Sun et al have prepared a manuscript describing the relationship between bacterial carriage during early life and the development of otitis media in children living in Western Australia. Although their study is relevant to the advancement of knowledge about the contribution of nasopharyngeal colonization to the development of OM, it requires some important clarifications and re-organization before it should be considered eligible for publication.

Introduction

- Major Compulsory Revision: The third paragraph in the introduction is hard to follow: the authors should more carefully describe the comparison group from the study conducted in the Northern Territory of Australia: was it no carriage, later-onset carriage, or something else?

- Major Compulsory Revision: The third paragraph in the introduction fails to provide a clear rationale for the current study. The authors should build a stronger argument to support their analysis; although it seems as though they aim to repeat the study from reference 3 using a larger sample size, the authors have not sufficiently explained why the results from the aforementioned study are interesting, or need repeating. This seems to be a missed opportunity, especially since the authors spend so much time focusing on their ability to stratify on Aboriginal ancestry.

- Minor Essential Revision: The term “general population” in the second sentence of the Introduction should be clarified: does this mean the general population in the world, in Australia, in Western Australia, etc.?

Methods

- Major Compulsory Revision: It is unclear from the authors’ description where NPA samples were collected: in clinical practice or in the field (“follow-up field visits” are mentioned in a subsequent sentence)? If it is in the field, this needs to be explained more clearly. Although this may be clear in the published literature where this was first described, the methods for this paper should provide the reader with this basic information.

- Major Compulsory Revision: The authors state that “…an ear, nose, and throat (ENT) specialist assessed ear health…”, and then start talking about some sort of “diagnosis” being established using otoscopy, etc. There is a disconnect
between these sentences: in general, ear health is not the same as a diagnosis, and it is unclear what, exactly, is being diagnosed. The authors should clarify what type of clinical information was actually collected.

- Major Compulsory Revision: There are some fundamental problems with the description of how the data for this study were analyzed. The most important problem is the authors’ failure to explicitly state their comparison group for each analysis: the aim of the study was to evaluate the association between colonization – compared to no colonization – and the development of OM. The authors need to make this explicit throughout their paper. This is particularly confusing because the authors present analyses stratified by Aboriginal ancestry; it is unclear throughout the paper if the authors are comparing colonization to no colonization, Aboriginals to non-Aboriginals, or early colonization vs. later colonization.

- Major Compulsory Revision: The second fundamental problem under Data analysis has to do with how the authors dealt with confounding in general. First, it is unclear how the authors selected potential confounders to control for: did the selected confounders actually influence the observed OR in a meaningful way? Or were they selected based on some other criteria? Second, the phrase “All analyses were adjusted for age, age-squared, sex, and the following…” is inconsistent with what is presented in Table 3. The results presented in Table 3 include several different models that each controlled for different variables. The authors need to substantially improve their description of their analysis of confounders/model building.

- Major Compulsory Revision: The authors failed to provide sufficient justification why they did not control for PCV7 status. They state that previous analyses showed no difference in colonization rates among vaccinated and unvaccinated children, and provide a reference. It is unclear as to whether this association (no difference) actually applies to the current study population – is this, in fact, the same population? Regardless of whether it is the same population, the authors’ rationale would be substantially strengthened if they could state that they evaluated PCV7 as a potential confounder in their models, but that PCV7 did not influence the point estimates. This is the only way to assess for the presence of confounding in practice.

- Major Compulsory Revision: It is unclear what is meant by “crowding index”. The authors need to explain what this variable is, and how it was measured.

- Minor Essential Revision: What is the difference between presence of specific pathogens at any time during (a) the first 3 months of life and (b) at age 1-<3 months only? Does the first definition include 0-1 months? If so, the authors should just say so. Also, note that the placement of the “(a)” is in the wrong (grammatical) location in the manuscript.

- Minor Essential Revision: The authors begin several of the methods subheadings with statements that the methods from this study have been previously described. This is important information, but it is redundant to state this over and over for each subheading. The authors should use this statement only once.
- Minor Essential Revision: The authors spend a lot of time describing the details of tympanometry testing. In general, this description is very lengthy, and should be made more concise.

- Minor Essential Revision: What, exactly, is age-squared? And why (e.g.; what was the rationale) was it evaluated as a confounder? This term should be eliminated or qualified.

Results

- Major Compulsory Revision: It is unclear what is meant by “Table 1 shows the carriage of … in children who had a diagnosis of OM at least once or never”. This should be clarified: Table 1 shows … in children with and without an OM diagnosis during the study period, etc.

