Reviewer's report

Title: Increasing Nontuberculous Mycobacteria Reporting Rates and Species Diversity Identified in Clinical Laboratory Reports

Version: 0 Date: 14 Dec 2017

Reviewer: Troels Lillebæk

Reviewer's report:

Comments (in order of appearance - not importance)
P3, L54-56: You write: "NTM illnesses are generally not communicable, although one case study has shown a disease cluster of M. abscessus subsp massiliense was most likely due to person-to-person 56 transmission" I suggest to include this important reference (which is "not only a case study"): Bryant JM et al. Emergence and spread of a human-transmissible multidrug-resistant nontuberculosis mycobacterium. Science 2016; 354(6313):751-757.

P4, L74-76: You write: "Having clinically significant NTMs isolated more frequently from environmental sources (specifically potable water) than in past studies suggests the potential that exposure to these NTM species is increasing." You do not know for sure if they are actually clinical significant, that is causing clinical disease in humans or at least to which degree they are. At least rephrase to "Having potentially clinically significant NTMs isolated more frequently from environmental sources (specifically potable water) than in past studies suggests the exposure to these NTM species might increase."

P6, L 93-94: You write "The methods used to speciate the recovered NTMs by all four states were largely mycolic acid analysis and DNA probes." This is a rather unprecise description of the methods used for species ID. I suggest to be much more precise; indicate specific diagnostic tests used and add the relevant references. E.g. add a column to table 1.

P6, L 101-103: You write "In this study, the 2014 data from the four states are compared to the 1994 NTM data from CDC's 1999 report in the U.S [14]. The 1994 positive result numbers by
species for each of the four states were combined for comparison with the 2014 totals." If I understand correct, you compare four states with total numbers for all of US. Can you justify this comparison? Are the four states representative for NTM overall distribution in all of US. How are the 4 states distributed geographically compared with the states included in the overall figure. It seems important to justify the conclusions of an "increase" in NTM from 1994 to now that this comparison is valid.

P6, L109-110: You write: "Over the past 40 years, methods used to recover NTM from a human specimen have not changed dramatically, what has changed is the procedures used for identification" I am not sure you are correct and what do you mean by "methods used to recover". Surely, over the past 40 years, there has been increasingly attention paid to NTM = much more samples analyzed on - as you write - increasingly improved diagnostic systems. Thus, simply because of more examinations (and better diagnostics) you will see more positive samples. However, that does not necessarily mean more NTM definite disease (the increasing nos. of positive samples can represent colonization - as see in e.g. Denmark and other countries - or just possible disease), see reference https://www.ncbi.nlm.nih.gov/pubmed/28751677

P6, L112-113: You write: "The DNA/RNA probes, and mycolic acid methods introduced in the 1990's are the methods employed by the State Labs, during the dates included in study" Same comment as P6, L93-94. It’s important for interpretation of results how much better, the recent diagnostic methods were compared to the 1990s methods, e.g. more sensitive.

P6, L130-131: You write: "The Mississippi, Missouri, Ohio, and Wisconsin disease network had 4,200 NTM reports submitted in 2014." How do you know that the "reporting habits" in 2014 reflects the "reporting habits" in the 1990s? It could be that laboratories are more likely to report NTMs in 2014 vs. 1990s, thus, increasing rates represent increasing reporting. Justify comparison in text

P6, L131-132: You write: "The overall NTM prevalence rate was 16.0 positive reports per 100,000 persons" As I read it you can have numerous reports per person? Likely more per person
in 2014 that 1990s? If numerous, the increasing rate could reflect more reports per person in 2014 because of easier access to diagnostics.

P8, L140-141: You write: "The five most common species isolated from human specimens were M. avium 705/4,200 (17%), M. gordonae 688/4200 (16%), …" I assume you did not include gordonae when reporting increasing rates? Gordonae nearly always represent contamination/colonization - not disease (except rare cases of immunocompromised patients). I suggest to leave out gordonae completely (if not already done) - it's highly irrelevant.

P8, L146-148: You write: "In 2014, there were 4,200 reports with a report rate of 16.0 reports per 100,000 persons (Table 1), in these four states. This rate is two times higher than what was observed 20 years ago. Based on the 2014 data, an estimated 50,970 NTM reports are projected nationally" In line with my previous comments. What does two times higher really represent? More samples examined? Better diagnostics? Changed reporting habits? Contamination? Colonization? Disease? This, is crucial for conclusions in the article. Important to discuss limitations for the study and not to conclude more than the data / method(s) justify.

P7, L151-153: You write: "The three complexes/groups that had statistically significant rate increases were: M. chelonae-abscessus group, M. fortuitum group and M. avium complex. All are clinically significant NTMs." This is highly arguable. Normally, you need to apply ATS-guidelines to decide if NTM causing disease (in brief, symptoms + Xray findings + >= 2 specimens positive and other diagnosis excluded). Or you can classify patients into three NTM disease categories using the modified American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) 2007 criteria based on microbiological data only: definite NTM disease, possible NTM disease, and NTM colonization (ref: Griffith, D. E. et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am. J. Respir. Crit. Care Med. 175, 367-416 (2007)). This method has been validated by Andrejak et al. in 2010 (Ref: Andrejak, C. et al. Nontuberculous pulmonary mycobacteriosis in Denmark: incidence and prognostic factors. Am. J. Respir. Crit. Care Med. 181, 514-521 (2010).).
You write: "There are strengths to using state NTM reports to investigate the epidemiology of a disease or microorganism. State NTM reports capture illness in both the young and elderly population as well as the poor and the uninsured." How do state reports capture illness. In line with section just above, how do you know the reports reflect illness?

These are limitations of the State data; more than one report may belong to one individual because more than one specimen was collected by a physician during the course of a single episode in order to evaluate treatment efficacy. These over counting instances can overestimate the burden of disease associated with NTM." Absolutely, but then why conclude there is an increase? Or at least modify conclusion considerably.

Numerous factors could have contributed to the increased prevalence between 1994 and 2014. They include increased awareness of NTM related infections/diseases, improved laboratory techniques, increases in underlying risk factors, an increase in human activities associated with water (aquatic or water therapy, water aerobics) and or soil (gardening), and changes in the modes by which people are exposed. Nonetheless, the number of reports, and two fold increase in positive reports indicate that NTM illnesses affect human health". I disagree with "the number of reports, and two fold increase in positive reports indicate that NTM illnesses affect human health". You cannot (in my opinion) conclude that from the data presented.

**Are the methods appropriate and well described?**

If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**

If not, please specify which controls are required in your comments to the authors.

Unable to assess

**Are the conclusions drawn adequately supported by the data shown?**

If not, please explain in your comments to the authors.

No
Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?

If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

Not relevant to this manuscript

Quality of written English

Please indicate the quality of language in the manuscript:

Acceptable

Declaration of competing interests

Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors'
responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal