Author's response to reviews

Title: Correlation of nasopharyngeal cultures prior to and at onset of acute otitis media with middle ear fluid cultures

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Author's response to reviews: see over
**Reviewer's report**

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

**Version:** 1  
**Date:** 24 June 2014  
**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.

   **Ans:** We disagree that the objectives are not clinically relevant and that a systematic review pre-empts the value of our submission. The recent publication by van Dongen is excellent. However, two of the authors (JC and MP) have published several meta analyses and systematic reviews and we are therefore aware of the strengths and the limitations of those types of analyses, and many are noted in the paper by vanDongen. Our manuscript is novel because the data have been collected prospectively during the recent pneumococcal conjugate vaccine era time frame. Secondly, our paper addresses a very important question under debate – since it would be much easier and convenient to collect NP samples when children are healthy as opposed to being limited to when they have onset of AOM, would samples collected when healthy reflect samples at onset of AOM just as well? For the first time with this study we provide a resounding NO to that question. We do agree that adding the predictive value for antibiotic resistance enhances our paper and we appreciate the suggestion. We therefore have revised the manuscript to include the objective of assessing antibiotic resistance (now noted in the Introduction and results, see below answers to comments 9 and 10).

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)?

   **Ans:** We recently published a comparison of nasopharyngeal wash and swab samples in the J of Infectious Diseases (PMID 24864122) and showed that NW has better detection rate compared to swab. In that report we also showed that all the pathogens detected by NP swabs were also present in nasal wash in virtually all cases. We added a sentence in the methodology section on page 5, line 7-8 to make it clear and cited the reference.

   “The detection rate of otopathogens from NW is higher than NP samples as shown recently by our group [18].
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.

Ans: Agree with the reviewer. Line 18-27 has been moved in the method section under statistical analysis on page 5 lines 17-24.

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?

Ans: We have added in the Methods on page 4 line 16-21:

“This is an ongoing prospective study where not all the children have completed the planned visits. In addition, although we request collecting samples at 6, 9, 12, 15, 18, 24 and at 30-36 months of age, most parents don’t consent for all the seven collection visits, especially since a venipuncture occurs simultaneously with the NP samplings. There is no statistical definable pattern of missing data [16].”

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?

Ans: We have revised and added the information as follows on page 6 line 6-10:

‘The distribution of sample visits at 6, 9, 12, 15, 18, 24 and 30-36 months was as follows: 402 (19.4%), 388(18.7%), 366(17.7%), 297(14.3%), 292(14.1%), 244(11.8%) and 82(4%) respectively. A total of 530 AOM visits occurred in 309 of the children. The mean age of children at the time of AOM episode was 13.7 months and median age was 12.’

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?

Ans:

To make this clear, the following information has been added in the legend of table 1

“Other bacteria were only sought when canonical otopathogens (S. pneumoniae, NTHi and M. catarrhalis) were not detected in the visits. They may have been present along with main otopathogens but were not included in the calculations.”

Regarding Staphylococcus aureus, 189 (9.1%) were isolated during healthy visits and 34 (6.4%) were isolated during AOM visits. MEF cultures were positive for Staph aureus in 5 cases (0.9%). The following information has been added in the result section on page 7 line 1-2.

‘Staphylococcus aureus was also detected in 189 (9.1%) cases during healthy visits and 34 (6.4%) cases during AOM 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.

Ans: Agree with the reviewer. Percentages were also calculated with total # of cases and this is now included in table 2.

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.

Ans: Agree with the reviewer. Table 3 has been modified to show 2-by-2 results.

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?

Ans: We also sought to assess whether the presence of potential otopathogens in NP samples obtained at healthy visits shortly prior to onset of AOM might substitute for MEF samples, reasoning that they might be more predictive of the etiology of AOM than samples obtained at healthy visits at longer time intervals prior to onset of AOM. We agree with the reviewer that
numbers in table 4 are small. We have removed table 4 from the manuscript and replaced the data with text (page 7 lines 10-15).

“We analyzed the correlation between concordance of MEF culture results and NP cultures taken at healthy visits 4, 3, 2, and 1 week prior to AOM onset. NP cultures taken 1 week prior to onset of AOM were more frequently concordant with MEF cultures compared to NP cultures taken 2 weeks prior to onset of AOM. As the time interval between NP culture sampling and onset of AOM lengthened the concordance became significantly lower (p< 0.05).”

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?

Ans: Agree with the reviewer. We have now analyzed agreement of antibiotic resistance in the NP and MEF samples and included this hypothesis in the introduction (Page 3 line 29-30), and results in the manuscript (on page 8 line 23-30 and page 9 line 1-2 and page 9 line 14-18) as well as included the discussion section (on page 10 line 11-16).

Page 3 line 29-30.
“Can antibiotic susceptibility of otopathogens collected from the MEF be predicted by otopathogen isolates obtained from the NP at healthy or at onset of AOM visits?”

page 8 line 23-30 and page 9 line 1-2 and page 9 line 14-18

“Antibiotic Susceptibility of Middle Ear Fluid Isolates Compared to NP Isolates at Healthy visits
Oxacillin resistance of Spn in the MEF from AOM visits was not significantly different from Spn isolates at healthy visits. 42 (27.6%) of 152 Spn isolates from MEF at AOM visits were oxacillin resistant compared to 187 (28.5%) of 656 Spn isolates from NP at healthy visits. Comparison of Spn with MIC of ≥2 µg/ml to penicillin in MEF isolates and NP isolates at healthy visits showed 17.5% of 103 MEF isolates of Spn were penicillin-resistant compared to 6.7% of 509 NP isolates at healthy visits, which was significantly different (p=0.001). 73 (37.2%) of 196 NTHi isolates from MEF at onset of AOM were β-lactamase positive compared to 57 (22.5%) of the 253 NTHi NP isolates during healthy visits (p =0.001).”

