Author's response to reviews

Title: The influence of Streptococcus pneumoniae nasopharyngeal colonization on the clinical outcome of the respiratory tract infections in preschool children

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Author's response to reviews: see over
Author's covering letter for re-submission

Title: The influence of Streptococcus pneumoniae nasopharyngeal colonization on the clinical outcome of the respiratory tract infections in preschool children

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Cover letter

Dear Editor-in-Chief,

Please find enclosed our manuscript entitled “The influence of Streptococcus pneumoniae nasopharyngeal colonization on the clinical outcome of the respiratory tract infections in preschool children” by Petraitiene et al which we would like to re-submit for publication as a research article to the BMC Infectious Diseases.

The manuscript was submitted on 21st January 2015. We received comments of the reviewers and we prepared our response to these comments.

Reviewer 1

Major compulsory revisions:

1. The biological hypothesis of this study is not clear. As stated by the authors, the pneumococcus commonly colonizes the nasopharynx of young children without causing disease. Therefore detection of the pneumococcus from a NP swab may be an indication of the pneumococcus being the cause of the RTI, or may not, and may be that the organism is just being carried. The authors did not test for any other common respiratory pathogens, and therefore cannot assume that the detection of pneumococcus in the nasopharynx is indicative of pneumococcal disease. Please can the authors clarify their biological reasoning behind how pneumococcal colonization is associated with recovery time, clinical symptoms, use of antimicrobials etc. It is hinted at in the discussion that the authors hypothesize that colonization with the pneumococcus increases ones risk of pneumococcal disease, and it is therefore assumed that the authors used pneumococcal colonization as a proxy for pneumococcal disease? Please clarify this in the manuscript, preferably in the earlier parts such as the introduction and methods. The limitations associated with this inference, need to be stated in the discussion.

Response: Hypothesis explanation and reasoning have been added to the Background section. Limitations have been added to the Discussion.

Background: Lines 75-81.

As we did not test for other pathogens in the nasopharynges of our subjects, we could not determine the aetiology of the disease. We hypothesised that the RTI of children colonized with SPn (who are at risk of developing pneumococcal disease) is longer and more severe than RTI of non-colonized children. Nasopharyngeal colonization with SPn increases one’s risk of developing pneumococcal disease [23]. The most common cause of RTI is viral, which may facilitate the conversion of asymptomatic carriage of SPn to a pathogen and pneumococcal disease may be more severe than that caused by other pathogens.
The major limitation of our study is that we did not test for other pathogens in the NP of our subjects, thus we could not determine the actual cause of the RTI. Nasopharyngeal colonization with SPn increases the risk of developing pneumococcal disease (either primary or as a complication of a previous RTI caused by another pathogen) and this is the most likely reason of our findings. An extended study with testing for other pathogens in the respiratory tract is necessary to confirm this. Another limitation is that we were unable to perform neither bile solubility nor latex-agglutination tests with negative NP samples and thus up to 3% of our negative samples were potentially false-negative.

2. Background, third paragraph: Day-care centre attendance and young siblings are known to be risk factors for pneumococcal colonization, and these studies should be referenced in the background section. However, as the pneumococcus is commonly asymptomatically carried, it is not clear how the authors propose that it may be associated with recovery time, symptoms etc. Please could the authors clarify their hypothesis.

Response: Line 73 – references regarding day-care centre attendance and young siblings [21, 22] have been cited in the Background section. Hypothesis has been clarified.

3. Results, Figure 1: Please can the authors explain their reasoning in this analysis – is it the assumption that if the pneumococcus is detected in the nasopharynx that it is the cause of the RTI? If so, this is not plausible as pneumococcus is known to be carried, specifically amongst the age group studied. If not, then please can the authors explain the relevance of figure 1.

Response: As we could not determine the aetiology of the RTI from the data we had obtained in our study, we hypothesised that the longer recovery duration in SPn-positive cases might be because SPn carriage increases the risk of SPn infection and the conversion of SPn from a commensal to a pathogen is often facilitated by a viral infection which is the most common cause of RTI. This may be the main factor for the longer recovery duration in SPn-positive cases and those with particular serotypes as shown in the revised Figure 1.

Hypothesis has been clarified in the Background section.

Lines 75-81.

4. Results: throughout results, data in the form of numbers (n/N) should be provided and not only the percentage results.

Response: Numbers have been added throughout the Results section.

5. Discussion, first paragraph, third sentence: please can the authors explain this sentence as the pneumococcus is commonly carried amongst healthy individuals. It is not colonization, but rather pneumococcal disease that affects health.

