Author's response to reviews

Title: Identification of non-tuberculous mycobacteria isolated from clinical specimens at a tertiary care hospital: a cross-sectional study

Authors:

Imran Ahmed (imran.haq@aku.edu)
Kauser Jabeen (kausar.jabeen@aku.edu)
Rumina Hasan (rumina.hasan@aku.edu)

Version: 4 Date: 13 October 2013

Author's response to reviews: see over
Editor,  
BMC Infectious Diseases

Dear Editor,

Re: Submission of revised manuscript. MS: 1909470607100992  
“Identification of non-tuberculous mycobacteria isolated from clinical specimens at a tertiary care hospital: a cross-sectional study”.

We would like to submit a revised version of our manuscript. You will find below a point-by-point response to the concerns raised by reviewers. We are also emailing (to BMCSeriesEditorial [BMCSeriesEditorial@biomedcentral.com] ) the full text of an article referenced by reviewer 2. Please make available it to the reviewer if needed.

Please do not hesitate to contact us should you have any queries.

Thank you

Imran Ahmed,  
Kauser Jabeen, and  
Rumina Hasan

Department of Pathology & Microbiology  
Aga Khan University Hospital  
Stadium Road  
Karachi-74800  
Pakistan
Response to reviewers’ comments

MS: 1909470607100992

Identification of non-tuberculous mycobacteria isolated from clinical specimens at a tertiary care hospital: a cross-sectional study

Imran Ahmed, Kauser Jabeen and Rumina Hasan

Reviewer 1: George M Viola

Reviewer’s report:

Ahmed et al, discuss an important topic, which is the frequencies and antimicrobial susceptibility panel of non-tuberculous mycobacteria from Pakistan, a developing nation. Although, the antimicrobial susceptibility panel is clinically useful, the following comments would need to be addressed.

Major comments:

1) Line 92: “Significance assessment of NTM isolation was carried out in cases where clinical information was available.” It appears that clinical information was available for only 52/104 patients (50%- Line 131). Line 90: “Clinical information is routinely collected at AKUH clinical microbiology laboratory as good clinical practice to serve this purpose.” What kind of information was collected? Why wasn’t information available for all 104 patients?

Line 95: mentions the American Thoracic Society/ Infectious Diseases Society of America diagnosis for pulmonary NTM. As all NTM are environmental, and as the authors correctly mention, their recovery not always denotes true infection, but can be reflective of bacterial contamination, why are so many of the NTM considered to be a cause for pulmonary NTM infection with 1 sputum specimen (the ATS/IDSA recommends 1 BAL specimen or 2 sputum
specimen)? From the 104 patients included in the study, clinical data was available for only 52 patients (50%), in which the authors state that of these, 12 had significant pulmonary symptoms and 5 had significant extrapulmonary symptoms (total 17/104 patients, Table 2). If we remove all those patients that only had 1 NTM sputum specimen we would only have 5 patients with significant pulmonary and 5 with significant extrapulmonary symptoms (total 10/104 patients. In summary, as less than 10 percent of the cases can be considered clinically significant, it is difficult to determine whether or not the recovered NTM are truly reflective of a true infection or not.

**Answer:** We appreciate the reviewer’s concern of unavailability of clinical information for all the NTM isolates included in the study. AKUH clinical microbiology laboratory receives specimens not only from its associated hospital (i.e. in-patients, registered with the hospital) but also from all over the Pakistan through its collection point units (i.e. out-patients who are not registered with the hospital). Clinical information was collected for in-patients by liaising with their doctors to determine the significance of the NTM at the time of isolation. However, it was not possible for all the out-patients’ specimens coming in from outside the AKUH. The kind of clinical information collected included presenting symptoms and its duration, occupation, co-morbidities (e.g. malignancy, HIV infection), smoking, previous tuberculosis or tuberculosis treatment history, chronic obstructive pulmonary disease, cystic fibrosis, immunosuppression (HIV, malignancy, chemotherapy, steroid intake), surgical procedure (as a risk factor for extrapulmonary NTM infections), and chest X-ray and/or CT scan findings. We could not include individual patient’s clinical information for the fact that this information was collected from laboratory’s records and not from direct interaction of authors with patients or their clinicians. Following statement has been added to the manuscript (line number: 91-96)
“This included presenting symptoms, occupation, co-morbidities (e.g. malignancy, HIV infection), smoking, previous tuberculosis or tuberculosis treatment history, chronic obstructive pulmonary disease, cystic fibrosis, immunosuppression (HIV, malignancy, chemotherapy, steroid intake), surgical procedure (as a risk factor for extra-pulmonary NTM infections) and chest X-ray and/or CT scan findings.”

It is rightly said that NTM are environmental organisms and their isolation does not always denote true infection, clinical correlation is necessary before considering an NTM isolate as significant. According to ATS/IDSA guidelines 2 ‘sputum’ samples are required to diagnose NTM pulmonary infection. The reason for including some of the patients with only one sputum culture positive for NTM was based on a strong clinical suspicion of NTM as the cause of patient’s symptoms and that most of these patients had AFB smear-positive from the sample (2nd sputum specimen could not be collected). Therefore, the diagnosis in these cases is not certain, but a potential one. Multiple factors increase the probability of clinical significance of NTM: “recovery from multiple specimens or sites, recovery of the organism in large quantities (AFB smear-positive specimens) or recovery of an NTM isolate from a normally sterile site” (ATS/IDSA statement). If we remove patients with only one sputum specimen positive for NTM, 5 pulmonary and 5 extra-pulmonary NTM isolates would be left as significant. This would in essence mean 20/52 significant NTM isolates from 10/42 patients, because clinical information was not available for the rest of 52 NTM isolates (52 patients).

Now line number 138-139 reads as follows:

“Clinical information was available for 50% of the isolates (52 NTM from 42 patients) (to assess significance of NTM isolated.”

Following statement has been added to the manuscript:
“The reason for including some of the patients with only one sputum culture positive for NTM was based on a strong clinical suspicion of NTM as the cause of patient’s symptoms and that most of these patients had AFB smear-positive from the sample (2nd sputum specimen could not be collected). Therefore, the diagnosis in these cases is not certain, but a potential one.” (line number: 140-144)

2) Line 127: “Among 54/71 (76%), and 19/33 (58%) SGM could be further identified.” That is a total of 73/104 (70%) patients had their NTM identified. As several NTM exists with diverse antimicrobial susceptibility panels, although difficult to obtain in resource limited nations, this information would enhance the usefulness of this paper.

**Answer:** This would have been a nice addition to the article. Unfortunately we do not have this information available for all the NTM species.

3) As NTM may be considered a contaminant, especially if multiple organisms are recovered from a non-sterile specimen, where any of the respiratory or extrapulmonary specimens, polymicrobial, or where they all monomicrobial? For example if 1 sputum specimen revealed NTM, candida, and alpha hemolytic streptococcus, it will be difficult to determine if the recovered NTM is a true infection or just a contaminant.

**Answer:** The information of other bacterial growth was not reviewed for this study. However, the isolates were referred to be significant only if they have been isolated in multiple cultures from a nonsterile site or from a sterile site with strong clinical correlation.

**Minor comment:**
Line 156: As it appears that several of these specimens were submitted to Aga Khan, a tertiary referral center, as NTM are environmental organism with a diverse ecological niche, it would be interesting to illustrate a map of Pakistan, where the different NTM were isolated.

**Answer:** A map of Pakistan with geographic location of NTM isolation is represented in figure 1.
Reviewer 2: Won-Jung Koh

Reviewer's report:

The authors present the epidemiology of NTM isolated from clinical specimens at a tertiary care hospital in Karachi, Pakistan.

Major comments

1. The authors described that this is the first study regarding NTM epidemiology in Pakistan. However, at least one abstract of previous study conducted in the same region (Karachi, Pakistan) is available in the PubMed database. Khanum T, Rasool SA, Ajaz M, Khan AI. Isolation-drug resistance profile and molecular characterization of indigenous typical and atypical mycobacteria. Pak J Pharm Sci. 2011 Oct;24(4):527-32. http://www.ncbi.nlm.nih.gov/pubmed/21959816

According to the above study, M. xenopi was the most common NTM isolates from clinical specimens. M. kansasii and M. thermoresistible were frequently isolated and M. fortuitum was relatively rare isolate. These results were very different from those of the current manuscript by Ahmed and coworkers. The authors need to comment this disagreement between two studies, although both studies were performed in the same region in Pakistan.

Answer: The study reported by Khanum et. al. was published in 2011 and can indeed be credited as being the first of its kind from the region. However, several points undermine its merits: 1. In the abstract, Khanum et. al. describe utilizing PCR based identification and differentiation of 16S rRNA gene(s). The authors did not discuss use of 16S rRNA any further in the materials and methods, results or discussion in the full text (full text article has been emailed to BMC editorial board). However, they describe using EZTBPCR kit (Bio Diagnostic Research Company, Malaysia). This kit differentiates mycobacteria as *M. tuberculosis* and nontuberculous
mycobacteria only, without further species differentiation of NTM. 2. Khanum et. al. paper describe subjecting 40 isolates to PCR based identification. With this, 37.5% were identified as typical mycobacteria and 25% were identified as atypical mycobacteria. These percentages do not add up to 100% and there is no explanation for this in the full text of the article. 3. In the discussion section, Khanum et. al. correlate their findings with an Indian study (Agarwal A, Jindal N. Isolation rates of non-tuberculous mycobacteria from Amritsar. Indian J Med Microbiol 2001;19:230-1) that their isolation of a lower percentage of atypical mycobacteri a than typical mycobacteria was in agreement with Agarwal et. al. Although partially right, but Agarwal et. al. had an isolation rate of 10.6% NTM, whereas Khanum et. al. describe it as 41.33% NTM which is a big difference between the findings of the two studies. Having said that, the difference in our results and the ones reported by Khanum et. al. are surprising and need further studies to confirm these findings. Khanum et. al. did not relate the isolation of NTM with the clinical findings. We have tried to make this link in the present study and this feature makes it the first study reporting such findings from Pakistan.

