Reviewer’s report

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

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Reviewer: George M Viola

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Ahmed et al, discuss an important topic, which is the frequencies and antimicrobial susceptibility panel of non-tuberculous mycobacterias 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.

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 there 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.

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.

Minor comment:
1) Line 156: As it appears that several of these specimens where 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 where isolated.

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

**Quality of written English:** Acceptable

**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