- Major Compulsory Revision: The paragraph beginning with “Table 3 shows the associations between early carriage…” should be completely re-written. First, the topic sentence of that paragraph should be summarized into something like: Table 3 shows crude and adjusted associations between early carriage of x, y, z, and OM. Second, the comparison group needs to be added to the description of the results. Third, it would be better to organize this paragraph by population (e.g.; stratified analyses: Aboriginal vs. non-Aboriginal) rather than by pathogen. Fourth, the authors state that there “was little change in odds ratios after adjusting for confounders” but fail to report crude OR (in the text or tables), and spend a lot of time describing specific results adjusted for different sets of confounders. The authors should add the crude ORs, and if they highlight the fact that the ORs were not substantially changed by adjustment, then they do not need to spend so much time describing these results in the text.

- Minor Essential Revision: The subheading Participants, specimens collected and diagnosis of OM is odd; it should be divided into more than one category: Study Population and Rates of Colonization.

- Minor Essential Revision: The description of the study population would be improved if the authors changed the sentence “In this sub-population of study participants…” to something more like: Among members of the study population who had NPAs and at least one subsequent clinical examination…”

- Minor Essential Revision: The authors state that “The carriage rates of individual pathogens were similar to those reported previously [7]”. The authors should save this type of comparison for the discussion section, except if reference 7 is describing a different study using the same study population, in which case, the authors should say this.

Discussion

- Major Compulsory Revision: In general, this section could be re-written to more carefully discuss the differences between Aboriginal and non-Aboriginal children. Although this is a major focus of the manuscript, the authors fail to provide a rationale as to why NTHi colonization appeared to increase the risk of OM in Aboriginal children, but not non-Aboriginal children; or why Mcat colonization appeared to increase the risk of OM in non-Aboriginal children, but not in
Aboriginal children. Without a plausible biologic rationale for this difference, it seems likely that these results are due to chance.

- Major Compulsory Revision: I do not follow the authors’ assertion that the lack of association between Pnc or Mcat and OM in Aboriginal children may be because Pnc and Mcat carriage preceded NTHi carriage. This needs more explanation.

- Major Compulsory Revision: The authors failed to provide a meaningful discussion of the limitations of their study. Although they acknowledge their small sample size, they fail to address the potential for bias or confounding, or the generalizability of their results.

- Major Compulsory Revision: The authors’ overall conclusion that “This study provides further evidence that early onset of bacterial carriage increases risk of OM…” is an over-simplification that does not accurately describe the results of this study: the results indicated that colonization with specific pathogens increased the risk of OM in different groups of children. The authors should change this conclusion to more accurately reflect their results.

- Major Compulsory Revision: The authors’ conclusion that “Early interventions to reduce the burden of OM are needed” is not relevant to the current paper, and reads more like a policy statement than a conclusion to describe the results of a scientific study. Nothing in the current paper suggests some type of early intervention, reduction of overcrowding, promotion of hand hygiene, avoidance of ETS, etc. listed in the conclusions will be effective in reducing OM burden. The authors should focus their conclusions on the prevention of NTHi or Mcat colonization, because they have some data indicating that this can lead to OM.

- Minor Essential Revision: The paragraph describing future studies seems unnecessarily long. This content may not be appropriate.

Tables
- Table 1
  o The title for Table 1 is not accurate; remove the word “Association”, and specify the study population (e.g.; participants who had NPAs and at least one subsequent clinical examination)

- Table 2
  o Discretionary Revision: Specify the study population in Table 2
  o Discretionary Revision: Use consistent language to describe exposure to ETS in the text and Table 2
  o Discretionary Revision: Add p-values describing the difference between the groups to Table 2
  o Discretionary Revision: It is unclear what is meant by the footnote; it is not possible to use any of the data presented in Table 2 to discern the number of missing observations, therefore, Table 2 is incomplete
  o Discretionary Revision: Table 2 appears is missing some description of age
- Table 3
  o Major Compulsory Revision: This table should be substantially altered to reflect the comments made in the text. In addition, the authors need to clarify the comparison group, clarify whether or not all associations are adjusted for age and sex and age-squared (again, no idea what this means), add crude ORs, consider re-organizing the table such that the stratification variable appears on the x-axis.
  
  o Major Compulsory Revision: It is particularly concerning that the information presented in this table – or the information presented in the preceding tables – cannot be used to calculate the crude OR in this study. At the very least, the number of Aboriginal and non-Aboriginal study participants in each category of exposure and outcome (e.g.: to build a 2x2 table) should be included in one of the tables such that the reader can calculate crude OR by hand. Exclusion of these results makes it appear as though the authors are hiding something.
  
  o Discretionary Revision: In general, this table (and the results presented in the text) has too many significant digits for the sample size. It is misleading to indicate this high level of precision.

Level of interest: An article of importance in its field

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

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests