Author's response to reviews

Title: Comparative study of bacteriological culture and real-time fluorescence quantitative PCR (RT-PCR) and multiplex PCR-based reverse line blot (mPCR/RLB) hybridization assay in the diagnosis of bacterial neonatal meningitis

Authors:

Yajuan WANG (cxswyj@vip.sina.com)
Gaili GUO (glhaiyang123@163.com)
Huixin WANG (huixinwang20096818@aliyun.com)
Xuefang YANG (yangxuefang0302@sina.com)
Fang SHAO (shaofg2002@163.com)
Fanrong Kong (Fanrong.Kong@health.nsw.gov.au)
Caiyun YANG (18910770953@163.com)
Wei GAO (yemengmeng99@hotmail.com)
Zhujun SHAO (shaozhujun@icdc.cn)
Jinjing ZHANG (candy0719zjj@126.com)
Jie LUO (luojie112723@sina.com)
Yonghong YANG (yyh628628@sina.com)
Bingqing ZHU (zbqzhu@126.com)

Version: 2
Date: 5 July 2014

Author's response to reviews: see over
Dear BMC Pediatrics editor,

We would like to request that you consider the enclosed manuscript for publication in BMC Pediatrics. The paper, entitled *Comparative study of bacteriological culture and real-time fluorescence quantitative PCR (RT-PCR) and multiplex PCR-based reverse line blot (mPCR/RLB) hybridization assay in the diagnosis of bacterial neonatal meningitis*, was developed and evaluated real-time PCR (RT-PCR) and a multiplex PCR-based reverse line blot (mPCR/RLB) hybridization assay for rapid detection of neonatal meningitis bacterial pathogens for potential use in routine diagnostics.

We feel that BMC Pediatrics is the ideal place for this paper to appear. We have written the manuscript in accordance with the guidelines of BMC Pediatrics.

As you previously suggested, we have successfully addressed both of the reviewers comments (see below for detail) and also made all the relevant changes in the manuscript.

I hope they are now acceptable by you and reviewers to be published in BMC Pediatrics.

We are pleased to answer more of your questions if there are any. If you have any questions or comments, please do not hesitate to contact me, the corresponding author, Yajuan WANG. Thank you and we look forward to hearing from you.

Yajuan WANG,
ABSTRACT

OBJECTIVE: Bacterial meningitis is more common in the neonatal period than any other time in life and the evidence based diagnosis is still a challenge. This study intended to seek the best strategy for neonates bacterial meningitis bacterial pathogens identification after evaluate three different we can access. Language translation is needed in order to clearly define the study objective.

The aim of this study was to seek the best strategy for bacterial pathogens identification of neonates bacterial meningitis after evaluate three different methods we can access.

Reviewer's report

Minor essential revisions
2. Most of the required corrections are editorial in nature. The reviewer has suggested many editorial corrections in a word edition of the manuscript. I have modified the required corrections according the reviewer’s suggestions. Details see the paper.

Discretionary revisions: None

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:
I declare that I have no competing interests.
Reviewer's report

Title: Using real-time fluorescence quantitative PCR (RT-PCR) and multiplex PCR-based reverse line blot (mPCR/RLB) hybridization assay assist culture in the diagnosis of bacterial meningitis in neonates

Version: 1  Date: 14 June 2014
Reviewer: Tinuade Ogunlesi

Reviewer's report:

MINOR ESSENTIAL REVISIONS

1. Abstract - In the Results, the statement "....One strain of .......Also cultures identified.....PCR techniques....." is repeated.

RESULTS: One strain of *S. epidermidis* and one of *E. faecalis* were identified using mPCR/RLB but not by RT-PCR. Cultures identified one strain of *S. pneumoniae* that was missed by both PCR assays.

The last sentence in the conclusion was not shown in the results.
The results were shown comparison of RT-PCR assay with mPCR/RLB assay and CSF bacterial culture and Table 8-9.

Keywords - "bacterial meningitis in neonates" cannot be listed along "neonates"
“bacterial meningitis in neonates” was changed to “bacterial meningitis”.

2. Introduction - Paragraph 1 Lines 7 and 8: Provide a reference.

Paragraph 2 Line 2: Signs and symptoms cannot be absent in meningitis.
The sentence “Signs and symptoms of neonatal bacterial meningitis may be subtle, nonspecific, vague, atypical or absent.” was changed to “Signs and symptoms of neonatal bacterial meningitis may be subtle, nonspecific, vague, atypical.”

Page 2 Paragraph 1 Line 4 - 8: These already show bias as they are supposed to be what the study should find eventually. They are better expunged.
The sentences “All the participants included had been diagnosed for bacterial meningitis. CSF was obtained for culture from fifty six neonates diagnosed with bacterial meningitis and admitted to the neonatal department in Beijing Children’s Hospital, affiliated to Capital Medical University in 2009.” were deleted.