Following additions to the manuscripts have been made:

“A study from Karachi, Pakistan, described M. xenopi as the most common while M. fortuitum as relatively rare NTM species among isolates collected from four laboratories in Karachi. These results are in contrast with the present study and need further studies to confirm the findings.” (line number: 173-176)

Now line numbers 32-33 and 244-245 read as follows:

“This is the first study reporting NTM species and their clinical significance isolated from clinical specimens from Pakistan.”
2. How could the authors divide the NTM isolates into RGM and SGM? The differentiation between RGM and SGM could be difficult for some NTM isolates which were cultured only in MGIT liquid media, not on the LJ solid media. (Kim CJ, et al. Differentiating rapid- and slow-growing mycobacteria by difference in time to growth detection in liquid media. Diagn Microbiol Infect Dis. 2013 Jan;75(1):73-6.)

**Answer:** Conventionally, mycobacteria are divided into RGM and SGM based on their growth rate on a standard solid culture medium. The same was performed in the present study. MGIT liquid media were used for specimen inoculation and not for growth rate determination. Briefly, a well isolated colony growing on a solid culture (i.e. Middlebrook 7H10 agar) was subcultured to 7H9 broth containing Tween-80 and incubated until slightly turbid. This suspension was then diluted 1:100 and a loopful of this was subcultured to Middlebrook 7H10 and streaked to get isolated colonies. Colonies appearing ≤7 days were considered RGM and those appearing after 7 days were SGM. (Reference number 13 in the manuscript).

Now, line 109-114 reads as follows after revision:

“**Growth rate (as determined by subculturing the organism onto Middlebrook 7H10 agar after growing a well isolated colony in Middlebrook 7H9 broth with Tween-80) and colony pigmentation were assessed and para-nitro benzoic acid (PNB) sensitive and thiophene-2-carboxylic acid hydrazide (T2H) resistant strains (M. tuberculosis ssp. bovis is T2H sensitive) were identified as M. tuberculosis complex and excluded from the study.”**

3. For the species identification of cultured NTM, the authors used conventional biochemical tests rather than molecular identification methods, because of “cost or lack or expertise”.

However, Khanum and co-workers performed the PCR based molecular identification method

**Answer:** This is one of the limitations of the present study and described in the manuscript (line number 237). However, as described in the answer to comment number 1 above, Khanum et. al. describe using 16S rRNA in the abstract of their study, but failed to discuss it in the materials and methods, results and discussion section of the article. Their description of the species, as we understand, is based on phenotypic identification. This disagreement has now been addressed in the present study. Please see answer to comment 1 above.

4. Clinical information was available in only 50% of patients. In addition, at least two positive sputum cultures were needed to diagnose the NTM lung disease. According to Table 2, however, many patients with clinically significant NTM isolates had only one positive sputum culture. These should be clarified.

**Answer:** Clinical information was available for 52 NTM isolates from 42 patients (revised line number: 137). According to ATS/IDSA guidelines 2 ‘sputum’ samples are required to diagnose NTM pulmonary infection.

“The reason for including some of the patients with only one sputum culture positive for NTM was based on a strong clinical suspicion of NTM as the cause of patient’s symptoms and that most of these patients had AFB smear-positive from the sample (2nd sputum specimen could not be collected). Therefore, the diagnosis in these cases is not certain, but a potential one.” (line number: 140-144)
Multiple factors increase the probability of clinical significance of NTM: “recovery from multiple specimens or sites, recovery of the organism in large quantities (AFB smear-positive specimens) or recovery of an NTM isolate from a normally sterile site” (ATS/IDSA statement).

If we remove patients with only one sputum specimen positive for NTM, 5 pulmonary and 5 extra-pulmonary NTM cases would be left as significant. This would in essence mean 20/52 significant NTM isolates from 10/42 patients, because clinical information was not available for the rest of 52 NTM isolates (52 patients).

5. Drug susceptibility tests were performed in only 28% (20/71) of RGM and 52% (17/33) of SGM.

**Answer:** This would have been a nice addition to the article. Unfortunately we do not have this information available for all the NTM species.

**Minor comments**

1. Some abbreviations (M, male and F, female) describe below Table 2 were not used in Table 2.

**Answer:** We thank you for mentioning this to us. The same have been deleted from table 2.

2. The year of publication was not available in some references (ref #6, #11, and #19).

**Answer:** We thank you for pointing out this omission. The year of publication has been included in the reference number 6, 11 and 20 (previously number 19).