Author's response to reviews

Title: Characteristics of pncA Mutations in Multidrug-resistant Tuberculosis in Taiwan

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Author’s response to reviews:

Dr. Roxane Rajabi
Editor, BMC Infectious Disease

Re: Characteristics of pncA Mutations in Multidrug-resistant Tuberculosis in Taiwan (MS ID: 1275184982533499)

Dear Dr. Rajabi

The manuscript has been revised in accordance with your suggestion and resubmitted in a revised form for your consideration to be published in the BMC Infectious Disease.

We thank for the opportunity to improve the quality of our manuscript and have attempted most of the suggestions from you and reviewers into our revision. We hope the manuscript in its present revised form can meet your approval of publication.

Sincerely yours,

Yu-Chi Chiu, MD
Chest Department
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Dear Reviewer 1: Elvira Richter

The manuscript has been revised in response to your comments.

Q1. DST: the authors should cite the guideline according to which DST was performed. Furthermore, they should cite the source for the use of the higher concentration (5µg/ml) for rifampicin, since this is not usual.

Ans: The guidance of standard proportion method for drug susceptibility test (DST) of tuberculosis was cited accordingly. In Taiwan, DST for rifampicin was performed in two concentrations (1 and 5 µg/ml) and resistance for rifampicin was defined as those resistant to low drug concentration levels (1 µg/ml). The higher concentration of rifampicin (5 µg/ml) was for clinical judgment as the clinician may choose to prescribe higher dose of rifampicin for rifampicin-resistant isolates. Please see reference 20 of the new manuscript.

Q2. pncA sequencing: Has the gene been sequenced in both directions? I.e. have the mutations been proved by both-side sequencing?

Ans: The pncA gene was sequenced as described in previous study (1). The sequencing was performed in one direction but the test was repeated twice and the results were identical.

Q3. The following sentence is not clear: ‘When taking the spoligotyping results into consideration, no clustered isolates were found…..’ How can they find clustered isolates if not confirmed by spoligotyping? Please reformulate and state the conclusion more precisely.

Ans: To be revised in accordance with your suggestion. Please see line 259 of the new manuscript.

Q4. Table 2 is very confusing and should be reorganized. For a reader the finding of sensitive strains is very difficult. The strains should not be gathered according to the spoligotyping pattern (which is not the topic of the analysis) but according to the mutations. Thus, most WT strains are in series. The spoligotyping result can still be included as result in a column. Furthermore, mutations are listed without a count in column No. of Isolates and without designation of PZA susceptibility. If these are cases with multiple mutations this should be indicated unequivocally.
Ans: The Table 2 was revised in accordance with your suggestion. The strains were stratified according to the results of pncA sequencing. The strains with concomitant multiple mutations were indicated specifically. Please see Table 2 of the new manuscript.

Your attention and kind advices are highly appreciated.

Yu-Chi Chiu, MD

References


Dear Reviewer 2: Christophe SOLA

The manuscript has been revised in response to your comments.

Q1. This paper (YC CHiu et al.) describes the characteristics of pncA mutations in MDR TB in Taiwan. Although it is technically sound it suffers some limitations:

-no presentation of genetic MDR-TB characteristics for rpoB and katG-inhA

-no comparison with pncA characteristics from fully susceptible isolates.

Hence the paper is a very preliminary report. There is indeed no scientific reason to separate genetic characterizaiton of pncA from rpoB and katG-inhA genetic characterizartion. Was this done too ?

Ans: The purpose of the present study was to evaluate the predictive value of pncA mutation in identifying PZA-susceptible isolates among MDRTB isolates. Therefore only MDRTB isolates were included for investigation and only pncA gene mutation were analyzed. The issue was mentioned in the section of limitation. Please see line 310 of the new manuscript.

Q2. -recommended critical concentration to discriminate between PZA susceptible to PZA resistant starts with 100 µg/ml. However higher cc are often used (300, 900). Please justify the use of this unique cc

Ans: The susceptibility of PZA was determined by the non-radiometric BACTEC
Mycobacteria Growth Indicator Tube (MGIT) 960 method (BD Biosciences, Sparks, MD, USA). This commercial kit used 100 µg/ml as critical concentration only. The critical concentration of 100 µg/ml for DST of PZA was also widely adopted in previous studies (1, 2, 4). Please refer to the references of this response letter.

Q3. -do not use millicentrigramme/ml this is not international MKSA system, use µg/ml.

Ans: To be revised in accordance with your suggestion. Please see line 117 of the new manuscript.

Q4. -suggest in discussion hypothesis why MDR-TB isolates with concomitant PZA resistance were more likely to be sputum smear negative.

Ans: In the present study, we found that MDRTB patient with concomitant PZA resistance were more likely to be sputum smear negative. The cause of significant correlation between PZA susceptibility and sputum smear results cannot be easily identified in the study design. A recent study showed that exposure of lowly active MTB bacilli to anti-TB agents led to the emergence of resistant mutants and it required higher drug concentrations to eliminate metabolic inactive mycobacteria (3). Therefore, the MDRTB isolates with PZA resistance are probably less metabolic active which may lead to the lower sputum smear positive rate. Further in-vivo and in-vitro studies will be needed to verify the issue and elucidate the underlying mechanism. Please see line 297 of the new manuscript.

Q5. -explain in discussion why the sensitivity of the PZase test is as low as 58,3% compared to 80-96% in other studies?

Ans: The sensitivity of PZase test reported in the present study is lower as compared to those reported in previous studies. The geographic factors may contribute to the differences as wide range of sensitivity in PZase test. Unlike previous studies that enrolled both MDRTB and non-MDRTB isolates (4), only MDRTB isolates were included for analysis in the present study. The different composition of MTB isolates, including various genotypes and drug susceptibility profiles, were possible causes of the discordant results. Meanwhile, the PZase tests of all the isolates were repeated twice and reported accordingly. Further studies with larger sample size will be needed to identify the impact of genotypes and drug resistance in PZase activity tests. Please see line 277 of the new manuscript.

Your attention and kind advices are highly appreciated.
References