Response: Sentence has been extended to note the risk of pneumococcal infection.
6. Discussion, first paragraph, fourth sentence: it is my understanding that colonization does not affect the course of a disease in the carriage state and that it will only affect the outcome if it develops into disease either as the primary or secondary infection. The authors did not identify the prevalence of other common respiratory infections in this study, and did not examine the difference in the course of the RTI in patients with and without pneumococcal colonization.

Response: Sentence has been removed. Information regarding other pathogens has been added as a limitation in the Discussion section.

Lines 286-290.

7. Discussion and abstract conclusion, first sentence: the statement that Spn nasopharyngeal colonization has a negative impact on the course of RTI need to be more clearly explained as it is not the colonization itself that causes the negative impact but rather that Spn colonization increases ones risk of pneumococcal disease, and this in turn, has a negative effect on the course of RTI.

Response: Sentences have been rephrased.

Lines 44-47 and 295-300.

SPn nasopharyngeal colonization has a negative impact on the course of respiratory tract infection, likely because of SPn being the cause of the disease or a complicating factor. It is also associated with and may be responsible for higher frequencies of bronchitis, pneumonia, acute otitis media, sinusitis and the need of antimicrobial treatment. Our results illustrate the negative impact of SPn colonisation on RTI (longer duration and absence from DCC) and indirectly – importance of pneumococcal vaccines as a measure to prevent SPn infection.

8. Abstract, results: Data in the forms of numbers (n/N) need to be provided to justify the statements made. Include only the most important results of the study in the abstract and provide all the data to support the results.

Response: Numbers have been added to the Abstract, Results section.

9. The manuscript would benefit from general language and grammar editing.

Response: We have done all our best.

Minor Essential Revisions

1. Background, second paragraph, first sentence: It is not clear what is meant by this sentence as the role of pneumococcus as a cause of severe disease is well understood and proven. Recent studies have been focused on the ability of the pneumococcal conjugate vaccine to prevent pneumococcal disease.
Response: We wanted to illustrate the distinction of our study. Sentence has been corrected as per suggestion.

Lines 58-60

Many recent studies emphasize on the SPn effects on severe diseases such as pneumonia, bacteraemia, meningitis [6-12], and AOM [13-17], and the ability of pneumococcal conjugate vaccines to prevent them.

2. Methods, first paragraph: Did the enrolment criteria include upper- and lower respiratory tract infections?
Response: Yes. This has been clarified in the criteria.
Line 93.

3. Methods, second paragraph: What duration of symptoms was used as a cut-off to meet the criteria of acute infection?
Response: 1 week. This has been added to the inclusion criteria list.
Line 95.

4. Can the authors clarify the use of the pneumococcal conjugate vaccine in their country – is it used as part of routine infant immunization program, what is the vaccine coverage in the country?
Response: The study was carried out before the introduction of universal PCV vaccination in Lithuania in October 2014. Information regarding this has been added to the Background section.
Lines 65-67.

5. Italicise names of bacterial organisms in the references
Response: Species names have been italicised.

6. Table 1: This table should be renamed as it does not give the demographic data of enrolled patients but rather number of patients and the prevalence of pneumococcal colonization by site. Alternatively, additional demographic data should be added to this table such as mean age of children by site etc.
Response: Table now includes additional data.

7. Results, second paragraph: The details of the results of the Poisson regression analysis need to be shown to qualify the last sentence in this paragraph.
Response: Table has been reworked as per Referee 3 suggestion. Details of the results have been added to Table 2.
8. Table 2: the actual result numbers (n/N) need to be added to this table to support the results obtained.
Response: Table has been reworked. Numbers have been added.

9. Figure 1 and Figure 2: Please clarify the y-axis on this figure as the title is Cases per serotype, and yet the axis is measured in percentages?
Response: Both figures have been reworked.

10. Results: Please clarify what “G+” as a serotype is?
Response: “G+” belongs to non-typable pneumococcus. All instances of “G+” in the manuscript have been changed to “non-typable”.

11. Figure 1 and 2 and corresponding legend: the asterisk denotes significant difference in what between the serotypes indicated and the spn-negative cases? This should be clarified in the figure legend.
Response: Both figures have been reworked and figure legends have been clarified.

12. Results, recovery paragraph, last sentence and Figure 1: It is not clear how serotypes 15, 23 and 6A were significantly associated with longer disease duration compared to spn-negative cases when all of the patients infected with these serotypes recovered within 4 weeks.
Response: Issue has been clarified in the legend and the corresponding results paragraph. The differences were tested between “1-2 weeks” duration group and “3-4 weeks” group.

