Reviewer's report

Title: Differences in nasopharyngeal cultures prior to and at onset of Acute Otitis Media and correlation with middle ear fluid cultures

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Reviewer: Thijs M.A. van van Dongen

Reviewer's report:

1. Research Question/Objective:
The research question/objective is clinically not very relevant. The first part of the question has been recently addressed in my systematic review and these results do not add much to the current evidence base. I am afraid I do not see how the second part of the question (predictive value of samples at 'healthy' visits) is of interest to clinical practice/research. What would be interesting however, is whether NP samples have predictive value for antibiotic resistance of microorganisms in the MEF, but this was not included in the objectives.

2. Methods:
P5, lines 5-6: ‘Microbiology data gathered from NW and NP are collectively represented as nasopharyngeal culture.’ Why not present them separately, or use only the true nasopharyngeal sample (because you have both for all children anyway)?

3. Methods:
P6, lines 18-27: I would describe these in the methods section instead of the results. Especially, since you do mention what the reference standard is (MEF) in the methods section. These are preferably next to each other.

4. Results:
I would like to see more information regarding missing data: 619 children were included, and according to protocol each child should have been sampled seven times (at 6, 9, 12, 15, 18, 24 and at 30-36 months of age). This would mean a total of 4,333 healthy visits, but the authors report on 2,071 visits. What happened to the other children/visits?

5. Results:
Table 1: I would like to see more data on the distribution of these visits (so: what proportion of these samples was taken at 6 months, 9 months, etc.), as we know that prevalence does change with increasing age (and as figure 1 shows). Also, what was the mean/median age of the children at the time of their AOM episode?

6. Results:
In table 1, the authors report that there was a large proportion of 'other bacteria' in the NP during healthy visits. Is it correct that these bacteria were no longer
present during the AOM visits? Staphylococcus aureus is being discovered more frequently in the NP of children with and without AOM since introduction of PCV. How large were the proportions of S. aureus during all visits?

7. Results:
Table 2 depicts only a minority of the total number of samples and I would prefer to see the total of 530 (or 529? (165+354)) used in the percentages calculations for the reader to be able to evaluate the actual proportions.

8. Results:
I would prefer to see all 2-by-2 results per microorganism in table 3. So numbers for: +/+ , +/-, -/+ and -/- . In this way, these data can be better used/extracted for future research/reviews.

9. Results/Discussion:
Next to the lack of relevance of these data, the numbers in table 4 are very small and do not benefit the article. The conclusions are quite straightforward/logical and in my opinion do not add anything to clinical practice/research. I would like to know what merit the authors see in these data?

10. Results:
The final part of the results section has much potential and shows important data! However, the authors present differences between NP and MEF, while I would like to see the agreement. There is not much (recent) data on predictive values of NP samples to predict antibiotic resistance of MEF pathogens. This is a question that could be of importance for clinical practice! Would the authors be willing to elaborate on this part of their manuscript?

11. Results/Statistics:
No confidence intervals are reported for the predictive values.
Table 1: Shouldn't the authors use a paired samples t-test instead of a Chi-square to compare the proportions np samples?

12. Discussion:
P9, lines 13-15: ‘We observed that Spn and NTHi NP colonization increased significantly with age but as children got older the relationship with detection in the NP with detection in MEF got weaker.’ Interesting point; where can I find these data?

* Are limitations of the work clearly stated?
A limitation worth mentioning would be the large proportion of missing data.

* Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
Yes

* Do the title and abstract accurately convey what has been found?
Yes

* Is the writing acceptable?
Yes

In conclusion:
Major Compulsory Revisions: 1, 2, 4, 5, 6, 9, 10, 11
Minor Essential Revisions:
Discretionary Revisions: 3, 7, 8, 12

**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