised version, we
categorized the age into six groups (<25, 25-34, 35-44, 45-54, 55-64 and 65+ years),
based on age categories from the WHO-global report [Multidrug and extensively
drug-resistant TB (M/XDR-TB) 2010, GLOBAL REPORT ON SURVEILLANCE AND RESPONSE].

What is the reference category for the place of residence?
Answer: In the revised version, we clearly indicated that south part was set as the
reference. The proportion of MDR-TB among new cases was higher in the central
(9.50%) or north part (9.57%) than that in the south area (4.91%). Compared with
patients living in the south part of Jiangsu province, those living in the central or north
part had 1.47-fold (95% CI: 1.01-2.13) or 1.42-fold (95% CI: 1.05-1.92) increased
risk, respectively.

Background
Tuberculosis (TB) is a leading cause of death due to an infectious agent: could the
authors specify which infectious agent?
Answer: We specified the infectious agent by modifying this sentence as
“Tuberculosis (TB) is a leading cause of death in humans due to an infectious agent
(mycobacterium tuberculosis, MTB) and it remains a major public health burden in
developing countries”.

Methods
P6: “the annual reported new sputum smear positive (SS+) cases were 23603 and
previously treated SS+ cases were 5524 (based on surveillance data in
2007)”. Could the authors provide a reference? What was the source of data? Were
the patients recruited in the study from the same source of data?
Answer: In China, a TB surveillance system has been built for several years. Patients
were registered in local TB Dispensary and reported to the upper level administrative
authorities. Individual information, onset of symptoms, date of diagnosis, sputum
smear test, chest X-ray examination, prescribed medication, as well as treatment
outcomes were recorded in the registry book. In this study, we used surveillance data
from Jiangsu Provincial Center for Disease Prevention and Control. The estimated number of annual reported TB cases was based on the surveillance data in Jiangsu, where the recruited patients came from.

P6: “the initial mono-drug resistance rate was set at 6% among new cases and 16% among previously treated cases based on the proportion of rifampicin resistant isolates from previous surveys”. To calculate the number of patients to be included in the study, the authors refer to previous surveys, could it be possible to specify a reference?
Answer: The estimated mono-drug resistance rate was referred to an unpublished pilot study.

The authors refer to “Cluster sampling method”? Could they further explain?
Answer: Cluster sampling is a sampling technique where the entire population is divided into groups, or clusters, and a random sample of these clusters are selected. All observations in the selected clusters are included in the sample. Cluster sampling is commonly used, rather than simple random sampling, mainly as a means of saving money when, for example, the population is spread out, and the researcher cannot sample from everywhere. The loss of effectiveness by the use of cluster sampling, instead of simple random sampling, is the design effect. The design effect is basically the ratio of the actual variance, under the sampling method actually used, to the variance computed under the assumption of simple random sampling. In this study, as recommended, the sample size was multiplied by the design effect to take into account the cluster sampling method.

A sample size was estimated based on the total number of reported new sputum smear. Does it mean that the patients were included until the sampling site reaches a minimum number of cases (67 SS+)? Are the patients randomly distributed over the 12 months of the study period?
Answer: All newly registered pulmonary TB patients with sputum smear positive tests in selected study sites were eligible for inclusion. Eligible patients should be continuously recruited since May 1, 2008, until the sampling site reaches a minimum number of cases.
Data collection

Are definitions of previously treated/new cases based on WHO guidelines? If so could it be possible to add a reference?

Answer: The definitions of new/previously treated cases referred to the WHO guidelines [7,11].

Reference:

Data analysis:

Are data case-based or aggregated? How are they entered in the DB?

Answer: After obtaining informed consent, a standard questionnaire was completed for each recruited patient to collect demographic data and the history of treatment. Data were double entered with EpiData 3.1 (Denmark) and discrepancies were checked against the raw data.

Results

Prevalence and pattern of drug resistance

The authors specify “A large part of them (97.81%) were Han Chinese”: could the authors specify the origin of the 2.2% remaining?

Answer: There are 55 Ethnic Minorities in China. The majority of the population belongs to the Han ethnic. In the present study, most of them (97.81%) were Han Chinese. The nationalities of remaining patients were Hui, Mongolian, Miao, Yi, Zhuang, Tu, Buyi, Bai, and Hani etc.

Area of residence: could it be possible to look at a smaller geographic resolution if a trend has really to be observed? Could it be possible to map the estimated rate of cases?

Answer: We added the figure 3 to illustrate the geographical variation of MDR-TB.

Risk factors:

Table 4 - Factors associated with multi-drug resistant tuberculosis
Could the authors specify the meaning of cOR and aOR?

