Author's response to reviews

Title: The influence of environmental exposure on the response to antimicrobial treatment in pulmonary Mycobacterial avium complex disease

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Version: 2 Date: 1 August 2014

Author's response to reviews: see over
August 1, 2014

Philippa Harris

Executive editor, BMC Infectious Diseases

Dear Professor Harris,

We heartily appreciate the opportunity to revise our manuscript, “The influence of environmental exposure on the response to antimicrobial treatment in pulmonary Mycobacterial avium complex disease.” Please find attached our revised manuscript, as well as a point-by-point response to the reviewers’ comments. The research protocol was approved by the institutional review board of Kyoto University. We believe that the changes we have made to the manuscript have satisfactorily addressed the comments raised by the reviewers. We hope that you will kindly consider the revised version of our manuscript for publication in BMC Infectious Diseases.

Sincerely,

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Editor’s comments:

(1) Did you analyze your data with soil exposure as a continuous variable (i.e., total hours per week)

As the Editor suggested, we analyzed our data with soil exposure as a continuous variable. The data were similarly significant with the categorized variable (≥ 2 hours/week or < 2 hours/week)

Characteristics of the patients with pulmonary *Mycobacterium avium* complex disease with or without sputum conversion

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with sputum conversion (n=52)</th>
<th>Patients without sputum conversion (n=20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure time to soil before treatment</td>
<td>1.7±4.2</td>
<td>3.3±9.3</td>
<td>0.73</td>
</tr>
<tr>
<td>Exposure time to soil during treatment</td>
<td>1.2±4.0</td>
<td>3.1±9.3</td>
<td>0.59</td>
</tr>
<tr>
<td>Exposure time to soil during observation</td>
<td>1.9±4.2</td>
<td>3.3±9.3</td>
<td>0.74</td>
</tr>
</tbody>
</table>

The data show the means ± standard deviations of total hours per week.

Characteristics of the patients with pulmonary *Mycobacterium avium* complex disease with or without a relapse

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients without relapse (n=37)</th>
<th>Patients with relapse (n=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure time to soil before treatment</td>
<td>0.9±1.7</td>
<td>3.8±7.1</td>
<td>0.03</td>
</tr>
<tr>
<td>Exposure time to soil after the start of treatment</td>
<td>0.5±1.2</td>
<td>3.5±7.0</td>
<td>0.001</td>
</tr>
<tr>
<td>Exposure time to soil during observation</td>
<td>1.9±1.7</td>
<td>4.3±7.0</td>
<td>0.004</td>
</tr>
</tbody>
</table>

The data show the means ± standard deviations of total hours per week.

Characteristics of the patients with pulmonary *Mycobacterium avium* complex disease with or without treatment success

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with treatment success (n=37)</th>
<th>Patients with treatment failure (n=35)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Environmental exposure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposure time to soil before treatment</td>
<td>0.9±1.7</td>
<td>3.5±8.3</td>
<td>0.07</td>
</tr>
</tbody>
</table>
Exposure time to soil after the start of treatment 0.5±1.2 3.3±8.3 0.02
Exposure time to soil during observation 0.9±1.7 3.7±8.3 0.06

The data show the means ± standard deviations of total hours per week.

Factors associated with treatment success in the patients with pulmonary *Mycobacterium avium* complex disease

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Exposure time to soil</td>
<td>0.64 (0.40-0.92)</td>
<td>0.003</td>
</tr>
<tr>
<td>Positive smear</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Negative smear</td>
<td>2.86 (1.09-7.87)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

The exposure time to soil (total hours per week) was analyzed as a continuous variable.

(2) how did you decide to dichotomize the soil exposure valuable at the two hours? Would you results be different if you had used three or four hours?

According to our previous study, the soil exposure value at two hours was the best point to discriminate between the patients with pulmonary MAC disease from those without pulmonary MAC disease (Ref 19. Fujita K, et al. *Clin Microbiol Infect.* 2013, 19:537-541). As the Editor suggested, we analyzed different times as follows. The two hour variable was also best in this study.

Characteristics of the patients with pulmonary *Mycobacterium avium* complex disease with or without a relapse

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients without relapse</th>
<th>Patients with relapse</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low soil exposure after the start of treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 hour per week</td>
<td>27 (73.0)</td>
<td>4 (26.7)</td>
<td>0.004</td>
</tr>
<tr>
<td>&lt; 2 hour per week</td>
<td>35 (94.6)</td>
<td>7 (46.7)</td>
<td>0.0003</td>
</tr>
<tr>
<td>&lt; 3 hour per week</td>
<td>36 (97.3)</td>
<td>9 (60.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>&lt; 4 hour per week</td>
<td>36 (97.3)</td>
<td>10 (66.7)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

The data show the numbers (%) of patients.
without treatment success

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients with treatment success (n=37)</th>
<th>Patients with treatment failure (n=35)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low soil exposure after the start of treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 hour per week</td>
<td>27 (73.0)</td>
<td>16 (45.7)</td>
<td>0.02</td>
</tr>
<tr>
<td>&lt; 2 hour per week</td>
<td>35 (94.6)</td>
<td>15 (57.1)</td>
<td>0.0002</td>
</tr>
<tr>
<td>&lt; 3 hour per week</td>
<td>36 (97.3)</td>
<td>24 (68.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>&lt; 4 hour per week</td>
<td>36 (97.3)</td>
<td>27 (77.1)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

The data show the numbers (%) of patients.

(3) re: your finding of low soil exposure during therapy and its association with the re-infection, did you control for the variable of “soil exposure to treatment” in your multivariate model?

Because the factor associated with an absence of relapse was low soil exposure alone (Table 3), we calculated the relative risk in a univariate analysis (OR 0.05, 95% CI 0.007-0.25, P=0.0001) (page 11, lines 163-167).

(4) what kinds of soil exposures did people say “yes”, to?, The list of examples should be fully detailed.

We questioned all the participants about their experiences with farming or gardening or with any activities involving soil exposure, such as digging or carrying soils, turning soil with a spade, mowing grass, planting flowers, and exposure to soil dusts (pages 7-8, lines 98-101).

(5) is there any environmental data from your region where soil has been examined for presence of M. avium? Can you cite or discuss this data even if not published?
We previously reported the isolation of *M. avium* and *M. intracellulare* from approximately one-half of the patients’ residential soils (Ref 19, Fujita K, et al. *Clin Microbiol Infect.* 2013, 19:537-541) and described it in the Background section. We reported that the patients with pulmonary MAC disease have significantly more soil exposure than the non-infected control patients after adjustments for host traits [15] and that approximately one-half of the patients’ residential soil contained MAC strains. The clinical and corresponding soil isolates with identical genotypes were identified among the patients with a high soil exposure [16] (page 6, lines 69-73).

(6) what was the total length of follow up time for the cohort? Did it differ between patients and did you control for follow-up time in your multivariate analysis?

We analyzed the total length of follow-up time. It did not differ between the patients (Table 1, 3 and 4 in the revised version) and was not included in the multivariate analysis.
Reviewer's report
Title: Influence of environmental exposure on response to antimicrobial treatment in pulmonary Mycobacterial avium complex disease
Version: 1 Date: 31 March 2014
Reviewer: Julie Philley
Reviewer's report:
This is a relevant article related to environmental exposure and antimicrobial treatment. I feel it adds some additional perspective to available environmental data related to NTM and appreciated the work of the authors. It is well written. I have no major revisions to suggest.

Minor revisions
1. Page 5, lines 58. better wording may read "A prospective, randomized, controlled study revealed a better microbiological response"

We rewrote this passage to include "a better microbiological response" (page 5, line 60).

2. It would be helpful to further understand high vs low soil exposure. (Ex. number of farmers vs gardeners? what type of soil used and what activities? rural vs suburban patients).

We described that twenty-five patients experienced high soil exposure during the observation. One patient was working as a farmer, and the remaining 24 patients were gardeners (page 10, lines 148-150).

3. It may be helpful for the authors to comment on species identification (M. avium vs M. Intracellulare) and the clinical significance (or lack thereof) related to environmental exposure and treatment response.

