Author's response to reviews

Title: Non-Typeable Haemophilus Influenzae and Streptococcus pneumoniae as Primary Causes of Acute Otitis Media in Colombian Children: A Prospective Study

Authors:

Alexandra Sierra (alexandra.sierra@yahoo.com)
Pio Lopez (piolo@emcali.net.co)
Mercedes A Zapata (nanazaz@hotmail.com)
Vanegas Beatriz (beatrixv07@hotmail.com)
Maria M Castrejon (maria.m.castrejon@gsk.com)
Rodrigo De Antonio (rodrigo.d.deantonio@gsk.com)
William P Hausdorff (william.p.hausdorff@gsk.com)
Romulo E Colindres (romulo.e.colindres@gsk.com)

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Author's response to reviews: see over
Sirs
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Dear Editors,

We were glad to receive your e-mail of November 8th, 2010 stating that our manuscript entitled “Non-Typeable Haemophilus influenzae and Streptococcus pneumoniae as Primary Causes of Acute Otitis Media in Colombian Children: A Prospective Study” has the potential to be published in The BMC Infectious Diseases Journal provided that some aspects are improved.

The authors appreciate the constructive comments and have attempted to modify the manuscript as requested within the limits of the study objectives. Wherever possible, we have made the changes and corrections suggested by the reviewers. Please find attached the revised version (with changes highlighted in yellow for your convenience) of the manuscript as well as a document containing our point-by-point answers to the comments made by the peer-reviewers.

Also, we have followed your recommendation for copyediting the paper to improve the style of written English.

We hope that the manuscript can be accepted for publication in The BMC Infectious Diseases Journal in its present version.

Yours sincerely,

PIO LOPEZ MD.
Point by point responses to peer-reviewers:

Reviewer 1: Oana Falup-Pecurariu

Comment/Question 1: Page 6, line 7: I would suggest to reformulate:

“AOM is one of the primary reasons for pediatricians to prescribe antibiotics”.

Answer 1: The text was updated

Revised Text:
Page 5:
AOM is one of the primary reasons for pediatricians to prescribe antibiotics

Comment/Question 2: Page nr.8: There may be interesting for the reader to know which were the antibiotics that the authors were supposed not to use.

Answer 2: There were no restrictions for antibiotic use following tympanocentesis. All subjects were treated according to local practice. The text was updated in the Methods section.

Revised Text:
Page 7:
There were no restrictions on antibiotic use following tympanocentesis. All children were treated according to local practices.

Comment/Question 3: The inclusion and exclusion criteria may be more clearly written and underlined.

Answer 3: Inclusion and exclusion criteria were re-written to be more clear

Revised Text:
Page 7:
The study included children visiting to the pediatrician with one of the general signs for AOM (otalgia/irritability, conjunctivitis, fever and either Paradise’s criteria (bulging, diffused or localized inflamed tympanic membranes) or spontaneous otorrhea (less than 24 hours). Patients identified for recruitment were either children with a new episode of AOM (less than 72 hours of onset) who had not yet received antibiotics for the episode (untreated group), or children who had a diagnosis of AOM within 48–72 hours prior to study enrolment and who received antibiotic therapy from a physician but remained symptomatic at the time of study entry (treatment failures). Children who received systemic antibiotic treatment for a disease other than AOM in the 72 hours prior to enrolment, and ones receiving antimicrobial prophylaxis for recurrent AOM, were excluded. Recurrent AOM was defined as ≥ 3 episodes in the past 6 months or ≥ 4 episodes in the past 12 months Children presenting (less than 24 hours) were included.
There were no restrictions on antibiotic use following tympanocentesis. All children were treated according to local practices.

**Comment/Question 4:** It would be very useful if the authors would clarify the following aspect: they defined 2 groups, one with no antibiotics and another with antibiotics (pag. 8 lines 8-11).

**Answer 4:** Following comment above, inclusion criteria were re-written indicating “Patients identified for recruitment were either children with a new episode of AOM (less than 72 hours of onset) who had not yet received antibiotics for the episode (untreated group), or children who had a diagnosis of AOM within 48–72 hours prior to study enrolment and who received antibiotic therapy from a physician but remained symptomatic at the time of study entry (treatment failures).”

**Comment/Question 5:** By the other hand, on pag.11 line, all 99 children included in the ATP analyses were untreated with antibiotics”.

**Answer 5:** The study intended to enroll not only untreated episodes but also treatment failures as defined in the inclusion criteria. Nonetheless, no treatment failures were included in the ATP cohort.