Answer: We specified the meaning of cOR and aOR in Table 4. “cOR, crude odds ratio; aOR, adjusted odds ratio, adjusted for sex, age, treatment history, area and cigarette smoking.”

For the choice of age categories, the authors decided to use tertile which is probably a rational way to do it in statistics, but what does these categories mean regarding the epidemiologically of TB in China and more particularly in this area? If the data allow (case-based data), could the authors re-do the calculation, using for example age categories from the WHO-global report, or any other categories that could be based on epidemiological hypothesis concerning the patterns of the affected population?

Answer: As mentioned above, we re-categorized age into six groups according to the reviewer’s suggestions.

P for trend:
- For age, was it calculated on categorical variables or continuous variables?
- For area of residence and alcohol drinking: is it meaningful to calculate a p for trend based on 3 categories?

Answer: Considering the reviewer’s comments and valuable significance, we deleted the results of trend test.

The authors state: “Drinking alcohol (#3 times/week) seems to be inversely related with the risk of MDR-TB with the OR(95% CI) of 0.57(0.34-0.97)”

Could the authors specify how the question was asked? Did they assess the quantity of alcohol absorbed by the patients? e.g. if 3 times/week and one glass each time, may not affect patient’s health.

Answer: The main objective of this study was to describe the current status of drug resistance among TB patients in Jiangsu province. Though we designed a questionnaire to explore potential risk factors for drug resistance, the information we collected was limited as we only designed one question to inquire the history of alcohol drinking. In the present study, patients frequently consuming alcohol were found to less likely have MDR-TB. This result is consistent with one previous report in Madrid, Spain. The potential reasons for this inverse relation are unclear and need further studies. Considering the limited information about alcohol drinking, we
excluded it in the revised version.

Discussion
Information provided in the first two paragraphs was already mentioned in the background section. Could the authors summarize it?

Answer: We refined these two paragraphs in the revised version.

Reviewer: PENG WU
1. In the multivariate logistic analysis which was used to identify potential risk factors possibly associated with MDR-TB, including patients’ age, sex, treatment history, residential location, tobacco use and alcoholic drinking, the authors mentioned that there was unexplainable relationship between alcoholic drinking habit and occurrence of MDG-TB. Meanwhile the association between tobacco use and MDG-TB was not detected in the study. I suggest the authors to look into the data and the statistical analysis carefully to try to find out what kind of reasons might lead to the results which were reflected as the odds ratios in Table 4. Age, treatment history and residential location were found to be associated with MDR-TB in the study, however could these associations and even the associations between tobacco use, alcoholic drinking and MDR-TB, be confounded by some interactions between different independent variables? Before the authors present the findings of analyses without interactions between the independent and dependent variables, it might be safer to carefully examine the data, conduct appropriate analyses, and then draw solid conclusion based on the findings from refined analyses.

Answer: We carefully checked our data and conducted statistical analysis again. According to other reviewer’s suggestion, we categorized age into six groups by using age categories from the WHO-global report. We also compared the proportion of drug resistance in different age groups and revealed that the frequency of MDR-TB was much higher in young adults and peaked at 35-44 years old, which was similar to the findings from MDR surveillance data in 13 countries of Central and Eastern Europe (CEEUR).

2. Most of the interpretation on odds ratio in the study is problematic, mainly in page 10 of the manuscript. Please check to make it correct.

Answer: We checked the manuscript and interpreted the OR in another way.
Minor Essential Revisions

3. There is undefined term “concierge DOTS” which might make the reader confusing. Would you please define it?

Answer: To avoid confusing the readers, we modified the sentence as ‘DST needs to be scaled up so that patients with susceptible TB can get DOTS, those with mono-drug resistance can get intensive DOTS to prevent multidrug resistance, and those with MDR can get the life-saving therapy they need’.

4. The authors mentioned “mono-therapy” in page 12. Does that sentence suggest that mono-drug therapy might be routinely implemented for drug-sensitive TB patients, but was wrongly prescribed to MDR-TB patients because of the lack of or delayed DST? If yes, I would suggest the authors to recheck the local guidelines for treating TB patients; if no, please re-organize the discussion here.

Answer: Page 12. Here, mono-therapy didn’t mean that mono-drug therapy was routinely implemented for some TB patients. Given that the MTB strains have been resistant to three first-line drugs, the effect of the combination of four anti-tuberculosis drugs was equivalent to mono-drug therapy. To avoid confusing the readers, we modified the sentence as ‘Even in some health facilities with necessary equipments, the results of susceptibility testing can take as long as several weeks, when the patients with MDR-TB have already been treated with INH and RIF for a long time’.

5. There are some typo and inappropriate words shown in the main text.

6. Some of the grammar in the main text definitely needs to be improved before resubmission.

Answer: We corrected these language errors.

Best wishes,
Jianming Wang