The relationship of environmental exposure with treatment response was similar in M. avium disease and in M. intracellulare disease. A statistical significance was observed in M. avium disease alone but not in M. intracellulare disease due to a small number of patients. We showed these results in the text (pages 14-15, lines 212-228).

**Microbiological outcomes and their related factors in M. avium disease**
Among the 56 patients with *M. avium* disease, 39 patients (69.6%) converted to negative sputum cultures, and 13 (33.3%) of these patients relapsed. Treatment was successful for 26 (46.4%) patients. No factors were associated with sputum conversion. More patients without a relapse had low soil exposure after the start of treatment than the patients with a relapse (24/26, 92.3% vs. 6/13, 46.2%, P=0.003). More patients with treatment success had low soil exposure after the start of treatment than did the patients without treatment success (24/26, 92.3% vs. 17/30, 56.7%, P=0.003).

**Microbiological outcomes and their related factors in *M. intracellulare* disease**

Among the 15 patients with *M. avium* disease, 12 patients (80.0%) converted to negative sputum cultures, and 2 (16.7%) of these patients relapsed. Treatment was successful for 10 (66.7%) patients. No factors were associated with sputum conversion, relapse or treatment success. More patients without a relapse had low soil exposure after the start of treatment than did the patients with a relapse (10/10, 100% vs. 1/2, 50.0%, P=0.17). More patients with treatment success had low soil exposure after the start of treatment than the patients without treatment success (10/10, 100% vs. 3/5, 60.0%, P=0.10).

4. Did the authors only suggest complete avoidance of soil exposure to patients in 2010? (Did they use masks?, etc)

We suggested the avoidance of soil exposure and the use of masks when the patients were exposed to soil (page 17 lines 268-269).

5. The authors did comment on the major limitation of not analyzing genotypes from relapsed patients. Clinically, the fingerprinting of isolates is essential information regarding soil exposure.

We analyzed the genotypes of the MAC strains stored from 6 of the 15 relapsed patients using variable numbers of tandem repeats. Of the four patients with a polyclonal infection, one had a high soil exposure. Two patients with a monoclonal infection had high soil exposure. One patient with *M. intracellulare* disease relapsed with *M. avium* disease and had high soil exposure. All the patients relapsed after the 12 months of treatment. We could not analyze the genotypes in the remaining 8 patients. As described, this is a major limitation in our study (page 17, lines 274-276).
Drs. Yutaka et al. seek to better understand if environmental exposure directly affects the antimicrobial response in MAI pulmonary disease in non immune compromised patients. They recruited 72 HIV negative patients with MAC and these individuals underwent a standard survey about risk factors for MAC. They were provided standard 3 drug therapy with clarithromycin, rif and EMB. We are not told, but assume medical adherence to this regimen.

Although the patients were recruited prospectively, this study is essentially a retrospective case control study, looking at patients that improved on therapy and the characteristics that were noted in each group.

1. The question is defined – they hypothesize that environmental exposures lead to more MAC (they have shown this before) and will influence antimicrobial treatments and response.

In accordance with Dr. Nyendak’s comment, we described the hypothesis in the Background section. We hypothesized that environmental exposures would lead to more MAC infection and influence the responses to antimicrobial treatments and relapse (page 6, lines 77-79).

2. The methods are ok, but there is no information on whether or not all of the cultures were macrolide susceptible. This could be a major confounder for relapse.

We did not routinely perform a susceptibility test for clarithromycin. We stored 33 strains isolated before treatment and all 15 strains isolated from each of the 15 relapsed patients. All of the 33 strains and the 15 strains were susceptible to clarithromycin. Therefore, macrolide susceptibility is unlikely to be a confounder for relapse (page 10, lines 144-145).

I found the terminology confusing: sputum conversion to me sounds like going from afb smear positive to smear negative – here the authors use sputum conversion to mean negative culture.
According to previous studies (Ref. 5, 7, 9, 11, 19), we defined sputum conversion as negative culture but not negative smear.

Sputum relapse is also confusing; they state two consecutive positive cultures are sputum relapse, but I don’t know the time interval here – is this within the 12 months of planned treatment?

