Author's response to reviews

Title: Viral-bacterial co-infection in Australian Indigenous children with acute otitis media.

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Author's response to reviews: see over
Dear Editors at BMC Infectious Diseases,

We resubmit this original research article “Viral-bacterial co-infection in Australian Indigenous children with acute otitis media” after consideration then amendment relative to the reviewer’s comments. All minor and discretionary revisions were addressed and/or the appropriate changes made to the text. Refer to the dot points below each requested revision in the following section titled Reviewer’s report. NOTE: All meaningful text changes are identifiable as track changes.

Yours sincerely,

Michael Binks, PhD Scholar

Reviewer’s report

Title: Viral-bacterial co-infection in Australian Indigenous children with acute otitis media.

Version: 1 Date: 6 April 2011

Reviewer: Christopher Ashhurst-Smith

Reviewer’s report:

Major Compulsory Revisions: NIL

Minor Essential Revisions:
1. p.2 Bold Results and Methods headings are the wrong way round.
   - Have rearranged “Methods” and “Results” headings

2. p.2 in methods, line 4: change “at” for “in”
   - Changed “at” for “in” – see point 3 below for larger correction.

3. p.2 in methods, line 5: “normal diagnosis” needs to be re-worded (such as "children with no evidence of AOM").
   - Changed “Geometric mean bacterial loads were significantly higher in acute otitis media (AOM) compared with normal diagnosis.” to “Geometric mean bacterial loads were significantly higher in children with acute otitis media (AOM) compared to children without evidence of otitis media.”

4. p.3 Background para 2, line 2: consider adding “limited education”.
   - “limited formal education [6]” added with reference – note the shift of all subsequent reference numbers

5. p.4 para 2: change "less vaccines" to "fewer vaccines”.
   - Changed “less vaccinees” to “fewer vaccinees”
6. p.5 para 2: comma after "children".
   - Comma inserted after “children”.

7. p.5 para 2: add "to" before "seasonal".
   - “to” added before seasonal

8. p.6 top para, line 2: comma after "summary".
   - Comma inserted after “summary”

9. p.6 lower para: add ("QRT-PCR") after "reaction".
   - Inserted RTQ-PCR after “quantitative real time polymerase chain reaction”. This was the acronym used in previous related publications by our group and throughout the rest of this paper.

10. p.6 lower para: messy! HMPV not stated in full on previous pages.
    - Expanded HMPV before brackets

11. p.6 remove dash, add brackets for HKU1 to NL63, also brackets for "WU and KI".
    - Brackets have been used in place of the dash

Authors own amendments – Tidying up paragraph.

p.6 para 3 reworded slightly
   - The PCR target genes were: 16S rRNA for total bacterial load [27], autolysin (lytA) for S. pneumoniae load [28], protein D (hpd) for H. influenzae load [3], and the outer membrane protein (copB) for M. catarrhalis load [29].

   p.7 para 3 reworded slightly
   - “to compare the proportions of virus and bacteria positive swabs related to categorical ear state diagnosis.”
   - changed to
   - “to compare the proportions of virus and bacteria positive swabs in each of the four ear states.

12. p.8 middle para: replace or delete "worst", and improve "6% of normal".
   - “worst” removed
   - Normal is qualified in the methods – “ear state was defined as follows: (a) Normal: normal or minor pathology (abnormal appearance or retracted drum),”
   - Changed paragraph from
   - “HAdV was present in swabs from children with the following worst ear diagnoses: 6% of normal, 7% OME, 19% AOM and 22% AOMwiP (Figure 1).”
   - To
   - “The proportions of HAdV detected in the nasopharyngeal swabs for each ear diagnosis were: 6% for Normal, 7% for OME, 19% for AOM and 22% for AOMwiP.”
13. p.8 last para: replace "elevated" with "high". ("elevated" is linked to a normal range, which is difficult to define here).
   • “elevated” changed to “high”

14. last para, line 3: comma after "Unexpectedly"
   • comma inserted

15. p.8 last line: "Normal (N)" is not very clear. Readers might confuse "N" with n numbers.
   • (N) removed. Paragraph altered slightly (see track changes).
   • Referring to the normal ear state group as “Normal” throughout.