13. Figure 1, Figure 2 and Table 3: please clarify the difference between serotypes 23F and 23.
Response: 23 stands for all non-23F serotypes. This has been clarified.

14. Table 3 and results, serotypes paragraph, first sentence: Please check this sentence as there were more 6B cases than 3 and 18C, and yet these have not been mentioned in the text.
Response: Mention of 6B has been added to the Serotypes paragraph. There were 4 cases of AOM with 6B, but this constituted only 6.9% of all 6B cases.

The most frequent serotypes were 6B (15.8%, n = 58), 19F (13.9%, n = 51), 23F (13.9%, n = 51), 15 (10.1%, n = 37), 14 (9.5%, n = 35), 6A (9.3%, n = 34).
15. Table 3 and results, serotypes paragraph, fourth sentence: This is because URTI was the most common diagnosis (n=592). The prevalence of serotype G was actually only 2% (13/592) amongst URTI cases.
Response: Sentence has been removed as being irrelevant.

16. Results, antimicrobial use paragraph, first two sentences: The relevance of the use of antimicrobials 1-6 months prior to sampling is not clear. This did not appear to be an objective of this study as all patients that had received prior antibiotics in the 1 month period prior to sampling were excluded from enrolment.
Response: Children who were treated with antimicrobials 1-6 months before enrolment were included in the study. An exclusion criterion was antibiotics within 1 month prior the study.

Discretionary Revisions

1. Methods, fifth paragraph: a reference should be provided for the standard culture methods used.
Response: Reference [26] has been cited.

Line 122

3. Results, DCC attendance paragraph: a more accurate term for “rate of spn colonization” is “prevalence of spn colonization”, as the term rate implies the speed at which it happens.
Response: Instances of “Rate” have been changed to “Prevalence” where appropriate.

4. A useful analysis would be to determine the predicted coverage of the pneumococcal conjugate vaccine against colonization, based on the serotypes detected.
Response: Data regarding this were published as separate paper – reference [20].

Line 68.
Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=21079
Reviewer 2

Major Compulsory Revisions

1. Throughout the manuscript the authors state that they demonstrate that Spn carriage negatively impacts on / influences / effects the course of respiratory infection. Here I think the authors would be better to emphasize that the nasopharynx is the reservoir for Spn (which underpins findings from other studies that have found that high Spn carriage rates (and density) are associated with disease) and instead talk about the association of carriage and infection and disease.

Response:

Lines 64-67

The nasopharynx is the reservoir for SPn and the carriage of SPn in children with acute RTI has not been studied widely. This study was undertaken to evaluate the circulation of SPn serotypes among children with acute RTI under six years of age in Lithuania before the introduction of universal pneumococcal vaccination in the country in October 2014 [19].


2. It would have been more valuable to see data around the cause of disease (viral/bacterial, Spn in particular). For children with Spn infection then you would expect Spn carriage rates to be higher. Observing the same phenomenon in children without Spn infection would speak to the issue of bacterial/bacterial or bacterial/viral interactions. These issues are not properly addressed and considered in the manuscript at present.

Response: Information regarding this has been added as a limitation of the study.

Lines 75-81 and 286-290.

3. The authors concluding statement “our results illustrate…” is true, but not relevant to the data presented.

Response: Conclusion has been rephrased.

Lines 298-300.

Our results illustrate the negative impact of SPn colonisation on RTI (longer duration and absence from DCC) and indirectly - importance of pneumococcal vaccines as a measure to prevent SPn infection.

4. It is unclear why the authors excluded those that had been previously vaccinated.
Response: Vaccination was considered as exclusion criteria and this is now stated at lines 101-102 as follows:

History of vaccination with any pneumococcal vaccine (because of the vaccine’s effect on reducing colonization with vaccine serotypes [24]).

5. The NP swabs were taken using a culturette with Amies transport media. Spn carriage should be assessed using a deep NP swab sample. The swab tip of the culturette appears quite large, are the authors confident this could reach the NP in children?

Response: We used special thin culturettes with Amies transport media. SPn were assessed according to the recommendations of using deep NP swabs. Reference [27] has been added. Line 122.


6. Similarly to the above, can the authors provide assurance by reference or their own data, that Amies media does not reduce recovery of Spn in comparison with the WHO guidelines for assessing pneumococcal carriage? (O’Brien et al. PIDJ 2003, Satzke et al. Vaccine 2013)

Response: Reference [25] has been cited in the Methods section: Hare et al. compared several methods for NP samples transport and found Amies transport medium adequate for SPn detection.