Two patients relapsed within the 12 months of treatment, and 13 patients relapsed after the 12 months of treatment (page 9, lines 143-144).

Treatment success also needs a time interval – how long were patients followed to indicate treatment success.

The patients completed treatment for more than 12 months and were followed for at least 2 years to define treatment success (page 7, lines 85-89).

The mean durations of follow-up in the patients with treatment success and failure were not different (53.8 months vs. 53.8 months, Table 4 in the revised version).

Another concern is that not all patients were tested for HIV – using ‘no obvious risk factors for HIV infection’ in my opinion not ideal as this has been shown to miss patients with HIV.

As Dr. Nyendak noted, we did not test for HIV infection in all of the patients and may have missed patients with HIV infection. All HIV patients must be reported to the government based on the law of infectious diseases in Japan. In 2013, 845 males and 25 females in all and 135 males and 5 females over 50 years old were newly diagnosed and reported as having HIV infection in Japan. In this study, 54 of 72 patients (75.0%) were females and 17 of 18 male patients were over 50 years old. Therefore, HIV infection is rare in the same population included in our study. Furthermore, our patients did not have other indicator diseases of AIDS. Therefore, it was unlikely that these patients had HIV infection.
The statistics section needs to be clearer. My understanding is that the outcome variable was relapse or not/ or treatment success or not? This would be a binary outcome and therefore logistic regression would be used. Need to clarify this in the methods although I do appreciate that this is mentioned in the results. Also need to clarify what is the reference in logistic regression – in table 2. That is, negative smear (no) is the reference? The text does clarify, but the table is not stand alone without referring to the text.

In accordance with Dr. Nyendak’s comments, we revised the statistical section and Table 2 and 5 as follows (page 9, lines 123-133):

Statistical analysis

JMP version 10.0 (SAS Institute, Cary, NC, USA) was used for all of the statistical analyses. We divided the patients into two groups; patients with sputum conversion and patients without sputum conversion; patients without relapse and patients with relapse; and patients with treatment success and patients without treatment success. The group comparisons were made using the chi-square test, Fisher’s exact test, and the Wilcoxon test. The comparisons of more than two groups were performed using an analysis of variance. Variables were included in the multiple logistic regression analyses if the probability values were < 0.05 according to the univariate analysis. Odds ratios (ORs) and their respective 95% confidence intervals (CIs) were computed as estimates of relative risk. A p-value of < 0.05 was considered statistically significant.

Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Positive smear</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Negative smear</td>
<td>4.58 (1.57-14.41)</td>
<td>0.005</td>
</tr>
<tr>
<td>Large cavity (≥2 cm)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Cavity (none or &lt; 2 cm)</td>
<td>4.29 (1.30-14.68)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 5

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>High soil exposure</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Low soil exposure</td>
<td>13.13 (3.27-88.87)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Positive smear</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>Negative smear</td>
<td>2.86 (1.09-7.87)</td>
<td>0.03</td>
</tr>
</tbody>
</table>
3. Is the data sound? I am unsure. We don’t have any data that tells us if patients who relapsed had macrolide resistant disease. This needs to be clarified.

We performed a susceptibility test for clarithromycin of the MAC strains stored from the 15 relapsed patients. All 15 strains isolated from each of the 15 relapsed patients were susceptible to clarithromycin (page 10, lines 144-145).

Table 1 tells us that 20 patients did not have sputum conversion. In their regression analysis, a lower burden seemed to fall out (negative smear) more likely to lead to negative cultures which makes sense.

Table 3 is confusing: there are 37 patients without relapse and 15 with. I thought there were 72 people in the study so the authors need to clarify where the other 20 patients are or why they are not in this table.

We divided 52 patients with sputum conversion into 37 patients without a relapse and 15 patients with a relapse. We evaluated the factors for a relapse among these 52 patients in Table 3. Treatment failed for 35 patients consisting of 20 patients without sputum conversion and 15 relapsed patients. The remaining 37 patients who achieved sputum conversion without a relapse were included in the treatment success group. We compared these 35 and 37 patients to evaluate the factors for treatment success in Table 4 and 5.

Again, knowing if culture/susceptibility was done will be important as a major confounder will be macrolide resistant disease.