16. p.9 line 2: Sentence starting "the total..." not clear. The total bacterial load was apparently high in both groups, and what is the definition of high?
   • Changed “The total bacterial load was perpetually high, showing no trend for ear state.” to “The total bacterial loads were all greater than 10^7, showing no trend for ear state.”

17. p.9 para 2, lines 4/5: add "Table 2" and "Fig.3". Also, this sentence is not reader friendly. Suggest rewording.
   Reworded as follows
   • The combined load of M. catarrhalis, H. influenzae and S. pneumoniae was greater when examined by presence or absence of any study virus (p=0.035) (data not shown). Of the individual pathogens, significantly higher loads were only seen for H. influenzae co-infected with any study virus (Virus +), HPyV and HCoV (Table 2) (Figure 3).

18. p.9 Section 2: "zero load"; change to "...to be undetectable...".
   • OLD “The loads of M. catarrhalis and H. influenzae increased significantly with age when children less than 3 months were included (more likely to have zero load for these bacteria).” changed to
   • NEW “The loads of M. catarrhalis and H. influenzae were significantly lower in children less than 3 months of age. This was in part due to the greater proportion of children without detectable OM bacteria at this early age.”

19. p.10 middle para: comma after "Importantly".
   • Comma added

20. p.11 in discussion end para 1: change "elevated" for "high".
   • “elevated” changed to “high”

21. p.11 para 2, sentence 2: Needs rewording or delete "still".
   • Deleted “still”

22. p.12 para 2: replace "may" for "might".
   • Changed to “might”
23. p.12 para 3, sentence 2: reword "and any virus" - not reader friendly when next to 2 viruses
   - changed to “any study virus”

24. p.12 same para: change "elevated" for "high".
   - Changed to “high”

25. p.13 top para, line 4: change "may" for "might".
   - Changed to “might”

26. p.13 para 2, line 2: Delete "the".
   - Removed “the”

27. p.13 The last para should be the conclusions. The content of the conclusions submitted is word for word for the last paragraph of the discussion!
   - Was some kind of editing error - fixed

28. p.14 top para: delete "truly".
   - Replaced with “actually”

Discretionary Revisions.
1. The make up of the control group needs to be explained in more detail. The number in the control group is low (5%). Perhaps explanation that obtaining a large enough control group from the population under study is difficult due to the high incidence of AOM.
   - See addition to background paragraph 1 “At any one time few as 8% of remote Indigenous children aged 6 - 30 months have bilaterally normal middle ears [1].”
   - Also in the discussion “In this study the high bacterial carriage rates, high bacterial loads, and small number of children with normal ears (5% of swabs), reflect the large burden of respiratory infection in Indigenous children who live in remote communities."
   - The above two quotes from the paper provide the supportive background for the limited number of normal ears found in remote Indigenous children.

2. Suggest adding p values to the data given at end p.8/top p.9
   - P values added

3. p.9 Additional explanation of the bacterial load when viruses were present, with reference to the tables.
   - Made some minor changes – see track changes in document
4. The tables are rather cluttered and might be difficult for readers not familiar with statistical data. Consider reducing or better layout.
   - The tables will obviously condense down considerably for the pdf version to fit on one page. Table 2 is large but does provide a snapshot of the known variables and their effect on load which is important in the context of the paper. However if the journal requests, it may be possible to incorporate this information into the text or add this table as supplementary data.
   - Removed the Age (days) row from Table 1.

5. In background, consider adding that some viral infections also induce new receptors for bacteria (Post et al., El Almer et al.).
   - Minor addition made with regard to RSV and Moraxella.

6. Add to discussion: Differences in binding is associated with the strain of M. catarrhalis in experimental studies (El Ahmer, 2003).
   - Only used this in background. There was no major difference in the bacterial load of M. catarrhalis with specific respiratory viruses other than for by linear regression analysis for more than 1 virus.

7. Pathology is associated with host responses and not simply the number/load of bacteria present.
   - True. However at this point we are unable to determine or quantify individual or population influenced host responses. Our primary focus was just to characterise the whether viral infection was involved with acute pathology with or without an increase in bacterial load. There is ongoing work at Menzies School of Health to better understand genetic factors that may contribute to different immune responses to infection in Indigenous populations. Nutritional status, birth weight, maternal smoking status and a long list of other issues, all affect immune capacity and are beyond the scope of this publication.

