Author's response to reviews

Title: Penicillin resistance and serotype distribution of Streptococcus pneumoniae in Ghanaian children less than six years

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Version: 2 Date: 12 July 2013

Author's response to reviews: see over

Thank you very much for considering the manuscript for publication in BMC Infectious Diseases. We appreciate the reviewers’ thoughtful comments and we are pleased to have the opportunity to address these in our revised manuscript. Our responses to the reviewers’ comments are below:

Editor's comment:

"Thank you for submitting your manuscript for reviews. Whilst the subject matter is interesting and relevant, the manuscript will need extensive revision to address the reviewer's concerns before publication could be considered. In particular, I think it important to clarify the subject selection (and justify the small number of children <2y) and the antimicrobial resistance testing. Please also check the references for accuracy (e.g. "pneumoniae" missing from ref 16 and spelling mistake ("pneumonia" not "pneumoniae") in ref 22). "

Answer: We have addressed the reviewers’ comments in particular the issue regarding the number of children below 2 years of age. The references have been checked.

Editorial comments:

After reading through your manuscript, we feel that the quality of written English needs to be improved before the manuscript can be considered further.

We advise you to seek the assistance of a fluent English speaking colleague, or to have a professional editing service correct your language. Please ensure that particular attention is paid to the abstract.

Reviewer number: 1

This is an interesting article with important results informing pneumococcal vaccination in Ghana. While worthy of publication, many issues need first to be resolved. The most important is probably the small number of children <2 years old, this being the main target group for pneumococcal
conjugate vaccines. This limitation needs to be addressed. Some of the other more important revisions are listed. However, suggested minor or discretionary revisions were too many to list, therefore a copy of the manuscript with tracked changes will be attached.

**Answer:**
We agree that our study is not representative for the children below 23 month and in particular below 11 month. However the reason for choosing nurseries and kindergarten children is that nurseries and kindergartens are characterized by overcrowding and are an optimal environment for horizontal spread of pneumococci (Skovbjerg et al 2013, Scandinavian Journal of Infectious Diseases, 2013; 45: 279–284). It is also in this environment the effect on introducing a PCV will have the largest direct effect on carriage, while in particular for the children below 11 month, it will be the heard immunity effect which will change the serotype. We have added a section in the discussion explaining these limitations.

**Major Compulsory Revisions**
1. Oxacillin is a screening test, so there is no need to refer to these results in the Abstract or Discussion. **Answer:** As suggested by the reviewer, we have deleted this.

2. The authors need to be consistent with abbreviations, i.e. PCV13 (or PCV-13) throughout the paper. **Answer:** We have looked through the manuscript and corrected it all to PCV-XX or PPV-23.

3. There was a lot of detail on climate in the Methods (first paragraph). Since this was not further discussed, I suggest it be removed (see attached file). **Answer:** As suggested by the reviewer, we have used the suggested correction.

4. The second paragraph of Methods describes the sampling of children 2-4 years from nurseries and 4-6 years from kindergartens. However, the Results include children <2 years of age (Tables 1 and 2). Where were these children from? **Answer:** All the children are from nurseries and kindergartens; we have corrected and specified the age descriptions in the methods paragraph.

5. The final paragraph of Results describes the higher coverage of PCV13 in children 0-2 years old. This age group is the main target group for vaccination with pneumococcal conjugate vaccines. The small number of children in this age group is a major limitation of this paper which has not been mentioned and needs to be addressed by the authors. **Answer:** It has been shown that children less than one year have a very high carriage rate, however the full effect of a PCV on carriage rate will properly first be on children older than 1 year. As previously mentioned the reason for choosing nurseries and kindergarten within the educational strata as sites for collecting nasopharyngeal specimens was because they are characterized by overcrowding and are considered to be sites with children who carry a large pool of different serotypes which can easily spread among other children in the classroom. It is also here the effect on introducing a PCV will have the largest direct effect on carriage, while in particular for the children below 11 month, it will be the heard immunity effect which will change the serotype. See also answer to previous question. Furthermore there has been added a paragraph in the discussion regarding this subject.
6. I am not convinced by the argument that geographical location is an important determinant of pneumococcal colonization (second paragraph of Discussion). The authors need to further analyse or explain the age distributions, and also comment on environmental risk factors, socioeconomic indicators, etc. before they can make this claim.

**Answer:** We have deleted the argument that geographical location is an important determinant of pneumococcal colonization. Comparing carriage rate for children should be in the same age group, in that it has been described that carriage rate in older children are reduced.

7. I am also not convinced that penicillin resistance is increasing (Abstract conclusions, third paragraph of Discussion and final conclusions). The data presented suggest that it may be but are not conclusive. If the authors are sure about this, they need to present the evidence more clearly.

**Answer:** We agree with the reviewer that our data on penicillin resistance are not conclusive but may only suggest a possible trend. We have deleted the sentence in the abstract and the conclusion, and modified the sentence in the third paragraph. Our data show a large group of intermediate resistant pneumococcal isolates, compared with the previous studies from Ghana. It is not as serious as the study by ref 29, however we do find a large group of intermediate resistant isolates, and we therefore think that in the future, Ghana should pay attention to penicillin use in the country.