Lines 119-120.


Minor Essential Revisions

1. Italicize species names in the reference list.

Response: Species names have been italicised.

2. Amend Table 3 to reflect standard serotype/group designations, e.g. “serogroup 15”, “23” should be e.g. “23A/B” etc. Also, please clarify what is meant by “G+” (presumably the serotypes/groups reaction for reagent G) and explain why these were not further distinguished.

Response: “G+” belongs to non-typable pneumococcus. All instances of “G+” in the manuscript have been changed to “non-typable”. Serogroup 23 (non-23F serotypes) and serogroup 15 have been clarified in the manuscript, Table 3, and figures.

3. How many isolates were unable to be typed?
Response: Of 367 SPn isolates 19 (5.2%) were non-typable.

Lines 186-187.

**Discretionary Revisions**

1. The study would have been much more informative with quantitative carriage data, can this be performed and added to the manuscript?

Response: We agree that the quantitative carriage data would have provided additional information; unfortunately, we were unable to perform this.

2. Why was the recovery data assessed as categorical rather than continuous data?

Response: Categorical variable has been selected for the ease of patients’ caregivers and participating physicians to report. From our previous experience this is usually reported in number of weeks even when requested for number in days. In contrast, day-care absence is tracked in a personal diary at the day-care, thus it was analysed as a continuous variable.

**Reviewer 3:**

**Major Compulsory Revisions**

Methods

Page 5, line 91 - Identification of pneumococci based on colony morphology and optochin susceptibility may only provide researchers with some unreliable data because some species of oral alfa-haemolytic streptococci are susceptible to optochin. A confirmatory test is necessary (bile solubility, latex agglutination).

Response: All positive samples were serotyped. Possible false-negative samples, unfortunately, were missed as we were unable to perform neither bile solubility, nor latex agglutination tests. We believe that these small numbers of potentially lost positive samples did not influence the accuracy of our results due to the large sample size of our study.

This information has been added to the Methods section. Reference [28] has been cited.

Lines 122-124.


Page 5, line 100 - What does "additional tests" term mean. Further details on the issue of question should be provided with as there are problems with understanding of statistic analyses made for associations of studied variables with particular serotypes/serogroups.

Response: Methods, Statistical analysis paragraph has been clarified. Results section has been reworked accordingly.
Results

Even though the results indicate no statistical significance it is important to show these data. Tables are a more readable form of presentation. I suggest using a sample table as below instead of Table 2 (in attachment).

Response: Table has been reworked as per suggestion. Poisson regression was used to yield all the neccessary results.

Page 6, line 130 and Figure 1 - As authors stated, recovery duration of disease was divided into 3 groups (1-2 weeks, 3-4 weeks, > 4 weeks). Thus, the question is why authors did not show these groups in Figure 1. Moreover, the information which statistic tests they used to obtain the significant differences is missing. Was a referred group chosen?

Response: Issue has been clarified in the Methods section. Figure 1 now displays all three recovery duration groups and p values. Figure 1 legend has been changed accordingly.

Lines 133, 143, and 480-482.

Page 8, line 163 and Figure 2 - While statistical analysis between two or more groups their quantities or means are compared. Why did authors depict only one group of compared variables in Figure 2? Again, I am curious which statistic tests they used to obtain the significant differences. What variables were compared with each other? Was a referred group chosen?

Response: Issue has been clarified in the Methods section. Figure 2 now displays both groups of subjects (those who had antimicrobial treatment and those who had not). Figure 2 legend has been changed accordingly.

Lines 141-145 and 488-490.

Minor Essential Revisions

Page 7, line 139 - Authors should be careful when they use terms serotype or serogroup . There is no serotype 23 or 15, these are serogroups.

Response: Serogroups 23 (non-23F serotypes) and 15 have been distinguished in the manuscript, Table 3, and both figures accordingly.

Page 7, line 140 - The term "Serotype G+" should be explained.

Response: “G+” belongs to non-typable pneumococcus. All instances of “G+” in the manuscript have been changed to “non-typable”.

We believe our findings would appeal to the readership of BMC Infectious Diseases. All authors have approved the manuscript and agree with its re-submission to the BMC Infectious Diseases.
We confirm that this manuscript has not been published elsewhere and is not under consideration by another journal.

The authors declare that they have no competing interests.

Authors have conceived and designed the study, participated in the data collection, statistical analysis, and interpretation of the data and writing of the manuscript. All authors read and approved the final version of the revised manuscript.

Looking forward to hearing from you.
With best regards,
On behalf of authors, Vytautas Usonis

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