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

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**
I declare that I have no competing interests.

**Addressing Reviewer’s report**

**Reviewer’s report**
This is an interesting report of “Viral-bacterial co-infection in Australian Indigenous children with acute otitis media”. I do not have comment for major compulsory revision but I do have several comments for minor essential revisions and discretionary revisions.

**Minor Essential Revisions**

In the second and third paragraph of abstract section, there is a subheading mistake of the Results and Methods subheadings.
- corrected

The author started the sentence with numbers in the second paragraph of the Abstract section (336 nasopharyngeal swabs…….), third paragraph of Results section (62% (228/366) of swabs amplified….). The sentence should start with word like “Three hundred and thirty six nasopharyngeal swab..”, or can edit like ” A total of 336 nasopharyngeal swabs….”.
- corrected

In Table 1. The authors showed the mean and median age in months and days of which both were significant. Thus showing one would be enough.
- Removed mean age in days from Table 2

In Results section, Bacterial load in the nasopharynx in relation to ear state subheading, the author stated “A dichotomous ranksum test revealed a significantly higher load at Normal (N) compared to AOM groups for *M. catarrhalis* (N= 5.42 x 104, AOM=2.19 x 106); *H. influenzae* (N= 1.33 x 105, AOM=1.09 x 106); *S. pneumoniae* (N=2.44 x 103, AOM=2.68 x 105).

However the data showed lower level in the normal compared to the AOM. The authors should correct this mistake.
- corrected

There is an improper reference format in Discussion section third paragraph “respiratory pathogen with studies showing occurrence of AOM in up to 50% of those children infected {Jacoby, 2007 #1964;Chonmaitree, 2008 #714;Alper, 2009 #1839;Moore, 2010 #877}”.
- Formatting fixed

There is a duplication of the last paragraph of discussion section and the conclusion section. The authors should delete the last paragraph of the discussion section.
- Removed duplication

**Discretionary Revisions**

A total of 366 nasopharyngeal samples were collected and tested from 114 Indigenous children. Therefore some samples were collected from the same child. If the sample from the same child is from different episodes it may be
reasonable to include both in the data analysis. However, if the samples were collected (with one month interval) before and after an antibiotic treatment of an episode, this will affect the bacterial load in the second sample. The authors should consider analyzing the data by excluding the result of samples after treatment of the same episode. The authors should discuss this in the discussion section.

- Antibiotics were used abundantly in this population. The reviewer makes a valid point with regard to episode, however we demonstrate that antibiotics had no effect on load (described page 10 para. 1 and discussed page 13 para. 2), even when considered by ear state (mentioned in results page 10, para. 1 and discussion on page 13, para. 3). As a result load comparisons throughout were not adjusted for antibiotic use.

In figure 2, the authors showed the significant difference between the normal and other groups. But if you look at the data carefully S.pneumoniae bacteria load for the positive samples in the normal group was relative high, quite similar to other groups (OME, AOM, AOMwiP). The S.pneumoniae bacteria load was significantly lower only because seven children in the normal group were negative for S.pneumoniae. The authors should discuss on this and its relation to antibiotic usage.

- The lower mean loads in the Normal group were indeed influenced by the number of negative RTQ-PCR results which was greater in the normal group. There was however NO influence of antibiotics on this finding. As shown in the regression table, age and viruses had the greatest effect on load.

The authors stated that “A panel of 17 respiratory viruses was screened by PCR in this study. Respiratory virus detection rate was 59% with Rhino 38%, polyomavirus 14%, adeno 13%, boca 8% and corona 4%. However other viruses like FLUA and B, PIV 1,2 and 3, HMPV, RSV and EV were only found in <9% collectively”.

Viruses associated with AOM may differ by region since different geographic background may have different prevalence of circulating virus associated with AOM or URI. It will be interesting to read discussion regarding this point in the paper.

- In the background we describe viruses commonly associated with OM in both Australia and overseas (page 4 para. 3). Our virus epidemiology was not dissimilar to that described. HRV and AdV, two of our most commonly encountered viruses, are further discussed with regards to disease in a broader context (page 12 para. 1, and para. 2). The major focus of this paper was to identify the impact of viruses on bacterial density and ear disease in this population. A broader take on the viral epidemiology deviates from the major aim of this work.