8. Figure 2 is a duplication of the data in Table 3 and should be removed.

**Answer:** As suggested by the reviewer, we have deleted figure 2.

9. In Table 1, 95% CIs need to be calculated using the exact method.

**Answer:** We have recalculated the CIs values using the exact method as described by Clopper and Pearson. The values in table 1 have been corrected.

10. Age groups need to be properly defined in Tables 1 and 2.

**Answer:** As suggested by the reviewer, we have re-defined the age groups.

11. Table 2 is too long – few readers will be interested in this level of detail. I suggest combining serotypes below PPV23 into ‘Other’ or ‘Serotypes not included in any vaccine’.

**Answer:** We have changed table 2 as suggested by the reviewer.

12. Table 3 headings need clarification, and columns for oxacillin resistant and penicillin susceptible isolates should probably be removed, leaving the data for penicillin nonsusceptible (intermediate resistant) and resistant pneumococci. The susceptible column is misleading as it does not include the oxacillin susceptible isolates that did not have MIC determined.

**Answer:** A new heading has been made, and the columns for oxacillin resistant and penicillin susceptible isolates have been removed.

13. There is confusion with respect to the % of isolates that were penicillin intermediate resistant (nonsusceptible). The text (Results paragraph three) and Table 3 indicate that 288 isolates were tested for oxacillin, and 44% (128/288) were resistant. It is reported in the Abstract that 45% of isolates were penicillin nonsusceptible (130/288 in Table 3 and paragraph three of the Discussion).
However, in the Results (paragraph three) it says 60 plus 57 (=117) of the oxacillin resistant isolates were penicillin intermediate resistant (41%). However, an additional 13 isolates were tested. Should the % of isolates that were penicillin nonsusceptible actually be 43% (130/301)?

**Answer:** In all 66 isolates (Accra) and 62 isolates (Tamale) were found to be oxacillin resistant. We then had 13 isolates from both Accra and Tamale, which were not tested for oxacillin. Because of the limited number, we decided to directly test the 13 isolates against penicillin using E-test. Because these 13 isolates were not tested for oxacillin, the correct percentage for oxacillin would be (62+66)/(288-13)= 46.5%. Because we have focused on the penicillin, we removed the percentage of oxacillin. The 13 isolates are included in the 288 isolates, so the correct percentage is 45% (67+63/288) for penicillin intermediate resistant. The numbers have been corrected in the manuscript.

14. I would suggest adding lines to indicate PCV7 and PPV23 serotypes in Figure 1 (it looks like serotypes are in this order anyway). Also one heading is in capital letters while the other is not.

**Answer:** We have added a line representing PCV-7, however due to serotype 6A, it is difficult to present PPV-23. We have added a comment regarding PPV-23 in the figure text. The typesetting has been corrected.

15. The conclusion needs to be rewritten after addressing the above points, and to avoid repetition.

**Answer:** The conclusion has been rewritten as suggested by the reviewer.

Minor Essential Revisions See attached Word document with tracked changes.

**Answer:** Thank you very much for the comments and corrections. We have more or less accepted all corrections, see tracked changes.

Discretionary Revisions
See attached Word document with tracked changes.

**Answer:** We have more or less accepted all corrections, see tracked changes.

Reviewer number: 2

Thank you for the opportunity to review this paper.

Abstract
1. In the abstract and in the discussion, the authors compare the vaccine coverage of their carriage strains by PCV-10 and PCV-13 to PPV-23. Coverage for invasive pneumococcal disease strains is more important than for carriage strains (because not all carriage strains are likely to cause invasive disease) which I think needs to be mentioned in order to give context to the discussion. Also, polysaccharide vaccine is not suitable for infants, the target group for vaccination, due to a lack of immunogenicity, and I think this is also relevant to this point. This could be expanded in the discussion and perhaps the comparison removed from the abstract. (Discretionary revision)

**Answer:** We agree that the PPV-23 is not an option for children less than two years, However it might be an interesting option to give a final booster with the PPV-23 vaccine in children as
suggested by Ota et al 2011 (Vaccine 29 (2011) 2999–3007). We therefore think that it is still appropriate to mention PPV-23 in the abstract.

Introduction
2. This study aimed to generate baseline data to inform vaccine and treatment policy in Ghana, but it is not clear whether sampling from nurseries and kindergartens in two cities achieves this; how representative of all Ghanaian children is this population? What is the urban/rural divide in Ghana? What is the socio-economic structure of populations attending nurseries and kindergartens compared to those who do not attend? (Major revision)

Answer: We agree that this study do not cover the whole of Ghana, but only two cities, Accra and Tamale. However we also believe that these two cities are main population centers in Ghana, and by looking at the pneumococcal serotype distribution in these two cities, we will have a representative idea of the serotype distribution in Ghana. The reason for selecting nurseries and kindergartens is that these centres are expected to be the site for a large pool of different pneumococcal serotypes. It will therefore be at these sites, which in our opinion will give the best estimation of what pneumococcal serotype to expect after the introduction of a PCV. See also answers to questions from reviewer 1.

