Reviewer's report

Title: Molecular epidemiology of pneumococci obtained from Gambian children aged 2-29 months with invasive pneumococcal disease during a trial of a 9-valent pneumococcal conjugate vaccine

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Reviewer: Raquel Sa-Leao

Reviewer's report:

General

The authors propose to describe the molecular epidemiology of invasive disease pneumococcal isolates recovered from vaccinated and unvaccinated Gambian children who participated in a 9-valent vaccine trial.

The writing is good and the title is appropriate. The abstract could be improved (detailed comments below). The aim is well defined and the methods used are appropriate.

Data deposition of novel MLST alleles and sequence types at the MLST pneumococcal database was not made and is strongly recommended for reasons indicated below.

The numerical results reported in the text and tables are not always consistent. Based on the data obtained the discussion could be better structured, focused and enriched (suggestions given below).

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1. Page 7, 2nd paragraph: Based on the observation that 3 of 6 meningitis isolates were of serotype 12F the authors suggest “serotype 12F has a high propensity to cause meningitis”. On the other hand, according to Table 1 a total of 12 invasive disease isolates of serotype 12F were recovered in the study making it the 2nd most abundant serotype. Of these 12 isolates only three caused meningitis. Based on this, I do not think there is firm evidence at this time to support the suggested “high propensity of serotype 12F” to cause meningitis.

2. Page 9, 2nd paragraph: The authors discuss capsular switching and use data retrieved from the MLST database to propose that “ST63 has a high propensity for capsular switching” and discuss other evidences of capsular switching by comparing their own data with data from the MLST database. According to information obtained from a previous database curator (personal communication) and my own reasoning, extreme caution should be used when extracting unusual serotype information from the MLST database to conclude on capsular switching propensity. While, all alleles stored at the MLST database have been through a process of realignment of original obtained sequences by the curator before they
could be accepted, other data on the isolates such as epidemiological data, serotypes and antibiotypes relies solely on the sender and, in general, has not been confirmed by independent laboratories. Therefore, it is impossible to be sure, whether there have been true capsular switching events or, on the other hand, there have been technical mistakes on serotyping. In fact, a recent paper has shown that technical problems can often lead to up to 5% wrong serotyping assignments (Konradsen HB. Validation of serotyping of Strpetococcus pneumoniae in Europe. Vaccine. 2005. 23(11):1368-73). I suggest that if the authors would like to discuss capsular switching events, they should do it within their own collection or be cautious in the conclusions they reach if using data retrieved from the MLST database.

3. Discussion: The discussion could be better structured and enriched. For example, although it is clear that a large number of novel ST were found in the study there is no discussion on the relationship between the Gambian isolates and those found elsewhere except for a few serotypes: 1, 12F, 14, and 6B. For the reader it would be interesting to know if, in general, the Gambian isolates are related or not with strains from other countries and which countries. A search of the MLST database for closely related sequence types should provide some answers to this. In particular, comments on serotypes 19A, 19F, 23F, 5 could increase the interest of the reader.

In addition, the authors have used BOX PCR to type their isolates, a method which is infrequently reported in the literature compared with MLST or PFGE. It would be interesting for the reader if the authors could provide information on the adequacy of this method for local surveillance based on their own experience as it is much cheaper than MLST. It would also be of interest a discussion on the general agreement on the concordancy between the two molecular typing methods used: BOX PCR and MLST. Will the authors continue to use BOX PCR? Do they suggest it could used as a single typing method or would they only use MLST in the future? This is relevant for readers which perform surveillance studies and may be working on tight budgets.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

4. Abstract: It is not obvious why did the authors give particular relevance to serotype 1 on the abstract among all the other serotypes studied. The conclusion may be too is general and could be more specific to this study.

5. Table 1: the table would read better if serotypes were sorted by number in ascending order or by ranking them in descending order.

6. Page 5, 2nd paragraph: Please check probable numerical inaccuracies

6.1. The top six serotypes account for 58.3% of the isolates according to table 1 (not 57.6%).

6.2. The ranking of serotypes among unvaccinated children should be revised as
discrepant results are found in Table 1.

6.3. The estimated coverage of PCV9 should be revised as discrepant results are found in Table 1.

7. Page 5, last paragraph: “...all the 127 isolates shared an identical or closely related profile...”. The meaning of this sentence should be clarified.

8. Table 2, Table 3, Figure 2, Supplementary Figure and last paragraph of page 6: In the paper the authors have given arbitrary numbers to the novel alleles and sequence types they found when doing MLST. From my point of view this can lead to confusion and even will impede other authors from citing the MLST findings described in this paper. I strongly suggest that the authors submit their data to the Pneumococcal MLST Database Curator in order to obtain allele numbers and sequence types which all the community will be able to recognize, compare to, and cite. This submission process follows an easy process described on the MLST website and an answer is usually obtained within a couple of days.

9. Page 6, 2nd paragraph: According to Table 2 there were 74 sequence types (not 76) of which 51 were novel (not 53).

10. Figure 2: The figure is cut on the right side (the image is incomplete) preventing full detailed analysis of it.

11. Legend of figure 2: What do the different types (light grey, grey, black) of dashed lines mean?

12. Page 8, first 16 lines: The authors discuss vaccine efficacy against serotypes 1 and 9V. I suggest that this discussion is beyond the scope of this paper, which has been defined by the authors as a description of the molecular epidemiology of the invasive disease isolates.

13. Supplementary figure: This figure contains a huge amount of relevant information. I suggest it should be part of the manuscript.

Discretionary Revisions (which the author can choose to ignore)

Page 4, last sentence: I suggest adding at this point the information that the 131 isolates described in this study were invasive.

Page 5: The subtitle “Microbiology” could be changed to “Antimicrobial resistance patterns”.

Page 12, Study Population: The authors had initially 132 isolates of which one was not viable after freezing which gives a total of 131. I suspect that there were four cases of disease from which two isolates (from different clinical sources) were obtained. However, after detailed characterization those were found to be identical and therefore the authors end up with a collection of 127 isolates. However, this is not clearly stated in the text and might lead the reader into some
confusion.

Page 13, Antimicrobial susceptibility testing. Are interpretation criteria the same as CLSI? If yes, the appropriate reference could be added.

10. Table 3 and Figure 2: Table 3 repeats the same information which can be read or directly extracted from Figure 2 and it is not very informative or relevant. I suggest Table 3 should be deleted.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

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

Quality of written English: Acceptable

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.