3. Results - Tables S3 and S4 should be cited before Tables 2 to 4.
Tables S3 and S4 were cited before Tables 2 to 4. I rearranged the tables from Table 1 to Table 9.

How many cases were actually positive by CSF culture? - Abstract and Tables
show 5 while the text under Clinical Microbiology shows 6. These need to be harmonized.

**Clinical Microbiology**

Five CSF (9%) bacterial cultures were positive: two *L. monocytogenes*, one each of *S. pneumoniae*, *E. faecalis*, and *S. epidermidis*.

The crux of this study appears to be the usefulness of the PCR methods when the CSF culture is negative in the presence of clinical suspicion of meningitis. The sub-section on Clinical Microbiology needs to be re-analyzed to answer the following queries: how many of the 38 cases with prior antibiotic exposure were positive or negative by culture? How many of the cases with negative CSF culture yield were positive by RT-PCR and mPCR/RLB assays?

How many of the 36 cases with prior antibiotic exposure were positive or negative by culture?
Only one case with prior antibiotic exposure was positive by culture.

How many of the cases with negative CSF culture yield were positive by RT-PCR and mPCR/RLB assays?

- 23 cases with negative CSF culture yield were positive by RT-PCR.
- 12 cases with negative CSF culture yield were positive by mPCR/RLB assays.

Table 3: 35.7% should be 3.5%. The displayed Chi-Squared results are wrong and should be cross-checked.
Table 3: 35.7% was changed to 3.5%.

\[ \chi^2 = 13.885, \quad P < 0.05 \]

4. Title should read: "Comparative study of bacteriological culture and real-time fluorescence quantitative PCR (RT-PCR) and multiplex PCR-based reverse line blot (mPCR/RLB) hybridization assay in the diagnosis of bacterial neonatal meningitis"
The title was changed to "Comparative study of bacteriological culture and real-time fluorescence quantitative PCR (RT-PCR) and multiplex PCR-based reverse line blot (mPCR/RLB) hybridization assay in the diagnosis of bacterial neonatal meningitis"

**Level of interest**: An article whose findings are important to those with closely related research interests

**Quality of written English**: Needs some language corrections before being published

**Statistical review**: Yes, and I have assessed the statistics in my report.

**Declaration of competing interests**: I declare that I have no competing interests.

Additional formatting request:
1. Copyediting: We recommend that you copyedit the paper to improve the style of written English. I have modified.

2. Tables as Figure files: You have uploaded the tables as figure files. Please remove them from the submission system and include the tables within the text file of the manuscript after the references. The tables should be formatted using the Table tool in your word processor. Please also move the table title to above the table and the legend to below the table, within the text. It is done.

3. Please upload the Supplementary tables and figures as Additional Files and not as Figure files.
OK.

4. Line Numbering: Please revise your manuscript to include line and page numbers. Authors are asked to ensure that line numbering is included in the main text file of their manuscript at the time of submission to facilitate peer-review. Once a manuscript has been accepted, line numbering should be removed from the manuscript before publication. For authors submitting their manuscript in Microsoft Word please do not insert page breaks in your manuscript to ensure page numbering is consistent between your text file and the PDF generated from your submission and used in the review process. It is done.

5. Competing Interests: Please be advised that manuscripts must include a ?Competing interests? section. This should be placed after the Conclusions/Abbreviations. If there are none to declare, please include the statement ?The authors declare that they have no competing interests.? Please consider the following questions and include an appropriate declaration of competing interests in your manuscript:

   Financial competing interests
   ? In the past five years have you received reimbursements, fees, funding, or salary from an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? Is such an organization financing this manuscript (including the article-processing charge)? If so, please specify. No.

   ? Do you hold any stocks or shares in an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future? If so, please specify.
No.

? Do you hold or are you currently applying for any patents relating to the content of the manuscript? Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript? If so, please specify.
No.

? Do you have any other financial competing interests? If so, please specify.
No.

Non-financial competing interests
? Are there any non-financial competing interests (political, personal, religious, ideological, academic, intellectual, commercial or any other) to declare in relation to this manuscript? If so, please specify.
For more information please visit the instructions for authors on the journal’s website.
No.

6. Authors' Contributions: For manuscripts with more than one author, all BMC Series journals require an Authors' Contributions section to be placed after the Competing Interests section.
WYJ, GGL, WHX and YYH were involved in the conception and design of the study. WYJ, GGL, WHX, YXF, SF, YCY, ZJJ and LJ contributed to acquisition of data. WYJ, KFR, GW, SZJ, YYH, and ZBQ contributed to analyses and interpretation of the data. WYJ, GGL and YYH were responsible for writing up of the paper while all co-authors reviewed the draft manuscript. All authors read and approved the final manuscript.