Author’s response to reviews
Title: Implication of Species Change of Nontuberculous Mycobacteria During or After Treatment

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Author’s response to reviews:
Dear Dr. Janice Leung
Thank you for giving us an opportunity to improve our manuscript. We are greatly indebted to the reviewers for their time and effort. We have revised the manuscript based on the reviewers’ comments, which we believe has strengthened the paper. We have summarized the changes to the paper below and these changes are underlined in the resubmitted manuscript.

Reviewer reports:
(Reviewer 1): This is interesting case series report regarding species changes of nontuberculous mycobacteria during or after treatment. Even though it is retrospective evaluation on a small number cases, it contains important massages to readers.

#1 P6 L17
Is that right? Did you use 3% Ogawa medium, not 2% or 1%. Please describe the reason for that and the possible effect made by these differences.

Response: The reviewer raised an important issue. Generally, 2% Ogawa media is used to shorten the incubation time as well as to increase the sensitivity of culture. However, in our
institution, 3% Ogawa media has been used to minimize the possibility of contamination considering the TB burden in South Korea. We have clarified this in the text (Line 15, page 6).

Do you think point mutation at positions 2058 or 2059 of the rrl gene is dominant mechanisms responsible for acquired resistance against CAM in MAA? While the citation #20 is invaluable they did not evaluate erm 41 in their study. It seems that many readers think acquired resistance against CAM would be mainly due to erm41-related mechanism. Please make sure this point by referring to recent reports on this topic.

Response: We understand the reviewer’s point. As the reviewer indicated, the inducible resistance based on the T28 sequevar of the erm (41) gene could be regarded as a kind of acquired resistance. Anyway, the sentence in question has been omitted in response to one of the 2nd reviewer’s comments. As suggested, we updated the references on the mechanism of resistance to clarithromycin (reference 19).

In connection with the description of P 11 L219=225, how many patients out of 7 patients who showed changes from MAC to MAA was during treatment? In other word, how many patients out of 7 patients who showed changes from MAC to MAA was after treatment?

Response: Four out of seven patients experienced changes from MAC to MAA during treatment and the other three patients experienced the same changes after treatment. Meanwhile, changes in the NTM species occurred during treatment in all five patients who exhibited changes from MAM to MAC. We have clarified this in the results section (Lines 5–9, page 8).

(Reviewer 2):
I reviewed a manuscript written by Lee et al. entitled "Implication of Species Change of NTM During and After Treatment. I found a few interesting findings in this manuscript, but there are several concerns I would like to ask the authors before published in the international journal.

Major Comment.
Although the authors define the "Newly isolated NTM species" as "disappearance of initially isolated NTM species and isolation of new species at least 2 times". This definition must have excluded the important cases such as who had positive culture at the same time during the treatment, and who had positive culture before the treatment initiation. Those important data
were needed to be explained. At least, they should indicate the number of the cases who had one isolation and clarify the clinical significance of it.

Response: The reviewer has raised an important issue. Among 164 patients with NTM lung disease, there were 54 patients (39 during treatment and 15 within 2 years of treatment completion) for which another species of NTM was isolated. Among these 54 patients, 16 satisfied the definition of a “change of NTM species”. We have clarified this in the text (Lines 18–21, page 7). Unfortunately, we do not have clinical data on the progression of NTM lung disease among patients for which another NTM species was isolated on one occasion.

Although the authors stressed that the proportion of the functional erm gene was high in this cohort, the readers should want to know the reason that the proportion of C28 sequevar was higher in the previous study they conducted than other reports.

Response: Thank you for raising an important point. The much higher proportion of MAA with inducible resistance to clarithromycin (T28 sequevar) could be explained by the hypothesis that MAA with the C28 sequevar might be eradicated by previous treatment with a regimen including a macrolide. We have clarified this in the text (Lines 20–22, page 10).

Minor comment.

L120: 4% sodium hydroxide (NAOH) seemed to be a higher concentration comparing to the other report. The method would have influenced on the clinical significance of the isolation.

Response: Because 4% NaOH solution is mixed with the same amount of sample, the final concentration of NaOH is 2%. We have clarified this in the text (Line 12, page 6).

The authors need to discuss more about the cases who had new MAC isolates during M. massiliense treatment. Why only one case developed CAM resistant?. Similarly, they need to discuss the acquired resistant in the case with M. abscesses. Most of the cases did not have acquired resistance, right? I think those are important findings.

Response: The reviewer raised an important point. We are convinced that the combinatorial use of macrolides, amikacin, and cefoxitin (or imipenem) minimized the possibility of the emergence of resistance. In fact, one patient in whom NTM lung disease recurred with clarithromycin-resistant MAC received azithromycin only while refusing IV therapy. We have clarified this in the text (Lines 9–10, page 9).