Methods
Sampling and study design
3. The author state that the participants were selected from “A list of nurseries (2 to #4 years-old children) and kindergartens (#4 to #6 year-old children)” which implies that no children <2 years old were included. In the results, there are some children <2 years old. This needs clarification. Additionally, the exclusion of children < 2 years old is expected to miss a significantly large proportion of the reservoir of pneumococcal carriage in Ghana, because the younger children are more likely to be carriers, and more likely to transmit. So I think this needs explaining in the study design, and discussing in the limitations section of the discussion.

Answer: We agree with the reviewer that there is a limitation with regard to children less than 2 years. For detailed response see answers to reviewer 1. We have added a paragraph in the discussion on this limitation of the study.

4. Why were children with URTI symptoms excluded? (Major revisions)

Answer: It is often a common practice among many parents to treat their children having URTI by visiting the drug store or pharmacy without doctor’s prescription in Ghana. It is only when the self-medication fails that they visit the hospital for treatment. To reduce the number of ”off the record” antibiotic treated children participating in the study, we excluded children with URTI.

Results
5. The 32% carriage prevalence is fairly low – this could be due to the exclusion of most children < 2 years of age but also perhaps suggests a relatively privileged (urban kindergarten-attending) population?

Answer: We agree that a general carriage rate of 32% is low, however if looking at each age group, you will see a high carriage rate mong the children in the youngest age group and drops as one grows older. An inclusion of newborn and exclusion of five years children and up would as suggested by the reviewer have an effect on the carriage rate. See also previous answers.
Although the study sites were focused on the cities Accra and Tamale, some nurseries and kindergartens were located in slums and some of the children came from surrounding villages to attend school in the cities.

6. 5% of pneumococcus carriers having multiple carriage is also low – this could also relate to the above points but could some cases of multiple carriage have been missed by the latex agglutination method performed on broth culture? These points could be included in the discussion. (Discretionary revisions)

**Answer:** Identification of pneumococcal species from swab samples was performed as described by ref 21, 22 and as Egere et al 2012 (ref 30). We do not expect to miss any isolates compared with other similar studies, instead the use of screening by latex agglutination was an extra test to look closer for multiple serotypes on blood agar plates.

Antimicrobial resistance

7. There is no mention made of the meningitis vs non-meningitis EUCAST breakpoints, and it might not be clear to some readers that the non-meningitis breakpoints are being used. This could be clarified. (Discretionary revision)

**Answer:** We have added a sentence in the antimicrobial resistance section specifying this.

Vaccine coverage

8. How are there children aged 0-2 years included? Please see my comments above re sampling methods.

**Answer:** We agree that particular children less than 1 year are not represented in the study. For a more detailed explanation see previous answers including answers to reviewer 1.

Discussion

9. The authors mention use of higher penicillin doses for pneumococcal disease treatment for meningitis if intermediate resistance is present, citing the CLSI guidelines. But they have used EUCAST for the susceptibility testing, not CLSI. CLSI only has an intermediate category for non-meningitis cases, stating that higher doses of penicillin can be used for non-meningitis cases. (Major revision)

**Answer:** We have rewritten the sentence from the discussion regarding the suggestion of using higher doses of penicillin. EUCAST guidelines are now the only guidelines presented in the manuscript to hinder confusions between the use of CLSI and EUCAST guidelines.

10. There could be some discussion of the limited time span of this baseline data. Fluctuations in baseline carriage prevalence can be missed with data from only a single time point. (Major revision)

**Answer:** Repeating carriage studies would be very good to have, and it is correct that over time there is a natural fluctuation in the pneumococcal serotype distribution. However we believe that this study will be able to provide some information on which serotypes will replace the vaccine serotypes after the introduction of the PCV-13 in Ghana. We do however agree that a new carriage study a couple of years after the PCV introduction would be good to have, to see the effect of the PCV introduction in Ghana.
Figure 1
11. Instead of two graphs each stratified by gender, one graph stratified by location could suffice. (Discretionary revision)
**Answer:** Because this study is not representative for the whole Ghana, but for two locations in Ghana, we do think it is more appropriate to keep the two graph’s.

Table 2
12. Probably not necessary because the information is in figure 1 except for the age breakdown, but there are not enough numbers to break down by both serotype and age anyway. (Discretionary revision)
**Answer:** We have changed table 2 as suggested by reviewer 1.

General
13. Are any of the authors Ghanaian? Was there any capacity building as part of this study to enable future surveillance post vaccine introduction? (Discretionary revision)
**Answer:** The first three authors are Ghanaian. This manuscript is furthermore a part of a larger project call ADMER. For further details on the project see this homepage (http://admerproject.org/), which also give some information on the issue with capacity building as part of this study. If the reviewer would like more information on the ADMER project, please send the authors an e-mail, and we will be happy to respond.