**Author's response to reviews**

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

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Editor-in-Chief
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Dear Sir,

We are herewith submitting a revised version of the manuscript entitled “Macrolide resistance determinants among Streptococcus pneumoniae isolates from carriers in Central Greece”.

We respond to the valuable suggestions of the reviewers.

Sincerely yours,

George A. Syrogiannopoulos, MD
Professor of Pediatrics
Reviewer 1:

Major Compulsory Revisions

1. The Discussion has been revised and shortened. We have tried to reduce the reiteration of the results. In some points we have tried to provide explanations or hypotheses. The issue of serotype replacement has been discussed.

Discretionary Revisions

1. The requested information on the isolation procedure has been added in paragraph 4 of the Methods. In the literature, it has been the standard procedure for the evaluation of nasopharyngeal (NP) pneumococcal carriage. We have been using it since 1995 and it is the one accepted by WHO (O’Brien et al. PIDJ 2003). The children can be colonized with more than one strain. Some cases of co-colonization may be missed, but it has not been considered a major problem (O’Brien et al. PIDJ 2003).

2. We do not have an appropriate collection of isolates recovered from children with invasive or non-invasive type of disease. There is limited recent information on this issue from Greece. According to the literature, the profile of the colonizing isolates is relatively close to that of pneumococci causing non-invasive disease, mainly acute otitis media (AOM) and possibly, although difficult to confirm, non-bacteremic community-acquired pneumonia (CAP). It is based on the comparison of NP isolates and those from AOM. In addition, the pathogenesis of all the types of non-invasive disease is through microaspirations of pneumococci from the NP niche. More differences on antibiotic susceptibility and serotype distribution exist between NP isolates and those causing invasive disease. The most important issue appears to be colonizing and non-invasive disease-causing isolates versus invasive disease-causing isolates. This is the so called “invasiveness” of different serotypes or clones. Actually, in several papers the authors place the nasal and NP isolates in the group of non-invasive isolates together with those obtained from the lower respiratory tract, ear and eye.

Minor Essential Revisions

1. Macrolide has been capitalized in Abbreviated title.

2. In the Results section of the abstract, the word “were” before “gradually” has been deleted.

3. Macrolide resistance determinants: The article “the” has been corrected according to the suggestion.

4. “macrolide-resistant isolates belonging to PCV7 serotypes” or “isolates of PCV7 serotypes” has been used throughout the manuscript.
5. As it is stated in item #4.

6. *mef* and *erm* have been italicized.

7. In paragraph 2 (revised manuscript) of the Introduction, the 3rd sentence starts with “The genetic determinant…”

8. It has been removed.

9. In paragraph 4 (revised manuscript) of the Introduction, “a” comes before “high prevalence”.

10. In paragraph 4 (revised manuscript) of the Introduction, “center” has been used.

11. MIC has been defined once.

12. In paragraph 5 of the Methods section, isolates were tested “with” levofloxacin and ciprofloxacin.

13. In the Methods section, the “s” has been left off the end of “primers” and it reads “primer pair”. Also in the same paragraph, the word “respectively” has been deleted.

14. In the 2nd paragraph of the Results section, “frequencies were” has been used.

15. In paragraph 4 (revised manuscript) of the Results section: PCV7 serotypes have been listed.

16. Paragraph 6 (revised manuscript) of the Results section: Yes, it is correct that the MIC50 and the MIC90 of the *erm*(B)-positive isolates both equaled 256 µg/ml.

17. In paragraph 8 (revised manuscript) of the Results section, Figure 3 has been referenced. Paragraph 9 (revised) of the Results section: Figure 3 has been referenced. Paragraph 10 (revised) of the Results section: Table 2 and Figure 2 have been referenced. Paragraph 11 (revised) of the Results section: Figure 2 has been referenced.

18. Paragraph 13 (revised) of the Results section: Table 3 has been referenced.

19. Paragraph 14 (revised manuscript) of the Results section: Only the information on isolates with an MIC to penicillin of 4 µg/ml has been kept and the wording has been changed.

20. Paragraph 15 (revised manuscript) of the Results section: The tested macrolide-resistant isolates were 265. The wording has been changed.

21. A distinction has been made, although, as discussed (stated) above in item Discretionary Revision #2, the profile of colonizing isolates is relatively similar to that of the isolates causing non-invasive disease.
22. Paragraph 5 (revised) of the Discussion section: “no longer” has been used, when this expression has been retained in the revised manuscript.

23. Paragraph 4 (revised) of the Discussion section: “may be changing due to clonal spread of S. pneumoniae of certain serotypes” has been used.

24. Paragraph 5 (revised) of the Discussion section: It has been removed as the whole paragraph has been extensively revised.

25. In paragraph 7 (revised) of the Discussion section: “gradually disappeared” has been corrected.

26. In Table 1, it has been corrected according to the suggestion. The denominator indicates the number of children with available information on recent antibiotic use. It has been clarified as a footnote.

27. In Figure 1, the Y-axis has been labeled “Percent of typeable isolates”. The corresponding numbers out of the entire set of typeable isolates as well as these out of only the macrolide resistant population of isolates have been added to the text. They have incorporated into two different paragraphs, 2 and 3, in order to be easier to follow.

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

29. In Figures 2 and 3 the years are on the x-axis and the macrolide resistance determinants have been color-coded.

Reviewer 2:

1. EUCAST breakpoints have been applied and results are presented in Table 3.

2 and 3. We were not able to test the susceptibility to additional agents such as telithromycin and moxifloxacin.

3. In the last paragraph of the Results section, the wording has been changed and we report only the MICs to ciprofloxacin without classifying the isolates as susceptible.

4. We have reduced the length of the introduction and modified the Discussion.