**Revised Text**

**Page 10:**
No treatment failures were included in the ATP cohort since none met the enrolment criteria.

**Comment/Question 6:** Page nr.9: What do you understand by careful sampling of spontaneous otorrhea?

**Answer 6:** Careful sampling was indicated for children who had spontaneous/accidental rupture of the tympanic membrane, removal and cleaning of the ear canal material was done, and deep aspiration of the MEF material through the perforation was attempted to minimize contamination and spurious results.

**Revised Text:**

**Page 8:** Samples of MEF for all children were collected by an ENT specialist by tympanocentesis or by careful sampling of spontaneous otorrhea “for children who had spontaneous/accidental rupture of the tympanic membrane. The latter entailed removing and cleaning the ear canal by deep aspiration of the MEF material through the perforation to minimize contamination and spurious results.

**Comment/Question 7:** It would be interesting to mention the serotypes that the authors found for the children that were fully vaccinated with PCV 7 and were positive cultured for *S. pneumoniae*. 
Answer 7: Following your recommendation this information was included under Results/Bacterial etiology & Serotype distribution of *H. influenzae* and *S. pneumoniae*. Page 13 and 14:

Revised Text:

Page 12:
The percentage of children who received at least one dose of heptavalent pneumococcal conjugate vaccine (PCV-7) was 30% (9/30) in children who were culture positive for *S. pneumoniae* (seven fully vaccinated and 2 partially vaccinated) and 35% who were culture positive for *H. influenzae* (11/31).

Page 12:
In fully vaccinated children, serotypes 19F (n=4), 19A (n=2) and 6A (n=1) were isolated. Serotypes 6A and 3 were isolated in the partially vaccinated children.

Comment/Question 8: Page nr.17 For conclusions it would be maybe better if the authors would stress their own findings for the population that they studied.

Answer 8: We have updated the manuscript with the serotype percentage coverage for all available formulations according to the results in this population, and then compare our results with other data in the region.

Revised Text:

Page 15/16:
From the reported pneumococcal serotypes in this study, 19F was the most frequently isolated pneumococcal serotype in this study, followed by 6A and 14. That four 19F cases were detected in fully vaccinated children appears consistent with the results of the PCV7 AOM efficacy trial conducted in Finland, where the lowest point estimate for all of the PCV7 serotypes was that of 19F, at 25% (95% CI -14 to 51) [21]. In this study, 3 and 19A together represented 13% of the pneumococcal AOM. Serotype 14 has previously been documented to be the most frequent in Colombia [20]. Similar to studies in Costa Rica [10,37] and Chile [11], 19F was the most frequently isolated pneumococcal serotype in this study, followed by 6A and 14. Global data shows the most common pneumococcal serotypes causing AOM are 3, 6A, 6B, 9V, 14, 19A, 19F and 23F [38] indicating that potential coverage for pneumococcal serotypes included in the available formulations ranged from 60-86% [37,39].

Reviewer 2: Robert Cohen

Comment/Question 1: The 5 last lines of the results section “Vaccine preventable AOM episodes » is not acceptable and to commercial and have to be deleted. There is no substantial evidence of the effectiveness of Haemophilus influenzae AOM by the PHIDCV.
**Answer 1:** We agree that the effectiveness of PHiD-CV against *H. influenzae* AOM has not been directly proven in a clinical efficacy trial. Similarly, we note that the efficacy of PCV-13 against AOM caused by the additional serotypes it contains beyond PCV-7—most notably serotype 3—remains to be demonstrated. Furthermore, even for those serotypes in PCV-7 where efficacy has been shown in each case, it has been less than 100%, though with herd protection the effectiveness may end up being substantially higher. The purpose of the original text and Figure 5 was to simply highlight the potential effectiveness against AOM of each of those vaccines whose formulations and target pathogens differ from one another. Nonetheless, we recognize that inadvertently we gave the impression that 100% effectiveness was proven for each of the formulations.

To avoid misinterpretation, we have removed Figure 5 and the accompanying text in the results section. As we believe highlighting the potential value of vaccine formulations targeted against multiple pneumococcal serotypes and/or *H. influenzae* is an important point, we now include the following text in the discussion section:

**Revised Text:**
Page 16:

The pneumococcal serotypes targeted by PCV-7 and PCV-13 comprise 47% (63% if 6A cross-protection is assumed) and 76%, respectively, of the pneumococci isolated from AOM samples in this study, and thus 14.1% (19.2% assuming 6A cross-protection) and 23.2% of all AOM cases sampled in this study [21,25]. The pneumococcal serotypes targeted by PHiD-CV also comprise 14.1% (19.2% assuming 6A cross-protection) of all AOM cases sampled; in addition, PHiD-CV also targets *H. influenzae* which represents an additional 27% of all AOM cases sampled. It is important to note, however, that neither clinical efficacy nor effectiveness data against AOM are available for either PHiD-CV or PCV-13, and so the magnitude of the clinical impact of each remains undetermined.