Reviewer’s report

Title: Macrolide resistance determinants in Streptococcus pneumoniae isolates from carriers in Central Greece, 2005-2009

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Reviewer: Jolene Bowers

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Major Compulsory Revisions
1. In general, the Discussion section lacks substance. The authors reiterate too many results without explaining or hypothesizing as to why they’re seeing these trends. Specifically, there is not even mention of why there is a shift to non-vaccine-type isolates. (Is it that protection from the vaccine is causing evolution of the S. pneumoniae population in the form of serotype replacement and/or serotype switching?)

Discretionary Revisions
1. In the Methods section, it might be a good idea to summarize the isolation procedure, even though it is “previously described”, because it is interesting that some children were colonized with more than one strain of S. pneumoniae. How was this determined? Is it a method that risks missing more strains within one child? As your trends and conclusions are based on this sample set, the sampling procedure is very important. Is it possible that the children were undersampled because only 1 or 2 isolates were taken from each child? Is it common for carriers to be carrying more than one strain?

2. This seems a valuable set of strains and some valuable data; there are not as many studies about S. pneumoniae carriage as there are disease-causing strains. It would be interesting to elaborate on the relationship between the trends seen in carried strains versus disease-causing strains. Are they the same trends? What might that mean? Or if they’re different, is the disease-causing population a subset of the carried strains?

Minor Essential Revisions
1. Macrolide should be capitalized in Abbreviated title.

2. In the Results section of the abstract, delete the word “were” before “gradually disappeared”.

3. Throughout the manuscript when writing about the macrolide resistance determinants erm(B), mef(E), and mef(A), the authors omit the use of the article “the”, for example “erm(B) gene dominated” rather than “The erm(B) gene dominated”. Either add “the” or take out the word “gene” to correct this.

4. Also throughout the manuscript, the authors misuse the wording to describe their isolates. In many places they are describing the serotype instead of the isolate. For example “macrolide-resistant PCV7 serotypes” and “non-PCV7
serotypes exhibiting macrolide resistance”. Serotypes are not macrolide-resistant, the isolates are.

5. Similarly, throughout the manuscript, the authors use “PCV7” to describe isolates. (In the abstract, in Figure 1, paragraph 7 of the Results section, etc) This is confusing as it could mean isolates of a serotype covered by PCV7 or isolates from children that have received the PCV7. Consider using terms like “isolates of vaccine type (VT)” and/or “non-vaccine type (NVT) serotypes”.

6. mef and erm are not and should be italicized in several places throughout the manuscript.

7. In paragraph 3 of the Introduction, the 2nd sentence should start with “The” as in “The genetic determinant…”

8. In paragraph 4 of the Introduction, it was impossible to make sense of “and are related with different characteristics of the strains harboring the respective genes.” What does this mean? Reword and clarify.

9. In paragraph 5 of the Introduction, “a” should come before “high prevalence”.

10. In paragraph 6 of the Introduction, “centers” should be “center”.

11. MIC should be defined once.

12. In the Methods section, paragraph 4 should read that isolates were tested “with” levofloxacin and ciprofloxacin, or tested “for levofloxacin susceptibility” not “tested to levofloxacin” or “tested to ciprofloxacin”.

13. In the Methods section, leave the “s” off the end of “primers” to read “primer pair”. Also in the same paragraph, it is unnecessary to use the word “respectively” in either place.

14. In the 2nd paragraph of the Results section, correct “frequency was” to read “frequencies were”.

15. In paragraph 3 of the Results section, it would be helpful for the reader if you point out which serotypes listed are vaccine types and which are non-vaccine types.

16. In paragraph 5 of the Results section, is it correct that the MIC50 and the MIC90 of the erm(B)-positive isolates both equaled 256ug/ml?

17. In paragraphs 7 and 8 of the Results section, the text seems to be describing trends illustrated in Figure 3 and the figure should be referenced. And in paragraphs 9 and 10, the text describes Figure 2 and should reference it. Also in paragraph 9, the text references Table 4 when there is no Table 4. Should this be Table 2?

18. Paragraph 12 should reference Table 3 not Table 2. In that same paragraph, observations jump from drug resistance back to serotypes (and data illustrated in Table 2). Consider putting these serotype observations with the rest of them (around paragraph 9?).

19. The meaning behind paragraph 13 is very unclear. What is the message here? And the last sentence does not make sense.

20. In paragraph 14, the isolates susceptible to levofloxacin and ciprofloxacin add
up to 264, but there were 265 macrolide-resistant isolates. If “All” were susceptible, where’s the last one?

21. In the Discussion, in the authors’ comparisons with other studies, more of a distinction should be drawn between carried versus disease-causing strains (for example in paragraph 1, the last sentence).

22. In the Discussion section, paragraph 4 and again in paragraph 7, the term “no more” is used when “no longer” reads better.

23. Paragraph 6 of the Discussion should read that the pattern “may be changing due to clonal spread of S. pneumoniae of certain serotypes.” The way it is written tells us that serotypes are clonally spreading. Serotypes don’t clonally spread, S. pneumoniae does.

24. In paragraph 8 of the Discussion, there is a misspelling of persistent that should be fixed. Also the first “the” should be taken out of the sentence “They represented the one fourth of the recovered…”

25. In paragraph 10, take the word “was” from before “gradually disappeared”.

26. In Table 1, first the title is missing a letter (enrollment). More importantly, the number of children with antibiotic use in the preceding 3 months is a proportion and percentage. Why does the denominator of the fraction not equal the number of enrolled children? Is the denominator the number of children that used antibiotics but not in the preceding 3 months? Please clarify.

27. In Figure 1, consider labeling the x-axis Percent of Typable Isolates. The graph was confusing at first because in the text where the authors describe the trends illustrated in Figure 1 (paragraph 2 of the Results) they’ve calculated numbers out of only the macrolide resistant population of isolates, where the graph shows numbers out of the entire set of typable isolates. Perhaps add these numbers to the text for clarification.

28. The legend of Figure 1 should read “percentage of attendees age-appropriately vaccinated with PCV7 at the time…”

29. Figures 2 and 3 might be easier to follow if the years were on the x-axis and the macrolide resistance determinants were color-coded.

**Level of interest:** An article of importance in its field

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

**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.