PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (**http://bmjopen.bmj.com/site/about/resources/checklist.pdf)** and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Cohort Study of Pulmonary Tuberculosis (COSMOTB) identifying drug-resistant mutations: Protocol for a prospective observational study in Korea
AUTHORS	Min, Jinsoo; Chung, Chaeuk; Lim, Jinsook; Park, Jong Hyock; Shin, Kyeong Seob; Jung, Sung-Soo; Lee, Ki Man

VERSION 1 – REVIEW

REVIEWER	Julio Croda Osvaldo Cruz Foundation, Campo Grande, Brazil
REVIEW RETURNED	18-Feb-2018

GENERAL COMMENTS	The number of drug-resistant TB patients selected will be 184 patients from a population of 2 hospitals. The calculation of the sample was based only on the prevalence of drug-resistant TB among patients with smear-positive pulmonary TB and not on the exposures they wish to analyze in the multiple logistic regressions to identify risk factors associated with unfavorable outcomes in the cohort study.

REVIEWER	Claudio Köser University of Cambridge, UK
REVIEW RETURNED	27-Apr-2018

GENERAL COMMENTS	I share the authors' concern that discordant results drug-susceptibility testing (DST) results between genotypic and phenotypic methods (in particular strains that are genotypically resistant but phenotypically susceptible) are undermining the faith of clinicians in DST. In fact, this is a priority area for the World Health Organization (WHO). I do not have any comments regarding the paper itself, apart from the fact that the authors should mention the sequencing technology that they plan to use in their study (e.g. Sanger sequencing). I would, however, would like to offer a few suggestions regarding the microbiological procedures for this study, which are beyond the scope of this study protocol: 1. WHO recently established, changed or withdrew 20 breakpoints for phenotypic DST (World Health Organization. Technical report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis [WHO/CDS/TB/2018.5]. Available from: http://apps.who.int/iris/bitstream/10665/260470/1/WHO-CDS-TB-2018.5-eng.pdf, additional supplementary data can be downloaded from https://www.finddx.org/publication/supplement-critical-

	concentrations-for-dst-for-tb-drugs/). Notably, the breakpoints for several fluoroquinolones and second-line injectables were lowered. Please ensure that all strains in this study are tested with these latest breakpoints. 2. For MIC testing, please use the 1% proportion method on either LJ, 7H10, 7H11 or with MGIT, otherwise the results will be of limited value. Please read the aforementioned WHO report with regards to minimising truncations of MICs and selecting standard two-fold dilution series (in this context, please also refer to the EUCAST SOPs for defining ECOFFs http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/EUCAST_SOPs/EUCAST_SOP_10.0_MIC_distributions_and_epidemiological_cut-off_value__ECOFF__setting_20171117.pdf). 3. Please note that the critical concentration (CC) for rifampicin in MGIT is likely too high (i.e. it is higher than the ECOFF), which explains why some mutations test susceptible, even though they confer elevated MICs (1). For this drug, it is therefore important that you test concentrations below the CC (1). In fact, WHO is in the process of conducting a systematic review of isoniazid and the rifamycins (rifampicin, rifabutin and rifapentine) to assess whether the CCs for some media have to be lowered. 4. Heteroresistance is a major reason for discordant MIC results. To capture minority populations that are below the limit detection of the sequencing technology that the authors propose to use, I would suggest that you sequence from the drug-containing medium rather than the drug-free tube to enrich for the resistant subpopulation (2). Should this not detect a resistance mechanism, please repeat the phenotypic test as false-resistance results are also a source for discordances, particularly for pyrazinamide and for in settings where the true prevalence of resistance is low and, consequently, the positive predictive value of phenotypic DST is poor (2, 3). References 1. Heyckendorf J, Andres S, Köser CU, Olaru ID, Schön T, Sturegard E, Beckert P, Schleusener V, Kohl TA, Hillemann D, Moradigaravand D, Parkhill J, Peacock SJ, Niemann S, Lange C, Merker M. 2018. What Is resistance? Impact of phenotypic versus molecular drug resistance testing on therapy for multi- and extensively drug-resistant tuberculosis. Antimicrob Agents Chemother 62:e01550-17. 2. Schön T, Miotto P, Köser CU, Viveiros M, Böttger E, Cambau E. 2017. Mycobacterium tuberculosis drug-resistance testing: challenges, recent developments and perspectives. Clin Microbiol Infect 23:154-160. 3. Simons SO, van Ingen J, van der Laan T, Mulder A, Dekhuijzen PN, Boeree MJ, van Soolingen D. 2012. Validation of pncA gene sequencing in combination with the mycobacterial growth indicator tube method to test susceptibility of Mycobacterium tuberculosis to pyrazinamide. J Clin Microbiol 50:428-34.

VERSION 1 – AUTHOR RESPONSE

Comment of Reviewer #1

The number of drug-resistant TB patients selected will be 184 patients from a population of 2 hospitals. The calculation of the sample was based only on the prevalence of drug-resistant TB among patients with smear-positive pulmonary TB and not on the exposures they wish to analyze in

the multiple logistic regressions to identify risk factors associated with unfavorable outcomes in the cohortstudy.
This is an important issue that was discussed while preparing the study protocol. As stated by Reviewer #1, the calculation of the sample number was based on the prevalence of drug-resistant TB. Because the overall aim of our study is to identify mutations related to drug-resistance in TB and their clinical implications, such calculation will cause some error in estimating sample size. However, the incidence of such mutations is not well known in South Korea; we could not find any reference study. We had mentioned this issue as our limitation in the Discussion section previously, and have attempted to elaborate on this.

Comment of Reviewer #2
Comment #2-1
I share the authors' concern that discordant results drug-susceptibility testing (DST) results between genotypic and phenotypic methods (in particular strains that are genotypically resistant but phenotypically susceptible) are undermining the faith of clinicians in DST. In fact, this is a priority area for the World Health Organization (WHO).
We are very thankful for Reviewer #2's comment about highlighting the importance of identifying discordant results of genotypic and phenotypic DSTs. With this study, we hope to achieve positive and meaningful results, which help shorten the current anti-TB treatment regimen.

Comment #2-2
I do not have any comments regarding the paper itself, apart from the fact that the authors should mention the sequencing technology that they plan to use in their study (e.g. Sanger sequencing).
I would, however, would like to offer a few suggestions regarding the microbiological procedures for this study, which are beyond the scope of this study protocol:
We have provided additional information and details about the methods of drug susceptibility testing in the 'study procedures' subsection. For determination of the MICs of anti-TB drugs, MYCOTB MIC plate (MYCOTB; Trek Diagnostic Systems, Thermo Fisher Scientific, OH, USA) will be used according to the manufacturer's instruction. Sanger sequencing will be adopted as a method of DNA sequencing for genes of drug resistance. The MIC testing and DNA sequencing will be performed at the National Institute of Health (Korea Centers for Disease Control and Prevention, South Korea).
We had incorrectly described method of culture based phenotypic DST, and have amended this. Phenotypic DST will be determined by the absolute concentration method using Lowenstein-Jensen media. We apologize for this mistake.

Comment #2-3
1. WHO recently established, changed or withdrew 20 breakpoints for phenotypic DST (World Health Organization. Technical report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis [WHO/CDS/TB/2018.5]).Notably, the breakpoints for several fluoroquinolones and second-line injectables were lowered. Please ensure that all strains in this study are tested with these latest breakpoints.
This is another limitation of our study protocol. We have prepared this study protocol in 2016, and have started to enrol the participants from December 2016. As the WHO report was published in 2018, we cannot adopt the critical concentration proposed by WHO.
Phenotypic DST in our study is performed at the Korea Institute of Tuberculosis (KIT), which is a member of the WHO TB Supranational Reference Laboratory (SRL) Network. We believe that the results of phenotypic DST performed at KIT is valid and reliable.
A comparison of the proposed critical concentrations of fluoroquinolones and second-line injectables in the WHO report with those adopted in our study protocol shows that only moxifloxacin has a different value of critical concentration. This WHO report proposes 1.0 μg/mL as the critical concentration of moxifloxacin, instead of 2.0 μg/mL, which is used in our study protocol. However, the MIC testing will be conducted for every isolates and will compensate for this issue.

For clarification, we have added drugs and their critical concentration used in the phenotypic DST in the 'study procedures' subsection.

Comment #2-4
2. For MIC testing, please use the 1% proportion method on either LJ, 7H10, 7H11 or with MGIT, otherwise the results will be of limited value. Please read the aforementioned WHO report with regards to minimising truncations of MICs and selecting standard two-fold dilution series.
We apologize for not stating the method of MIC testing to be used in our study protocol. For determination of the MICs of anti-TB drugs, MYCOTB MIC plate (MYCOTB; Trek Diagnostic Systems, Thermo Fisher Scientific, OH, USA) will be used according to the manufacturer's instruction. MYCOTB is known to be a rapid, convenient, quantitative and accurate method for testing both first- and second-line anti-tuberculosis drugs.

Comment #2-5
3.Please note that the critical concentration (CC) for rifampicin in MGIT is likely too high (i.e. it is higher than the ECOFF), which explains why some mutations test susceptible, even though they confer elevated MICs (1). For this drug, it is therefore important that you test concentrations below the CC (1). In fact, WHO is in the process of conducting a systematic review of isoniazid and the rifamycins (rifampicin, rifabutin and rifapentine) to assess whether the CCs for some media have to be lowered.
We are again thankful for this comment and suggested article. In the suggested article, the authors compared the utility of genotypic DST assays (Xpert MTB/RIF, line probe assay, and whole genome sequencing) with phenotypic DST and performed further analysis if discrepancies between the various methods were due to flaws in the genotypic or phenotypic test using the MIC results.The goal of our study is very similar to that of the Reviewer's suggested article. We are concerned and want to describe how discrepant results of phenotypic and genotypic DSTs in our study will affect the anti-TB treatment regimen. We added the results of the suggested article in the first paragraph of the discussion section.

Comment #2-6
4. Heteroresistance is a major reason for discordant MIC results. To capture minority populations that are below the limit detection of the sequencing technology that the authors propose to use, I would suggest that you sequence from the drug-containing medium rather than the drug-free tube to enrich for the resistant subpopulation (2). Should this not detect a resistance mechanism, please repeat the phenotypic test as false-resistance results are also a source for discordances, particularly for pyrazinamide and for in settings where the true prevalence of resistance is low and, consequently, the positive predictive value of phenotypic DST is poor (2, 3).
We wish to thank the Reviewer for suggesting another article, which reviewed the important issues regarding anti-TB drug resistance testing. Of the many points illustrated in the article, we are interested in the drug-resistance cases without identified genetic mutations. Although we are not sure about how many such cases will be found, we hope to obtain some answers using MIC testing. We have provided a new second paragraph for discussing this issue.

After adding the comments described above, the manuscript was sent to an English editing service agency for elimination of any grammatical errors. Other minor mistakes were also corrected.

VERSION 2 – REVIEW

REVIEWER	Claudio Köser University of Cambridge, UK
REVIEW RETURNED	26-Jun-2018

GENERAL COMMENTS	I only have some minor comments: Please change "because the critical concentration is extremely high" to "because some critical concentrations are too high" and “and its clinical outcome will be evaluated” to “and their clinical outcomes will be evaluated.” Provide a reference for the pyrazinamidase test used. Please adjust the position of the references throughout the manuscript (e.g. “success rate of 44–75% [5].” instead of “success rate of 44–75%.[5]” Please change "Lowenstein-Jensen" to "Löwenstein-Jensen" and also add the missing umlauts to the references, where applicable (e.g. "Schön" instead of "Schon"). Please italicise gene and species names throughout the manuscript and adjust the capitalisations of words in the references. Please italicise the “plus” in “MTBDRplus”.

VERSION 2 – AUTHOR RESPONSE

I have responded specifically to each comment below.

(Comment #1) Please change "because the critical concentration is extremely high" to "because some critical concentrations are too high" and “and its clinical outcome will be evaluated” to “and their clinical outcomes will be evaluated.”
I modified according to the reviewer’s comment.

(Comment #2) Provide a reference for the pyrazinamidase test used.
I added a reference regarding pyrazinamidase test.

(Comment #3) Please adjust the position of the references throughout the manuscript (e.g. “success rate of 44–75% [5].” instead of “success rate of 44–75%.[5]”
I modified according to the reviewer’s comment.

(Comment #4) Please change "Lowenstein-Jensen" to "Löwenstein-Jensen" and also add the missing umlauts to the references, where applicable (e.g. "Schön" instead of "Schon").
I modified according to the reviewer’s comment.

(Comment #5) Please italicise gene and species names throughout the manuscript and adjust the capitalisations of words in the references.
I modified according to the reviewer’s comment.

(Comment #6) Please italicise the “plus” in “MTBDRplus”.
I modified according to the reviewer’s comment.