We did not routinely perform a susceptibility test for clarithromycin. We stored 33 strains isolated before treatment and all 15 strains isolated after relapse. All of the 33 strains and the 15 strains were susceptible to clarithromycin. Therefore, macrolide susceptibility is unlikely to be a confounder for relapse (page 10, lines 144-145).
I also would be interested to know more about why we are looking both at relapse as an outcome and then later at treatment success as an outcome? This seems duplicative since the authors state that treatment success is no relapse.

Sputum conversion is considered to be associated with a patient’s severity, such as the presence of a positive smear and cavity from our data (Table 1 and 2). In contrast, we hypothesized that a relapse after sputum conversion was associated with reinfection from environmental exposure and showed the association of relapse with soil exposure (Table 3). Table 4 and 5 showed that both the patient’s severity (negative smear) and the environmental exposure (low soil exposure) were associated with overall treatment success.

Table 4: Treatment success and patients without treatment success. How long were patients followed to define ‘patients without treatment success’? Instead of this wording, should you call ‘patients without treatment success ‘treatment failure’? Is it microbiologic failure, is it chemotherapeutic failure? There is nothing in the discussion that goes into other types of failure (were they absorbing the drug?)

The patients completed treatment for more than 12 months and were followed for at least 2 years to define treatment success. The mean durations of follow-up in the patients with treatment success and failure were not different (53.8 months vs. 53.8 months, Table 4). As Dr. Nyendak suggested, we call “patients without treatment success” “patients with treatment failure” (page 8, lines 113-115).

This study was aimed to define the microbiological outcomes, and treatment failure means microbiologic failure. We did not include the adherence to this regimen as a variable for these microbiological outcomes. However, all patients had regularly visited our clinics and completed this regimen for more than 12 months (page 17, lines 277-280).

For the radiographic findings – why is it characterized as (none) or (none or < 2). Seems like this latter category is confusing (and in the discussion they talk about large cavitary lesions but these are not in the tables). I worry that there may be some confounding if the persons without treatment success had a higher burden of disease to begin with and could explain the findings.
In accordance with Dr. Nyendak’s comments, we categorized the radiographic findings as any size of cavity and a large cavity (≥2 cm) (Table 1, 3 and 5). Patients with a large cavity were associated with no sputum conversion in the univariate analysis and were likely to confound with a high burden of bacteria. However, a large cavity was not significantly associated with a positive smear (P=0.06).

Is there an interaction between negative smear and low soil exposure?

There was no interaction between a negative smear and low soil exposure before treatments (P>0.99), after the start of treatments (P=0.57) or during the observation (P=0.89) (page 10, lines 150-152).

Minor essential revisions

This paper could be strengthened by ensuring that the two groups (relapse/no relapse) are as similar as possible prior to the analysis. The confusing way the radiographic findings are presented are also of note. Is the relapse/no relapse table and results univariate? Please clarify why this is presented with the treatment success data.

The radiographic findings are shown as the presence of a cavity (any size of cavity or a large cavity (≥2 cm)). Table 3 showed the univariate analysis. Because only soil exposure was statistically significant, we did not perform a multivariate analysis. We evaluated the factors for a relapse among the patients with sputum conversion (n=52). In contrast, the treatment success data were analyzed among all of the participants (n=72), including patients with sputum conversion (n=52) and without sputum conversion (n=20).

The authors might comment on the width of the confidence intervals as well, which gets at the small sample size in the groups.

As Dr. Nyendak’s suggested, we comment on the width of the confidence intervals as a limitation of our study (page 17, lines 276-277).
It does seem entirely plausible that ongoing soil exposure in patients with abnormal lung architecture are at risk for relapse.

Although underlying lung disease and the presence of cavitary disease were not significant risk factors for a relapse (Table 3), all of the relapsed patients had abnormal lung architecture due to underlying lung disease or preexistence of pulmonary MAC disease. Therefore, we also consider that ongoing soil exposure in patients with abnormal lung architecture is a risk for relapse.

With respect to the writing, while grammatically ok, with the re-write, would make sure the introduction is more streamlined.

We rewrote the introduction.