Author's response to reviews

Title: Predominant porB1A and porB1B genotypes and correlation of gene mutations with drug resistance in Neisseria gonorrhoeae isolates in Eastern China

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Author's response to reviews: see over
Dear Prof. Rajabi,

We would like to thank you and the reviewers for having further read our revised manuscript (No. 2890120823526510) and give us good advice.

In the second editor’s letter, we are asked to return our re-revised manuscript by 11 August 2010. However, we did not receive the letter before 10 September 2010. We do not know why. After asked the editor office, we got the second editor’s letter in 11 September 2010. Besides, the associate editor and reviewers ask us to perform an assay to detect the beta-lactamase production of tested gonococcal isolates. Please understand we send the re-revised manuscript so late.

As for the reviewers’ comments before, we also think our previous MICs are relatively higher, but we do not know where is wrong. We asked a clinical laboratory to check our previous result of drug sensitivity test, and they gave us significantly lower MICs. Finally, we luckily found the purity of the previously used penicillin and tetracycline made by Chinese companies is much lower, which resulted in the previous high MICs. Thus, we purchased the antibiotics from bioMérieux Co. (France) to repeat the drug sensitivity test for three times, and the relatively lower MIC values were obtained. Furthermore, in our study only the non-beta-lactamase producing gonococcal strains that screened out using paper acidometric test by the clinical laboratories of hospitals were used, and we are sorry it was not stated in the previous manuscript. All the additional data were added in the re-revised manuscript.

We confirm again that the content in the re-revised manuscript has not been published or submitted for publication elsewhere. We also state that our experiments are ethically and legally acceptable. The authors have no conflicting financial interests.

Thank you for your consideration and we look forward to hearing from you.

With best regards,

Sincerely yours,

Prof. Jie Yan, M.D.

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Associate editor’s comments:

They must use the phrase “correlate with resistance” instead of conferring resistance. Importantly, they must also test their strains for beta-lactamase production given the high MIC values. We would be grateful if you could address the comments in a revised manuscript and provide a cover letter giving a point-by-point response to the concerns.

In addition, please can you state whether your research conformed to the Helsinki Declaration (http://www.wma.net/e/policy/b3.htm), and to local legislation. Please add a paragraph in your methods section stating this, and name the ethics committee which approved the research. Please also indicate whether patients gave informed consent to participate in the study.

Please also highlight (with 'tracked changes'/coloured/underlines/highlighted text) all changes made when revising the manuscript to make it easier for the Editors to give you a prompt decision on your manuscript. Please also ensure that your revised manuscript conforms to the journal style (http://www.biomedcentral.com/info/ifora/medicine_journals). It is important that your files are correctly formatted.

We look forward to receiving your revised manuscript by 11 August 2010. If you imagine that it will take longer to prepare please give us some estimate of when we can expect it.

You should upload your cover letter and revised manuscript through http://www.biomedcentral.com/manuscript/login/man.asp?txt_nav=man&txt_man_id=2890120823526510. You will find more detailed instructions at the base of this email.

Please don’t hesitate to contact me if you have any problems or questions regarding your manuscript.

With best wishes,
Ms Roxane Rajabi
The BioMed Central Editorial Team

Reply: We added an assay using nitrocefin discs as the second reviewer’s request, and added Ethics Statement in Methods in the re-revised manuscript. We addressed our reply to the reviewers’ comments by point-to-point described as below.

Reviewer: Lori Snyder
Reviewer’s report:
Minor Essential Revisions

1. As stated previously, it is important not to overstate the correlation between the gene mutations discovered and antimicrobial resistance. Until further experiments are done, you do not know that the mutations discovered are the reason for the resistance to the antimicrobials. As another reviewer said, these mutations may simply be a clonal marker. In the Abstract it is stated: “Multiple mutations in porB genes associated with penicillin and tetracycline resistance include novel A121 and N122 deletions which confer high antibiotic resistance.” Rewording the sentence would generate a statement that is supported by the data presented: “Multiple mutations in porB genes, including novel A121 and N122 deletions, are associated with high levels of penicillin and tetracycline resistance.” All other similar statements have expressed this point well, including the final sentence of the Introduction and the Conclusions.

Reply: We think the reviewer’s opinion is absolutely reasonable and thank you for your suggestion how to revise the sentence. The overstatement is mainly due to our lower English level. For example, we can not accurately distinguish the difference between “confer” and “associated”, and between “associated” and “correlated”. In the re-revised manuscript we revised the sentence as the reviewer’s advice.

We also know the MICs in the previous manuscript seems higher, but we do not know where is wrong at that time. As mentioned in the letter to editor, we finally found that the purity of two antibiotics (for research) made in China is much lower. So we repeated the drug sensitivity test for three times using the antibiotics from bioMérieux Co., and the relatively lower MICs were presented in the re-revised manuscript. Here, we are grateful to the reviewer’s opinion which resulted in our finding of the low purity of antibiotics used before.

2. It should not be ignored that plasmid-mediated resistance made account for the very high MICs of these antimicrobials. This should be included in the Discussion at the top of page 13.

Reply: We discussed it in the Discussion.
Reviewer: Robert Nicholas
Reviewer's report:
The revised manuscript by Sun et al. is much improved over the initial submission. That said, I still have concerns over the very high PenG MICs reported and am unsure why they do not fall in the same range as most chromosomally mediated strains in which penB mutations are important. Perhaps if the authors simply tested their isolates for beta-lactamase expression (nitrocefin disks are readily available) and reported the finding, this might make these numbers more reasonable (i.e. perhaps the beta-lactamase producing strains would have the higher MICs).

Reply: As mentioned in the letter to editor, in this study only the non-beta-lactamase producing gonococcal strains that screened out using paper acidometric test by the clinical laboratories of hospitals were used. We are sorry it was not stated in the previous manuscript. According to the opinion of the reviewer and the editor, in the re-revised manuscript we added the test using nitrocefin disks to recheck the production of beta-lactamase. Also as mentioned in the letter to editor, we luckily found that the purity of two antibiotics (for research) made by Chinese companies is much lower, which caused the abnormal higher MICs in the previous drug sensitivity test. We purchased the antibiotics from bioMérieux Co. (France) to repeat the drug sensitivity test for three times, and the relatively lower MICs were obtained. We must thank the reviewers for their mentioning which leads us to find the low purity of antibiotics used before.

Specific Points:
1. The last sentence in the Abstract is still much too strong given the lack of analysis of isogenic strains. The last sentence states “Multiple mutations in porB genes associated with penicillin and tetracycline resistance include novel A121 and N122 deletions which confer high antibiotic resistance.” First of all, I would replace “associated” with “correlated”, but most importantly, the statement that “novel A121 and N122 deletions confer high antibiotic resistance” is not supported by any experimental evidence and should be removed. It is certainly possible that these mutations confer high antibiotic resistance, but again this would require experiments to examine this phenomenon.

Reply: The reviewer’s opinion is right. We revised the statement and used “correlated” instead of “associated” in the re-revised manuscript.

2. N. gonorrhoeae only has PorB, while N. meningitidis has both PorA and PorB. The correct way to refer to PIA and PIB is PorBIA (PorB<sub>IA</sub>) and PorBIB (PorB<sub>IB</sub>).

Reply: We used PorB1A and PorB1B in the re-revised manuscript.

3. Gene names (i.e. porB) should be italicized, but as described in specific point 2, the correct notation is porB1A or porB1B (i.e. <ital>porB<sub>1A</sub></ital> or <ital>porB<sub>1B</sub></ital>).

Reply: We used italicized porB1A and porB1B in the re-revised manuscript.