Reviewer’s report

**Title:** Heavy Metal Exposure and Nasal Staphylococcus aureus Colonization: Analysis of the National Health and Nutrition Examination Survey (NHANES)

**Version:** 0 **Date:** 11 Oct 2017

**Reviewer:** Pete Kinross

**Reviewer's report:**

**Brief summary:**

This study investigates the association between blood concentration of Pb or Cd and nasal carriage of S. aureus that is either sensitive or resistant to meticillin, in the 2001-2004 subset of the US NHANES dataset, adjusting for various things including dietary factors, smoking and proxies of socio-economic status.

**Major comments:**

1. The conclusions and recommendations do not follow from the collected data nor from the stated aims of the project. The aims, methods and results examine the association of Pb and Cd exposure with nasal carriage of MRSA, whilst

   a. the conclusions state: "Results from our analysis of the association between Pb and both MRSA and MSSA suggest that current population exposure to Pb is associated with antibiotic resistance". There is no examination of association between detected blood Pb or Cd concentrations and the relative proportions of MRSA/MRSA carriage.

   b. the abstract contains one recommendation, i.e. "additional research to highlight heavy metal exposures as a source of antibiotic resistance is needed". This does not follow from the data as the study does not investigate of differences in the proportion of S. aureus that was resistant or non-susceptible to meticillin (plus oxacillin etc, depending on the case definition for MRSA). By contrast, the first paragraph of the discussion section is based on the collected data, and had sufficient information to form conclusions and recommendations.
2. Certain conclusions in the results/discussion section require additional (minor) statistical analysis. For example, rather than stating that there is a trend (i.e. a "dose response" on line 223), there should be statistical test for trend, e.g. between the quartiles in tables 4 and 5.

3. Some interpretation of statistical data is not accurate. For example, statistical tests should be used to assess whether an association is significant, rather than "the confidence level is no longer significant" (line 223).

4. The level of technical detail varies between sections. The methods and first paragraph of the discussion section contain tight scientific text, whilst other text is less technical. For example,
   a. in the abstract:
      i. it is not obvious that both MRSA carriage data and Pb and Cd data were available from the 'National Health and Nutritional Examination Survey', rather than an unspecified data source.
      ii. the sentence regarding 'blood Cd level' being 'significantly protective' lacks quantitative data for the reader to judge for themselves.
      iii. Is Q4 the highest (i.e. p76-p100)?
   b. In table 3, the term 'veggies' could be replaced, considering this journal's international readership.

5. Why was there no investigation of clinical infections with MRSA or MSSA, which are clearly the more important public health problem - if we assume that people captured in this database with nasal carriage are at higher risk of subsequent infection. On line 315, such investigation is recommended for future studies, but the limitations section does not state why such analysis was not included in this study, e.g. which data were (or were not!) available.

6. There could be clearer statement in the background regarding the reasons why it is simply bad to have too much Pb or Cd in the blood, other than an indirect link to carriage (rather than infection) with S. aureus. The discussion section could repeat this briefly.
Minor comments:

1. Tables 1-3 can be amalgamated.

2. It is fine that the data are from 2001-2004, but there should be a brief description of the other available data sources for 2014-2017, and perhaps, briefly, why they are insufficient for such a study.

3. Why is Race tabulated? It is unclear what the relevance is to the study. The background section does not state that the authors postulate any genetic basis a different propensity for S. aureus carriage - especially noting that the groupings are extremely non-specific, in terms of their geo-evolutionary history e.g. "white", "black", as compared to "Mexican". If these US data are a proxy for socioeconomic status, then surely other proxies for this can be added, in addition to education and income, e.g. State, etc. Relevantly, it appears that Race did not make it into the adjusted models, presumably due to insufficient evidence for association with nasal carriage of S. aureus.

4. The text in the background section regarding human immunity is interesting and generally important, but not directly related to the methods section, results section or the interpretation of the results in the conclusion (e.g. Th1 vs Th2 immune responses).

5. The conclusions regarding resistance of S. aureus to the toxic effects of Cd does not consider the intermediate factors relating to plasma concentrations of these metals and carriage, e.g. immunomodulation.

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