Author's response to reviews

Title: Nasopharyngeal carriage, serotype distribution and antimicrobial resistance of Streptococcus pneumoniae among children from Brazil before the introduction of the 10-valent conjugate vaccine

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Author’s response to reviews: see over
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To the Editor in Chief
BMC Infectious Diseases

Dear Sir,

Enclosed, please find the revised version of the manuscript MS: 3878626279032506 (“Nasopharyngeal carriage, serotype distribution and antimicrobial resistance of Streptococcus pneumoniae among children from Brazil before the introduction of the 10-valent conjugate vaccine”).

We have accommodated the reviewer’s suggestions. The major change was the separation of the “Results and Discussion” into two distinct sections, as requested by reviewer #2. Several additional changes/revisions were incorporated in the revised manuscript and the point-by-point responses to each reviewer are presented below.

We would like to thank the valuable comments and suggestions, which were helpful to improve the manuscript.

Sincerely,

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ANSWERS TO REVIEWER’S COMMENTS

MS: 3878626279032506

Reviewer #1: Yin-Bun Cheung

Reviewer’s report:
This reports the carriage and characteristics of Streptococcus pneumoniae in children recruited from a day care center and the emergency medicine department of a paediatric hospital in a Brazilian metropolitan area. This provides useful background information to understand the epidemiology and potential impact of pneumococcal conjugate vaccine.

Authors’ comment
Many thanks for the insightful comments.

Major compulsory revisions

1. The participants were recruited between March and June 2010. PCV10 was included in the Brazilian Immunization Program in May 2010. The authors need to explain the timing of the introduction in this metropolitan. Otherwise the title “before the introduction of the 10-valent conjugate vaccine” is not understandable.
Response: This information was missing in the original version of the manuscript, and it has been included in the revised version, on the basis of the comments. Although the PCV-10 has been incorporated into the Brazilian Immunization Program in March 2010, it was gradually introduced in different regions of the country, being available in October 2010 in Niterói city, where the present study was performed.

2. The statistical inference is based on unjustifiable assumption of independence between subjects. This is clearly wrong in the present setting of carriage in day care center. Previous researchers used e.g. robust standard error to allow for clustering (e.g. Hill et al. CID 2006, 2008). Since the study only included one day care center and one paediatric emergency medicine department, it is difficult to see how a valid statistical inference can be made (without replicates of day care center and paediatric emergency depart). I would suggest the authors to remove all the p-values and confidence interval and provide a purely descriptive report of their findings from the two sites.
Response: We agree with your comments on statistical analyses. To obtain the p-values, there is an assumption of independence between subjects and, although always assumed, it is difficult to confirm that this supposition is valid. However, even though no sample size calculation was achieved, we have made a statement in the Methods section – item “Statistical analyses”, emphasizing that the statistical inferences made in the revised version of the manuscript are restricted to the population analyzed in the present study (one DCC e one emergency room of a pediatric hospital), not necessarily extrapolating to the general population. Also, we calculated the crude odds ratio, which has a more descriptive character, in an attempt to fulfill your requirement concerning the descriptive analysis.
It is noteworthy that the two factors more associated with pneumococcal carriage among the analyzed population (day care attendance and cohabiting with siblings under 6 y old) were also reported in other international studies (Hsieh et al., 2012; Wroe et al., 2012), reinforcing our findings.

3. The “Estimated Risk” in Table 1 is a wrong column title. What is presented is actually the odds ratio. The $p=0.030$ on page 7 about having young siblings as a risk factor disagrees with the $p=0.003$ in Table 1.

**Response:** In fact, in Table 1 the column “Estimated risk” was “Crude odds ratio”. Also, the $p=0.030$ on page 7 about having young siblings as a risk factor was the correct one and the mistake was in made Table 1. Such information has been corrected in the revised version.
Reviewer #2: Rosa Prato

Major Compulsory Revisions

Abstract
The abstract lacks of the Methods section.

Response: The lacking of the Methods section in “Abstract” is due to the instructions for authors available at the BMC website, as follows: “The Abstract of the manuscript should not exceed 350 words and must be structured into separate sections: Background, the context and purpose of the study; Results, the main findings; Conclusions, brief summary and potential implications”.

Introduction
Paragraph 2: this point is poor of other epidemiological data on the incidence (or an estimation of it through hospitalization) of IPD, CAP and AOM in children under 5 years.

Response: Unfortunately, these data are very limited or even unavailable for most Brazilian locations. The available data have been described in this paragraph, and we have also included additional information. Regarding AOM, there is only one study in Brazil (Sih, 2001), reporting \textit{S. pneumoniae} as the prevalent agent, which is consistent with findings of other geographical regions, and this reference has now been included in the revised version of the manuscript.

Paragraph 4: at this point I want to read something more on how PCV10 is offered in Brazil (universal childhood free of charge vaccination? Other?). Mainly, it is necessary to state the rates of vaccination coverage reached for PCV7 and more recently for PCV10 if the authors want to assess the actual and future impact of vaccination on colonization.

Response: PCV10 is now offered in Brazil as a free of charge universal childhood vaccination and it was gradually introduced in different months for each Brazilian state. In Niterói city, it was available in October 2010, after the period that this study was performed.

In relation to PCV7, it is difficult to evaluate the rates and impact of vaccination coverage reached in Brazil, since it was only available in private clinics. However, taking into account the price of the vaccine, we can deduce that only a small portion of the population had access to PCV7 vaccination.

Our aim was to assess the future impact of PCV10 vaccination, since the subjects included in the study did not receive any dose of this vaccine, by the time the collection of specimens for the present study was performed.

Paragraph 5: can we speak of pre-vaccine era when we refer to the period before the introduction of PCV10? How many children have received PCV7 for considering that it had not any effect on NP carriage?

Response: In fact, this statement was referred to pre-PCV10 era, since six children of the present study had received at least one dose of PCV7. Thereby, the term “pre-vaccine era” was changed to “pre-PCV10 era” in the revised version of the manuscript.

Results and Discussion
These two sections must be absolutely kept separate. The readability of this text is not so good and I think that this is not the best way to write a scientific article.

**Response:** These two sections have been separate in the revised version, according to the recommendations.

Paragraph 4: 88.4\% is the proportion of ...  
**Response:** This information was not correct. The correct is 96.7\%, which is the proportion of isolates serotyped (117/121). Proper change has been done in the revised manuscript.

Paragraph 7: how the theoretical coverage of PCV10 and PCV13 has been estimated? Also by the two settings, it is not clear.  
**Response:** The vaccination coverage has been estimated simply by the sum of frequency of vaccine serotypes found among the children. This estimation is likewise performed in related studies.

Paragraph 9: the findings of this study must be better discussed at the light of the availability of PCV10 and of the unavailability of PCV13 in Brazil.  
**Response:** PCV13 is also approved for use in Brazil, although currently available only in private clinics. This information was included in the revised version of the manuscript to make the discussion such aspect clear.

Conclusions
Paragraph 1: that analyzed in the study is not a closed community but the authors must better discuss their findings (day care attendance, households contacts) at the light of the opportunities offered by vaccination.  
**Response:** In fact, “ household contacts” was also a factor associated with pneumococcal carriage and this has been included in the revised manuscript. Nevertheless, even though PCV10 vaccination is believed to reduce NP carriage, the estimated impact on the analyzed population was low (45.5\%).

Table 1
Is sex a risk factors for carriage? If yes, this must be discussed.  
**Response:** No. The \( p \)-value for sex was 0.530 and the level of statistical significance was defined as a \( p \) value less than 0.05.

