Author's response to reviews

Title: The Association of Pathogens Causing Community-Acquired Pneumonia with Age and Season among Children in Nanjing, China

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Author's response to reviews: see over
Dear Dr. Puja Myles and Mr Proel Vargas

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript, we appreciate editor and reviewers very much for their constructive comments and suggestions on our manuscript entitled “The Pathogens and Epidemiology of Community-Acquired Pneumonia among Children in Nanjing, China”. (MS: 1753985982140863). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches.

We have studied reviewer’s comments carefully and have made revision which marked in red in the paper. We tried our best to revise our manuscript according to the comments, including title and language, and we hope these revisions can meet with approval. Attached please find the revised manuscript, which we would like to submit for your kind consideration.

We would like to express our great appreciation to you and reviewers for comments on our paper. Looking forward to hearing from you.

Thank you and best regards.

Yours sincerely,
Keping Chen

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Dear Reviewers:

Thanks for your comments concerning our manuscript entitled “The Pathogens and Epidemiology of Community-Acquired Pneumonia among Children in Nanjing, China” (MS: 1753985982140863). These comments are helpful for revising and improving our paper and provide important guidance to our research. We have studied the comments carefully and made corrections which we believe will meet your approval. Revised portions are marked in red in the paper. The main corrections in the paper and the responses to the reviewers’ comments are as follows:

**Reviewer 1: Paramita Sengupta**

**Reviewer's report (boldface letter) and point-by-point response**

A table on the socio-demographic profile of the study children, depicting the age-groups, place of schooling/no schooling (staying at home/in daycare/in formal school) type of house (overcrowding/no overcrowding), socio-economic condition, number of siblings, etc may be added for more clarity as the title has the word 'epidemiology' in it but descriptive data is not there, and also because there are associations with age, crowded places etc in the 'conclusion' section, which is not supported.

We have added a table depicting the socio-demographic profile of our study children, which can explain and support our conclusion. Please see Table.1
Table 1. Socio-demographic profile of the study children

<table>
<thead>
<tr>
<th>Age group</th>
<th>age</th>
<th>Place of schooling</th>
<th>House condition</th>
<th>Number of sibling*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants group</td>
<td>newborn ~1 year old</td>
<td>at home</td>
<td>spacious</td>
<td>0</td>
</tr>
<tr>
<td>toddlers group</td>
<td>&gt;1 ~3 years old</td>
<td>at home</td>
<td>spacious</td>
<td>0</td>
</tr>
<tr>
<td>preschooler group</td>
<td>&gt;3 ~6 years old</td>
<td>In daycare</td>
<td>crowded</td>
<td>0</td>
</tr>
<tr>
<td>school children</td>
<td>7 ~14 years old</td>
<td>In boarding school</td>
<td>crowded</td>
<td>0</td>
</tr>
</tbody>
</table>

*according to Chinese family project, there is just one child for one family. With the development of the economy and the rising cost of living, parents are inclined to feed one child, especially in metropolitan areas, such as Nanjing.

Major Compulsory Revision:

1. How does the study sample drawn from a single hospital in Nanjing representative of the whole city of Nanjing? Does all the CAP cases in Nanjing seek hospital treatment? If yes, do they come to Zhongda hospital for treatment? If not, the word 'Epidemiology' may be removed from the title.

According to the gathered information, we cannot make sure that all the CAP cases seek hospital treatment and all the patients come to Zhongda Hospital for treatment. All the co-authors have reconsidered the title, which may be not appropriate. We have removed “epidemiology” from the title according to the reviewer’s comments and changed the title to “The Association of Pathogens Causing Community-Acquired Pneumonia with Age and Season among Children in Nanjing, China”.

2. Please mention the study design in the title/methods section

The study design is followed:

We selected 1204 children to participate our study from August 2011 to August 2013, and Serum samples of patients were separated from venous blood drawn from every child and tested with the Pneumoslide IgM kit.
Children were divided into different groups according to different age or season. The data was analyzed by using Statistical Package for the Social Sciences for Windows to analyze the positive rates of pathogens, and further analyze if the positive rates had association with age or season. P value <0.05 was considered to be statistically significant.

We have made corrections according to the reviewer’s comments and these corrections are marked in red in the Material and Methods section.

Minor essential revisions:

1. **The background does not clearly state the objectives of the study in the manuscript.**

   We added the following statements in order to state clearly the objectives of study. These statements are marked in red in background section.

   The present study analyzes the positive rates of pathogens of CAP treated in Zhongda Hospital, and further analyzes if the positive rates have association with age or season, which can help plan better therapeutic and prevention strategies to prevent the spread of the pathogens in susceptible age groups during peak season.

2. **The research question is not defined.**

   To make the research question clear, the following statement is added to the end of background section.

   The present study analyzes the positive rates of pathogens of CAP treated in Zhongda Hospital, and further analyzes if the positive rates have association with age or season among children.

3. **References which are outdated can be removed/replaced with**
newer ones (eg ref-18)

We have updated references 7, 18 and 19 with newer ones (published in 2012, 2002 and 2003, respectively) and made correction in the revised manuscript. At present, there are 19 references in the revised manuscript, including 8 references published within the past 5 years.

4. Line no. 270-71 in page 13 about 'crowded places may be the major reason for the spread........' is not conclusive or supported in the manuscript.