“To calculate whether antibiotic resistance of MEF pathogens can be predicted from NP isolates obtained at onset of AOM, we compared oxacillin resistance in 127 paired NP and MEF Spn isolates and found the PPV to be very high at 95.3%. Similarly comparison of β-lactamase activity of 170 paired isolates of NTHi showed the predictive value at 96%.

page 10 line 11-16
“Can antibiotic susceptibility of otopathogens collected from the MEF be predicted by otopathogen isolates obtained from the NP at healthy or at onset of AOM visits? Our data show poor correlation in predicting the antibiotic resistance of microorganisms in the MEF compared to NP samples taken during healthy visits. In comparison we found antibiotic susceptibility of otopathogens collected from the MEF can be predicted by otopathogen isolates obtained from the NP at onset of AOM visits.”

11. Results/Statistics:
No confidence intervals are reported for the predictive values.

Ans: Agree with the reviewer, 95% confidence intervals for the predictive values have been included in table 3.

Table 1: Shouldn't the authors use a paired samples t-test instead of a Chi-square to compare the proportions np samples?

Ans: Table one contains different healthy visits and AOM visits. After consultation with a biostatistician we were advised that a paired sample t-test is not the appropriate choice, especially since the responses are binary and the pairing isn't balanced (not all subjects contribute to both visit types). To compare the proportions in NP samples we have used now a logistic regression model and included in the data in the methods section under statistical analysis and results section.

Page 5 line 24-29.

“Bacterial otopathogens between healthy vs. AOM visits were compared using logistic regression with bacterial presence as a binary outcome and visit type factor variable as predictor. A subject level random effect was included to model within-subject correlation. The function glmer() from the R package lme4 was used to calculate the model [19]. Estimates of the bacterial otopathogen presence rate and the visit group odds ratio were calculated directly from the model.”

Page 6 line 18-30.
Data show that NTHi is more prevalent at AOM visits (Odd Ratio = 2.72). Co-colonization with multiple otopathogens distribution among healthy vs. AOM visits are also shown in Table 1. The ratio of frequency of NTHi (AOM/healthy) is about 200% and this estimate does not depend on the co-pathogens. On the other hand, the ratio of frequency (AOM/healthy) of Mcat alone or Spn alone are about 50-60% (ie. less prevalent in AOM visits). For Spn-Mcat [no NTHi] the ratio of frequency (AOM/healthy) is about 100%, ie. equally prevalent. When Mcat or Spn is co-colonized with NTHi, there is an NTHi ratio of frequency of about 200%, indicating NTHi seems to dominate. Otopathogen patterns change during the transition to AOM. For example, a Mcat-alone colonization is more likely to co-colonize during the transition to AOM than NTHi-alone colonization would be. This would explain the under-representation of Mcat-alone colonizations among AOM visits, without having to assume that Mcat-alone has a smaller transition rate to AOM.
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?
Ans: Figure 1 on page 13 shows the distribution of *S. pneumoniae*, *NTHi* and *M. catarrhalis* bacteria at 6, 9, 12, 15, 18 and 24 months of age during healthy NP colonization and their presence in MEF during AOM. We draw conclusions from that 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

**Assessment of the manuscript “Differences in nasopharyngeal cultures prior to and at onset of acute otitis media and correlation with middle ear fluid cultures”**
1. Is the question posed by the authors well defined?
   - Yes
2. Are the methods appropriate and well described?
   - Methods used are appropriate from previous works on the same subject by the authors and in the present study
3. Are the data sound?
   - Yes
4. Does the manuscript adhere to the relevant standards for reporting and data
deposition?
- Yes
5. Are the discussion and conclusions well balanced and adequately supported by the data?
- Yes
6. Are limitations of the work clearly stated?
- Yes and well addressed by the authors
7. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
- Yes
8. Do the title and abstract accurately convey what has been found?
- Very well
9. Is the writing acceptable?
- Yes, Acceptable

Minor Revisions (editorial)

Abstract
Objective:
Author to rephrase the sentence in line 3 of the objective as “…as determined by middle ear fluid (MEF) cultures obtained by tympanocentesis”

Ans: Agree with the reviewer and all suggestions have been incorporated as suggested.

Methods:
Authors to recast the statement in line 1 of the methods as “During a 7 year prospective study of 619 children aged 6 – 30 months from June 2006 to August 2013, NP cultures were obtained at healthy visits…..”

Ans: The statement has been recast in the method section on page 4 line 24-26 under sampling as suggested by the reviewer.

“Nasal wash (NW) and nasopharyngeal swab (NP) samples were collected over 7 years (June 2006 to August 2013), prospectively from healthy children at 6, 9, 12, 15, 18, 24 and 30-36 months of age.”

Conclusion:
In the last line, “tympanocentesis” should be replaced with “MEF”

Ans: According to the reviewer’s suggestions last line has been modified as below:

We will continue to collect MEF at our otitis media research center for the coming years and collect NP cultures in order to provide results to the health care community for review and consideration in recommendations for AOM management.

Level of interest
The findings of this article are important to those with closely related research interests

Quality of written English
- Acceptable

**Statistical review**
- The manuscript does not need to be seen by a statistician.

**Declaration of competing interests**
- I declare that I have no competing interests

- **Accept without revision**

**signed**

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