The manuscript has some good points, also considering the study setting, but it was written in a very confusing manner. I strongly recommend to the authors to rewrite in particular the results and discussion and submit a new version of the article.  
**Response:** Following your recommendations, the Results and Discussion section has been separated and more information has been included to make our findings clearer.
Reviewer #3: Maria Cristina Brandileone

Reviewer's report:
General comments:
The manuscript 'Nasopharyngeal carriage, serotype distribution and antimicrobial resistance of Streptococcus pneumoniae among children from Brazil before the introduction of the 10-valent conjugate vaccine' submitted by Felipe PG Neves et al. reports the prevalence of S. pneumoniae in nasopharynx of Brazilian children, the serotype distribution and characteristic of antimicrobial resistance of isolates before the 10-valent pneumococcal conjugate vaccine. Some researchers belonging to the group of authors (citing Lucia Martins Teixeira) have great experience on Streptococcus investigation field.

About the issue, few studies on colonization by pneumococcus in Brazilian population as children were published. Considering that Brazil was the first country in the world to introduce PCV10 in their national immunization program, it is important to get information of pneumococcal colonization in a period before PCV10 introduction and report it, to assess the direct impact of this vaccine on pneumococcal carriers. So the theme of the study is important.

The study is well written using simple talking, including well-defined objective, clear and direct; the methodology and laboratorial techniques (classic and molecular, CLSI criteria for define antimicrobial resistance etc.) are appropriate and well described, including those currently and internationally standardized for studies on pneumococcal colonization (STGG transport medium). The data analysis of risk factors is also appropriated.

Tables and figure are very clear. The results of the study comprising the pneumococcal carrier rates (overall rate 49.2%), risk factors associated with colonization (care attendance and cohabiting with siblings under 6 y old, p=0.001), the distribution of serotypes and antimicrobial susceptibility characteristics sounds well and are compatible with the data found in other international studies. In the discussion, the data found on this study are compared with the results found in international publications of important contributions in state of the art on the epidemiology of pneumococcus. The discussion is rich, well-developed and evolved. The conclusions are completely supported on the findings of the study, and answer the question proposed by the study.

Authors’s comment
Many thanks for your encouraging comments.

Major Compulsory Revisions

- It is unclear whether the subjects of study were or were not vaccinated with PCV10. Since PCV10 was introduced in (Brazil) or Rio de Janeiro State in May 2010 (?), and sampling was carried out from March to June 2010, this point needs to be clearer. Because the PCV10 was gradually introduced in immunization routine for children in Brazil in March 2010, and since this occurred in different months for each Brazilian State along the 2010, it is necessary to clarify the information. When was the PCV10 introduced in Niteroi, the city of sampling?

Having taken the PCV10 vaccine was considered an exclusion criterion for selection of subject? Risk factors displayed on Table 1 considered “Received at least 1 dose of PCV7” for analysis. And about PCV10? The study was carried out in Niteroi city.
Response: In fact, PCV10 was included in Brazilian immunization program in March 2010 (not in May, as it was informed in the original version of the manuscript). However, the analyzed subjects did not receive any dose of the PCV10, since this vaccination was introduced in Niterói city only in October 2010. Thereby, having taken the PCV10 vaccine was not considered an exclusion criterion for selection of subjects. This information was missing in the manuscript, but it has been included after revision.

Discretionary Revisions
- If it is possible, the authors could give some information about the population attended in the hospital and in the day care were sampling were collected. Public or private centers? The two institutions participating in the study were located in the vicinity or in the central part of the city? Because high level of pneumococcal colonization rates is associated with poor leaving conditions and crowding, it is important to characterize the subject included in the study.

Response: Such data have now been included in the manuscript in two sections (“Methods” and “Discussion”). One institution is located in the periphery and another in the central part of the city, but both are public, near to slums and deliver services to low income population, since the average family per month income of such subjects are ≤ two Brazilian minimum wages.

-I suggest to include the site below in the reference number 15.

Response: The site was included in the revised version of the manuscript as recommended