We have added a table “socio-demographic profile of the study children” to depict the age, place of schooling/no school, house condition and number of siblings. Preschool and school-aged children, who lived in crowded spaces (such as daycare or boarding school), were susceptible to infection with MP, INFB, and PIVs, compared to infants and toddlers who lived in spacious space (p<0.001). We can conclude crowded spaces may be the major reason for the spread of MP, INFB, and PIVs. Please see Table 1 in the revised manuscript.
Reviewer2: Pratap Kumar

**Reviewer's report** (boldface letter) and **point-by-point response**

In order to improve our manuscript, we added a table to depict the socio-demographic profile of our study participants, which can explain and support our conclusion. The new table is marked in red in revised manuscript.

<table>
<thead>
<tr>
<th>Age group</th>
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*according to Chinese family project, there is just one child for one family. With the development of the economy and the rising cost of living, parents are inclined to feed one child, especially in metropolitan areas, such as Nanjing.

1. **Minor essential revision:** Line 34. It is unclear who has undervalued the role of viral and atypical pathogens, or why this is so. The title and background section should clearly state the limitation of this study to the detection of atypical bacteria and viruses.

Without the appropriate techniques to detect viral and atypical bacterial pathogens, physicians previously could not diagnose some pneumonia caused by these pathogens. However, the doctor can diagnose some pneumonia caused by bacteria, because bacteria are easy to culture, compared to viral and atypical bacterial pathogens. As such, clinicians often undervalue the role of viral and atypical bacterial pathogens in respiratory tract infections. Of course, there is a difference under the different medical condition.

To state clearly the question, we have made some change, which are marked in red in abstract section.
Regarding the limitations of this study, authors had addressed the question in the Limitation section. Of course, the Pneumoslide IgM test does not have the same sensitivity for all 9 viral and atypical bacterial pathogens. However we think the low positive rates of CP and COX are associated with the sampling time, because antibody levels for CP and COX rise later compared to other pathogens. We can test multiple serum samples with different sampling times to improve the positive rates of CP and COX. We have supplemented the limitation for the detection of CP and COX in the Limitation section.

2. Minor essential revision: Line 159 reads "624 were positive for a positive rate of 51.83%", however from summing the numbers in Table 1 we arrive at 704 positives for a positive rate of 58.47%.

Of a total of 1204 samples tested, 624 were positive for a positive rate of 51.83%, which means that 624 samples were infected by pathogens. Some samples were mixed infection, which were infected by more than one pathogen, so we find 704 pathogens in Table 1 (Table 2 in the revised manuscript). In order to make the statement clear, the statement is corrected as “624 samples were positive for a positive rate of 51.84%”. The revised portion is marked in red in revised manuscript.

3. Major compulsory revision: The overall positive rate using Pneumoslide IgM ranges from 24% in infants to 47% in toddlers, to 79% in pre-schoolers to 81% in school children. This is a significant difference suggesting high degrees of possible bacterial pneumonias in infants that cannot be detected by the Pneumoslide technology. This variation is not mentioned or discussed by the authors.

As we stated in the Limitation section, Pneumoslide IgM could not detect the bacterial pathogens, so we could not exclude the possibility of
bacterial infection resulting in the significant difference between four groups. However, we think the possibility is less likely. On the one hand, viral pathogens are the most common cause of CAP in children younger than 2 years of age, accounting for 80% of cases, and bacterial pathogens are more common in older children [ref. 8]. On the other hand, taking Chinese conditions into account, we think crowded spaces may be the key reason. Please see the newly added Table 1 about socio-demographic profile of our study children.

We also discuss the significant difference in our revised manuscript, and marked them in red in results section.

4. Minor essential revision: Lines 161-162 read “pathogens ranking second to fourth place were INFA.” This should be INFβ, as listed in Table

We have made changes, which are marked in red in revised manuscript. We appreciate the reviewer’s attention.

5. Major compulsory revision: The use of the Chi-squared test is not clear to suggest associations with age and seasons. What distribution do the authors assume in the null hypothesis to test against what is measured? P-values must be reported as <0.0001 and not as 0.000 in Table 2.

Every pathogen has a different positive rate in different age groups and seasons. We made simple statistical analyses to attain p-values and to analyze the association between the positive rates of pathogens and age or season. The present study is our primary results, and further research has been underway in our clinical laboratory, hopefully providing some important results for the future. We have made the required changes for p-values and reported them as <0.001 in the new Table 3.
6. Major compulsory revision: In lines 209-210, there is no statistical basis to suggest "patients infected with MP were susceptible to other infectious pathogens." Mixed infections with INFB and PIVs are reported. Could the higher rates of mixed infections with MP be just a result of higher rates of infection with MP?

To validate the Pneumoslide IgM test, Sally et al. (Ref. 10 in manuscript) concluded that 13.3% of patients with lower respiratory tract infections had evidence of mixed infection. MP and RSV were both present in 3.3% patients and the rest (10%) had mixed infections with MP and ADV. Sally, et al. put forth two hypotheses to address mixed infection: the first suggested that one pathogen simply facilitates the penetration of the other; the second hypothesis states that both pathogens act independently to cause respiratory tract infection.

According to our present results, we cannot conclude that “patients infected with MP were susceptible to other infectious pathogens” and further research is needed to address the question. As to the reviewer’s comments, the higher rates of samples infected with MP and other pathogens may be just a result of higher rates of infection with MP. We, therefore, cannot exclude the possibility and agree this comment, and we changed the statement to “patients infected with MP may be susceptible to other infectious pathogens. However, neither the present study nor previous findings could explain these results, so further research is needed to elucidate the question.”